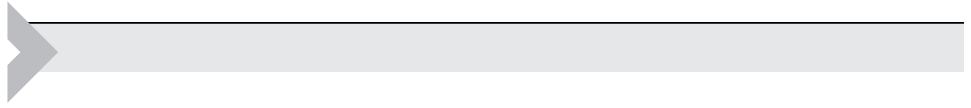Douglas G. Capone
Deborah A. Bronk
Margaret R. Mulholland
Edward J. Carpenter

Nitrogen in the Marine Environment

2nd Edition

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NITROGEN IN THE MARINE ENVIRONMENT

2nd Edition

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NITROGEN IN THE MARINE ENVIRONMENT

2nd Edition

DOUGLAS G CAPONE
DEBORAH A BRONK
MARGARET R MULHOLLAND
EDWARD J CARPENTER

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DEDICATION

We dedicate the second edition of Nitrogen in the Marine Environment to our spouses, Linda Duguay, Bill Norton, Skip Stiles, and Gerdi Weidner, for their constructive input to this volume and to our children (Jennifer and Rebecca Capone, Zach, Zane and Zoë Norton, Maeve and Patrick Mulholland Stiles, Daniel Carpenter and Rachel Poccia) for enduring our endless conversations revolving around nitrogen and the oceans.

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Foreword

A Timely Book for Interesting Times

Since the first edition of this book in 1983, several revolutions have occurred vis à vis scientific studies of the marine nitrogen cycle. Just consider some of the words and phrases, that appear in this edition but were unknown or seldom-used by marine scientists in 1983. Here are just a few: anammox, archaea, polymerase chain reaction, isotopomer, trace metal limitation. In 1983, we were in the early stages of just being able to properly enumerate the bacteria occurring in sea water. Now we talk about their inherent genetic capabilities of bacteria and archaea, and we are beginning to understand that viruses also play an important role. With respect to the oceanic fixed-N budget several source and sink terms have been increased several-fold, suggesting much more rapid turnover than envisioned in 1983. Thus this book appears at an ''interesting time'' vis à vis changes in our understanding of nitrogen in the marine environment.

While these massive changes in understanding would be more than sufficient to justify an update of the first edition, the articles in this book have perhaps a more important raison d'etre, because of the crucial role that nitrogen cycling plays in atmospheric chemistry and in aquatic productivity. In 1983, we were well aware that anthropogenic greenhouse gas emissions were likely to cause global warming, but it is probably fair to say that the prevailing view was of a process that involved gradual change. Now, we have ice-core data suggesting the possibility of massive climate change on sub-decadal time scales, and we are witnessing great perturbations in the marine ecosystem. In my view, the time is not far off when every scrap of understanding about marine ecosystems will be needed to help adapt to and mitigate global change. Because nitrogen plays such a key role, the chapters in this book are timely as a vehicle for accelerating understanding.

The editors and authors are to be commended for taking on the difficult task of providing a comprehensive update of the deliciously complex subject of nitrogen in the marine environment, and producing a timely book for interesting times!

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Louis A. Codispoti
Horn Point Laboratory,
University of Maryland Center for Environmental Science,
Cambridge, Maryland

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Preface to Second Edition

The debut of the first edition of Nitrogen in the Marine Environment in 1983 chronicled the tremendous growth in knowledge concerning the cycling of nitrogen in the sea to that point and heralded the explosive expansion in work on oceanic nitrogen cycling that quickly followed (Fig. 1). Nitrogen in the Marine Environment, second Edition (long overdue!) is the natural witness to that growth. The page count alone has doubled relative to the original edition, and this edition is still insufficient to cover all the threads of ongoing research on nitrogen in the sea.

Unprecedented discoveries over the last decade have changed the way we view the marine nitrogen cycle. These include the recognition that phytoplankton in the sea are not uniformly limited by nitrogen, and that the critical limiting nutrient or nutrients (we now recognize co-limitation) vary within and among systems as a result of differences in external forcings and the biogeochemically important functional groups present and consequently selected. The critical role of picoplankton in marine ecosystems and biogeochemical cycles (including nitrogen dynamics) is now well established.

Published Items in Each Year
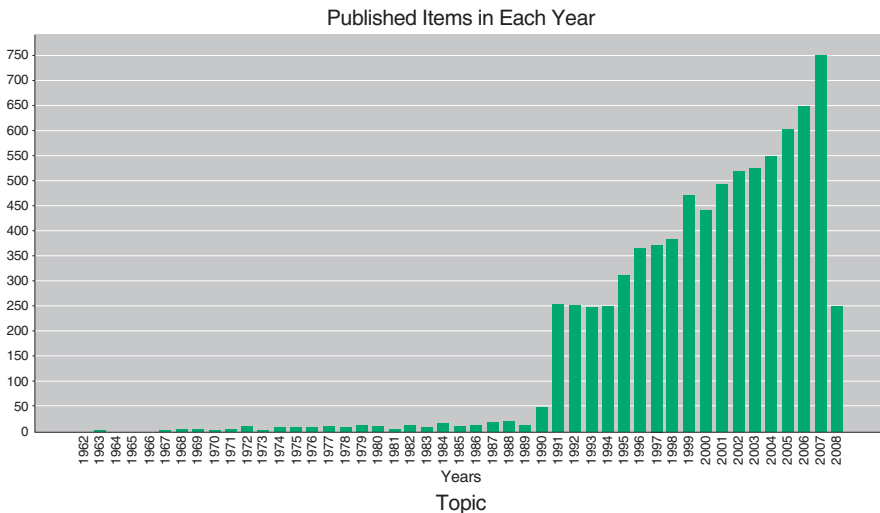
Figure 1. Number of papers by year with topics including "nitrogen and marine or nitrogen and ocean" from the Web of Science database. We assume the jump in citation rate from 1989 to 1991 represents changes in the data base itself (e.g., new journals included) rather than being a result of publication of the first edition of Nitrogen in the Marine Environment. The steady growth from 1992 is evident, and the number of papers doubled from 1993 to 2003.

We have gained a new appreciation of the diversity and the quantitative importance of marine nitrogen fixers. We now recognize the significance of Archaea and the processes they mediate (e.g., nitrification) and the importance of anammox bacteria in some marine ecosystems. In addition, our traditional view of just who does what in the marine nitrogen cycle is shifting as we discover that taxonomic groups often don't fit squarely into the functional group they've been assigned and furthermore that the biogeochemical roles of bacteria and phytoplankton often overlap. Even our understanding of what constitutes the labile fraction of the dissolved nitrogen pool has changed. We have identified and begun to quantify how many previously unrecognized biotic and abiotic processes affect the marine nitrogen cycle (e.g., extracellular enzymes, use of novel dissolved organic nitrogen compounds, photochemical processes, etc.). This illustrates just how much we have learned and are still discovering about the marine N cycle. We could go on—that's why the book is so long!

Numerous factors have fueled this revolution. Perhaps first and foremost is the appreciable growth of practitioners in this field bringing new tools and unique perspectives. This growth has occurred in tandem with the arrival of new technologies enabling us to address questions we couldn't even imagine or articulate two decades ago. Advances in molecular biology, analytical techniques, remote sensing, sensor technology, and computational methods and power have all had an impact. Moreover, with the burgeoning availability of time-series data sets, these diverse factors have synergistically led to the steady expansion of data collection and synthesis over the last twenty plus years. We have developed the capacity and infrastructure to measure more things in greater numbers and at lower cost.

The pace of discovery continues to accelerate. So what does the future hold? We believe we can safely predict that our resolution of the temporal and spatial scales of variability of the populations and processes relevant to understanding the nitrogen cycle will steadily increase with the improvement and deployment of moored arrays and remote observation systems with novel nutrient and biological sensors. Numerous metagenomic surveys are underway and the prospect that novel organisms and pathways will be revealed is practically a given. Such novel observations will inform and stimulate a new generation of experimentalists as well.

Alarmingly, the sea itself is changing at an unprecedented rate, in large part due to human influences. Increasing CO_2 concentrations, temperatures, coastal eutrophication, atmospheric nitrogen deposition, and decreasing pH will continue or even accelerate over the upcoming decades. What does this portend for the marine nitrogen cycle? What feedbacks within the system may minimize or aggravate the nature and rate of changes in marine ecosystems? Can, and should, the marine nitrogen cycle be manipulated to mitigate these changes? These are all questions on the table to be addressed by current and future cohorts of marine "azotonauts."

We expect the third edition will necessarily follow soon!

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Douglas G. Capone
Deborah A. Bronk
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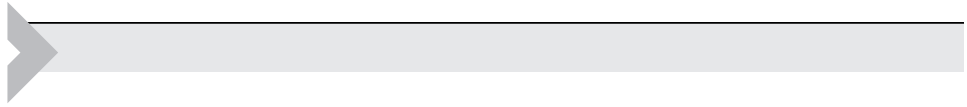
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Contributors

Lihini I. Aluwihare
Geosciences Research Division, Scripps Institution of Oceanography, La Jolla, California

Iris C. Anderson
School of Marine Science, College of William and Mary, Gloucester Point, Virginia

Hermann W. Bange
Forschungsbereich Marine Biogeochemie, IFM-GEOMAR, Leibniz-Institut für Meereswissenschaften, Kiel, Germany

John A. Berges
Department of Biological Sciences, University of Wisconsin – Milwaukee, Milwaukee, Wisconsin

Ilana Berman-Frank
Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel

Robert R. Bidigare
School of Ocean and Earth Science and Technology, Department of Oceanography, University of Hawaii, Honolulu, Hawaii

Elizabeth W. Boyer
Department of Environmental Science, Policy, and Management, University of California, Berkeley, California

Walter R. Boynton
Chesapeake Biological Laboratory, Center for Environmental Science, University of Maryland, Solomons, Maryland

Deborah A. Bronk
Department of Physical Sciences, The College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, Virginia

Douglas G. Capone
Department of Biological Sciences and Wrigley Institute for Environmental Studies, University of Southern California, Los Angeles, California

Edward J. Carpenter
Romberg Tiburon Center, San Francisco State University, Tiburon, California

Yi-Bu Chen
Norris Medical Library, University of Southern California, Los Angeles, California

James R. Christian
Fisheries and Oceans Canada, Institute of Ocean Sciences, Sidney, British Columbia, Canada
Canadian Centre for Climate Modelling and Analysis, University of Victoria, Victoria, British Columbia, Canada

Matthew J. Church
School of Ocean and Earth Science and Technology,
Department of Oceanography, University of Hawaii, Honolulu, Hawaii

William P. Cochlan
Romberg Tiburon Center for Environmental Studies, San Francisco State University, Tiburon, California

Richard C. Dugdale
Romberg Tiburon Center, San Francisco State University,
Tiburon, California

Allan H. Devol
School of Oceanography, University of Washington, Seattle, Washington

John E. Dore
School of Ocean and Earth Science and Technology,
Department of Oceanography, University of Hawaii, Honolulu, Hawaii

Paul G. Falkowski
Institute of Marine and Coastal Sciences, Rutgers University,
New Brunswick, NJ, USA
Department of Geological Sciences, Rutgers University, New Brunswick, New Jersey

Katja Fennel
Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada

Michael J. Follows
Department of Earth, Atmospheric, and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts

Peggy Fong
Department of Ecology and Evolutionary Biology, University of California, Los Angeles, California

Rachel A. Foster
Ocean Sciences Department, University of California, Santa Cruz, California

Fei-Xue Fu
Department of Biological Sciences and Wrigley Institute for Environmental Studies, University of Southern California, Los Angeles, California

Jed A. Fuhrman
Department of Biological Sciences and Wrigley Institute for Environmental Studies, University of Southern California, Los Angeles, California

Eric D. Galbraith
Department of Geosciences, Princeton University, Princeton, New Jersey

Yuan Gao
Department of Earth and Environmental Sciences, Rutgers University, Newark, New Jersey

Anne E. Giblin
The Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts

Edna Granéli
Department of Marine Sciences, Kalmar University, Kalmar, Sweden

Wilhelm Granéli
Department of Limnology, Lund University, Lund, Sweden

Nicolas Gruber
Institute of Biogeochemistry and Pollutant Dynamics, Department of Environmental Sciences, ETH Zurich, Zurich, Switzerland

Waldemar Grzybowski
Institute of Oceanography, Gdansk University, Gdynia, Poland

Dennis A. Hansell
Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida

John A. Harrison
School of Earth and Environmental Sciences, Washington State University, Vancouver, Washington

Ian Hewson
Ocean Sciences Department, University of California, Santa Cruz, California

Raleigh R. Hood
Horn Point Laboratory, University of Maryland Center for Environmental Science, Cambridge, Maryland

Charles S. Hopkinson
Department of Marine Sciences, University of Georgia, Athens, Georgia

Robert W. Howarth
Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York

David A. Hutchins
Department of Biological Sciences and Wrigley Institute for Environmental Studies, University of Southern California, Los Angeles, California

Bethany D. Jenkins
Department of Cell and Molecular Biology and Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island

Samantha B. Joye
Department of Marine Sciences, University of Georgia, Athens, Georgia

David M. Karl
School of Ocean and Earth Science and Technology, Department of Oceanography, University of Hawaii, Honolulu, Hawai

W. Michael Kemp
Horn Point Laboratory, Center for Environmental Science, University of Maryland, Cambridge, Maryland

Raphael M. Kudela
Ocean Sciences Department, University of California—Santa Cruz, Santa Cruz, California

Ricardo M. Letelier
College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, Oregon

Fredric Lipschultz
Bermuda Institute of Ocean Sciences, St. George, Bermuda

Michael W. Lomas
Bermuda Institute for Ocean Sciences, St. George, Bermuda

Claire Mahaffey
Department of Earth and Ocean Sciences, University of Liverpool, Liverpool, UK

Matthew D. McCarthy
Ocean Sciences Department, University of California, Santa Cruz, California

Karen J. McGlathery
Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia

Travis Meador
Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

Allen J. Milligan
Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, USA

Joseph P. Montoya
School of Biology, Georgia Institute of Technology, Atlanta, Georgia

Margaret R. Mulholland
Old Dominion University, Norfolk, Virginia

S. Wajih A. Naqvi
National Institute of Oceanography, Dona Paula, Goa, India

Gregory D. O'Mullan
Lamont–Doherty Earth Observatory, Columbia University, Palisades, New York

Judith M. O'Neil
Horn Point Laboratory, University of Maryland Center for Environmental Science, Cambridge, Maryland

Hans W. Paerl
University of North Carolina at Chapel Hill, Institute of Marine Sciences, Morehead City, North Carolina

Thomas F. Pedersen
School of Earth and Ocean Sciences, University of Victoria, Victoria, British Columbia, Canada

Michael F. Piehler
University of North Carolina at Chapel Hill, Institute of Marine Sciences, Morehead City, North Carolina

Anton F. Post
The H. Steinitz Marine Biology Laboratory, The Interuniversity Institute for Marine Science, Coral Beach, Eilat, Israel

Rebecca S. Robinson
Graduate School of Oceanography, University of Rhode Island, Rhode Island

Grace K. Saba
The College of William and Mary, Virginia Institute of Marine Science, Virginia

David J. Scanlan
Department of Biological Sciences, University of Warwick, Coventry, United Kingdom

Sybil P. Seitzinger
Department of Earth and Environmental Sciences, Rutgers University, Newark, New Jersey

Daniel M. Sigman
Department of Geosciences, Princeton University, Princeton, New Jersey

Deborah K. Steinberg
Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, Virginia

Lars Tranvik
Limnology, Department of Ecology and Evolution, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden

Bess B. Ward
Department of Geosciences, Princeton University, Princeton, New Jersey

Frances Wilkerson
Romberg Tiburon Center, San Francisco State University, Tiburon, California

Jonathan P. Zehr
Department of Ocean Sciences, University of California, Santa Cruz, California

THE MARINE NITROGEN CYCLE: OVERVIEW AND CHALLENGES

Nicolas Gruber

Contents

1. INTRODUCTION

The marine nitrogen cycle is perhaps the most complex and therefore the most fascinating among all biogeochemical cycles in the sea. As a limiting element for biological productivity, nitrogen occupies a central role in ocean biogeochemistry, exerting a significant influence on cycles of many other elements, in particular carbon
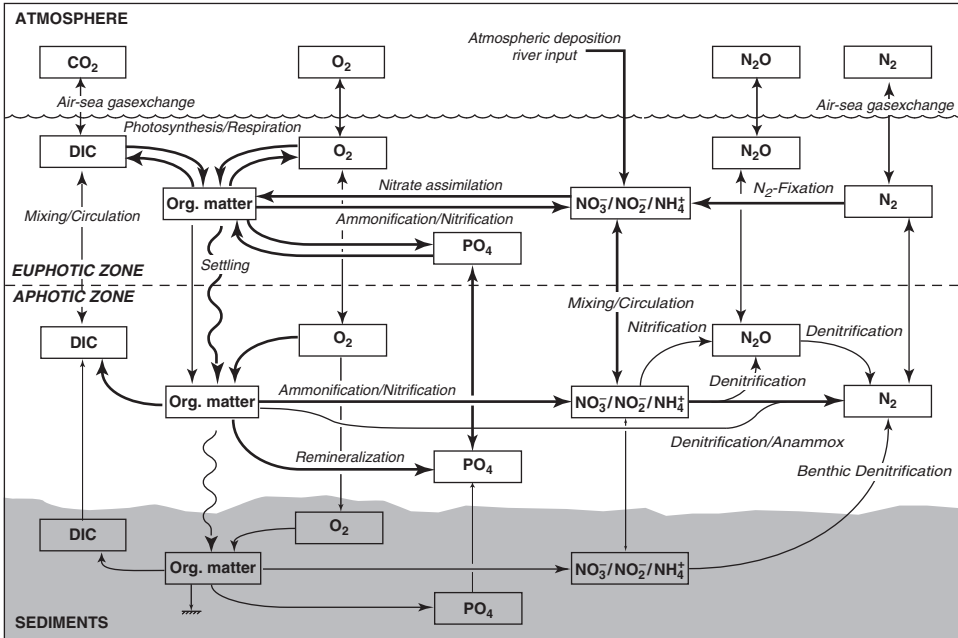
Figure 1.1 Schematic representation of the marine nitrogen cycle and its coupling to the marine cycles of oxygen, phosphorus, and carbon. Of particular importance are the processes of nitrogen fixation and denitrification, which make the fixed nitrogen content of the ocean open to biologically mediated changes.

and phosphorus (see Fig. 1.1). Nitrogen exists in more chemical forms than most other elements, with a myriad of chemical transformations that are unique to this element. Nearly all these transformations are undertaken by marine organisms as part of their metabolism, either to obtain nitrogen to synthesize structural components, or to gain energy for growth. Although most chemical forms of nitrogen in the ocean are bioavailable, the most abundant chemical form, dissolved nitrogen gas (N_2), is generally not. To emphasize this distinction, one often refers to all forms of nitrogen, except N_2, as *fixed* nitrogen. The general unavailability of N_2 for marine organisms gives the two biological processes of N_2-fixation, i.e. the conversion of N_2 to organic nitrogen, and denitrification, i.e. the conversion of nitrate to N_2, a particular importance. The balance of these two processes determines to first order the size of the oceanic inventory of bioavailable nitrogen, and consequently marine productivity. Marine biological processes in interaction with ocean transport and mixing control also the spatio-temporal variations of the various forms of fixed nitrogen within the ocean.

The primary engine that drives these ocean interior variations is the photosynthetic fixation of carbon into organic matter by marine phytoplankton in the light-illuminated upper ocean (euphotic zone) (see Fig. 1.1). Along with carbon, nutrient elements such as nitrogen, phosphorus, iron and many others are taken up and assimilated. Most of the resulting organic matter is either respired or remineralized

in the euphotic zone, but a fraction of it escapes remineralization and is exported into the dark interior ocean (aphotic zone). There, it is remineralized back to its inorganic forms. Finally, these inorganic forms are transported back to the euphotic zone by ocean circulation and mixing, where they can fuel new growth. This coupling between biological and physical processes thus forms a large-scale biogeochemical loop that controls the distribution of nearly all biogeochemically active chemicals in the ocean. I use the expression ''loop'' here instead of the more commonly used term ''biological pump,'' as ''loop'' emphasizes the coupling between the biologically driven downward transport of organic matter and the physically controlled upward transport of inorganic constituents. As a result of this loop, surface concentrations of the inorganic forms of chemicals, particularly limiting nutrients, are low, while the deep ocean is enriched in these inorganic forms. Concomitantly, the concentrations of organic forms, both particulate and dissolved, are high in the near-surface ocean and tend to decrease rapidly with depth.

This biologically driven biogeochemical loop is fundamental for Earth's climate, as it is one of the processes that determines the concentration of CO_2 in the atmosphere. If this biogeochemical loop were eliminated today, atmospheric CO_2 would raise by more than 200 parts per million (ppm) (Gruber and Sarmiento, 2002). Conversely, if biology became completely efficient in drawing down surface nutrients and exporting the fixed nutrients and carbon to depth, atmospheric CO_2 would fall by more than 100 ppm. Therefore, not surprisingly, changes in the strength of this loop have been invoked from the very beginning as a possible explanation of the large swings in atmospheric CO_2 that occurred over the past million years in association with the glacial–interglacial cycles (e.g., Brzezinski *et al.* (2002); Martin (1990); Sarmiento and Toggweiler (1984); Siegenthaler and Wenk (1984)). Given the key role of nitrogen as a nutrient that limits biological productivity, the hypothesis that alterations of the total amount of fixed nitrogen in the ocean have caused changes in productivity and consequently atmospheric CO_2 is tantalizing (Altabet *et al.*, 1995, 2002; Broecker and Henderson, 1998; Falkowski, 1997; Ganeshram *et al.*, 1995; McElroy, 1983). However, as will be discussed later in this chapter, there are several arguments casting doubts on this hypothesis (see also Gruber (2004) and Chapter 34 by Galbraith *et al*, this volume).

Interest in the marine nitrogen cycle has soared in the last decade, owing to several converging factors. First is the growing recognition that the immense acceleration of the global nitrogen cycle by humans, largely driven by the fabrication of nitrogen fertilizers from atmospheric N_2 and its subsequent application on agricultural soils (Galloway *et al.*, 2004), is changing not only terrestrial ecosystems and freshwater systems, but increasingly also coastal ecosystems (e.g., Beman *et al.* (2005)). The magnitude of this acceleration becomes evident when one considers that the amount of N_2 fixed each year by industrial processes is of similar magnitude as the amount of nitrogen fixed biologically (Falkowski *et al.*, 2000; Galloway *et al.*, 1995, 2004).

A second factor that has furthered interest in the marine nitrogen cycle is the solidifying evidence that the magnitude of marine N_2 fixation is much larger than was thought 20 years ago when the first *Nitrogen in the marine environment* book was published (see e.g., Capone (2001), Capone *et al.* (2005), Gruber (2005), and Mahaffey *et al.* (2005)). The most recent estimates for global N_2 fixation imply a mean residence

time of fixed nitrogen in the ocean of a few thousand years only (Gruber, 2004), an order of magnitude shorter than that of phosphorus, whose residence time is of the order of several tens of thousands years (Delaney, 1998). This makes the marine nitrogen cycle very dynamic and at the same time susceptible to substantial changes, unless there exist strong negative feedbacks that prevent the marine nitrogen cycle from undergoing large swings. A second consequence of the high rates of N_2 fixation is the recognition that the supply of new nitrogen by N_2 fixation to nitrogen stressed regions of the surface ocean could rival that supplied from below by physical processes (Karl *et al.*, 1997; Capone *et al.*, 2005). This makes N_2 fixation an important player for structuring marine ecosystems in such regions, with substantial implications for how carbon is cycled through the system and what fraction of it is exported.

The third and last factor is the growing concern over how marine biology and the marine nitrogen cycle may respond to future climate change and by their response either accelerate (positive feedback) or decelerate global change (negative feedback) (see e.g., Gruber *et al.* (2004), Gruber and Galloway (2008) or Chapter 35 by Berman-Frank *et al.,* this volume). Of particular concern is the likely decrease of the ocean interior oxygen concentration, which is bound to increase denitrification, and through the resulting decrease in the oceanic nitrogen inventory will lower marine productivity. This would cause a release of natural CO_2 from the ocean, thereby accelerating the CO_2 increase in the atmosphere and the resulting warming. In addition, a decrease in the ocean interior oxygen content will also likely increase the production and release of nitrous oxide, which is a much more powerful greenhouse gas than CO_2. Therefore, since both these changes would lead to an acceleration of the warming, the marine nitrogen cycle could act as a significant positive feedback factor in a warming world.

This chapter serves as an introduction to the rest of this book, and in many places, the reader is referred to relevant chapters for further details. The focus here is on providing an overview of the most important pools of nitrogen, their transformations, their distribution, and their connection to the cycling of other biogeochemically relevant elements, primarily carbon, oxygen, and phosphorus. The scale is global, as subsequent chapters (Chapters 11–22) cover individual ocean basins and systems. I will also address a series of nitrogen challenges, such as the question of how the marine nitrogen cycle appears to be able to maintain a relatively well established homeostasis, i.e., a balance between gains and losses of fixed nitrogen. Finally, I will discuss the anthropogenic perturbation of the marine nitrogen cycle. As we will see, however, the answers to many of these questions remain elusive, reminding us how little we know about the marine nitrogen cycle, and how much still remains to be discovered.

2. Overview of Forms, Pools, and Reactions

2.1. The electronic diversity of Nitrogen

With 5 relatively stable oxidation states in the marine environment (Fig. 1.2), nitrogen is very distinct from most other nutrient elements. For example, phosphorus, which has the same number of valence electrons, exists in the marine
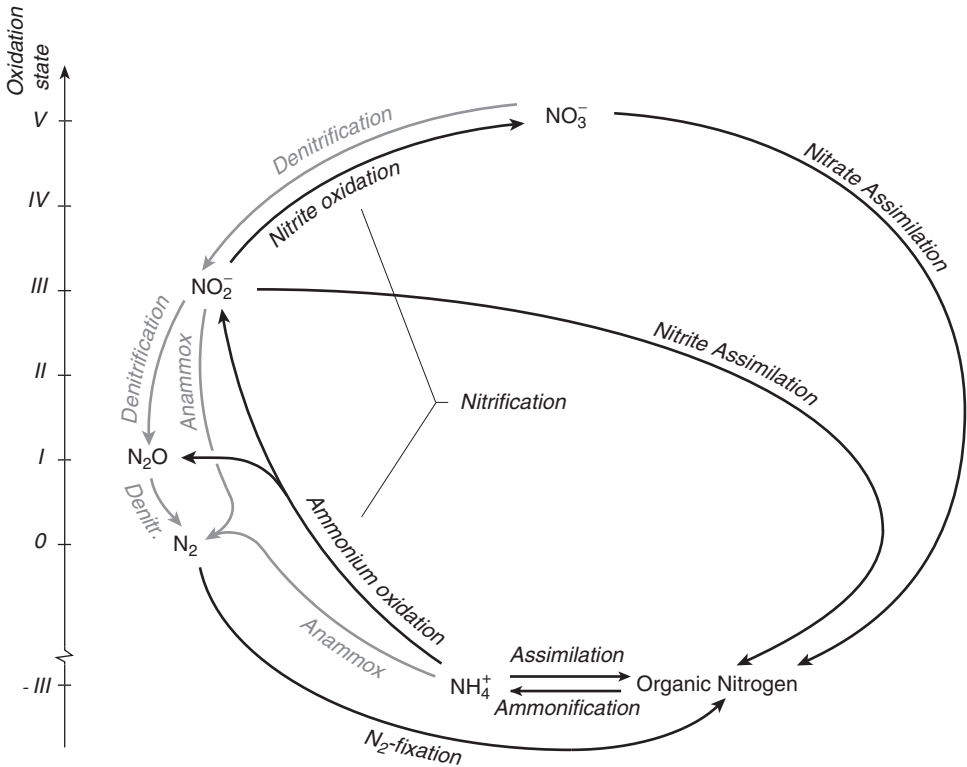
Figure 1.2 Major chemical forms and transformations of nitrogen in the marine environment. The various chemical forms of nitrogen are plotted versus their oxidation state. Processes shown in grey occur in anoxic environments only. See text and Table 1.1 for further details.

environment almost exclusively as ortho–phosphate, i.e., as PO_4^{3-} with an oxidation state of +V. Silicon also exists in the ocean primarily in the +IV oxidation state, i.e., as $Si(OH)_4$ or SiO_2 (opal). By contrast, nitrogen can be found in the marine environment as nitrate, NO_3^-, with an oxidation state of +V, nitrite, NO_2^- (+III), nitrous oxide, N_2O (+I), molecular nitrogen, N_2 (O), and ammonia, NH_4^+ (-III). In addition, there are a myriad of organic compounds containing nitrogen, most often in the form of amino–groups in the -III oxidation state (see also Chapter 3 by *Aluwihari and Meador*, this volume). Only carbon exhibits a similar range of oxidation states, from -IV in CH_4 to +IV in CO_2. The basis for the large number of stable oxidation states for nitrogen is its electron configuration, with two of its five valence electrons filling up the 2s orbital and each of the remaining three valence electrons occupying one of the three 2p orbitals. This gives a total electron configuration of $1s^2\ 2s^2\ 2p^3$. Stable oxdiation states, i.e., a noble gas-like electron configuration, can therefore be achieved if either all five valence electrons are "removed," giving an oxidation state of +V, or if five electrons are added, giving an oxidation state of -III. The ionization energy for the removal of the five valence electrons is relatively small, so that the gain from forming co–valent bonds can compensate easily for this energy. This provides an explanation for the relative stability of NO_3^-, and NH_4^+, but it does

not explain the stability of species such as NO_2^-, N_2 or N_2O. However, when considering the reactivity of a compound in the natural environment, one also needs to take into account its bonding energy as well as the activation energy needed to overcome an existing covalent bond.

For nitrogen, the basis for forming stable covalent bonds with other atoms is in most cases the hybridization of its outer s and p orbitals to form either four sp^3 hybrid orbitals or three sp^2 orbitals. In the sp^3 case, the 5 electrons are distributed such that a pair of electrons occupy an unshared sp^3 orbital, while the other three are shared with another atom through covalent bonds. In the case of the sp^2 orbitals, the third p orbital can be used to form π orbitals, which then permits the atom to form double and triple bonds. It is this flexibility, plus the much higher strength of the covalent bonds in the compounds that have double or triple bonds, such as occurs in NO_2^-, N_2 and N_2O, that make so many species of nitrogen stable in the marine environment. Phosphorus shares many of the same characteristics as nitrogen, but because it has 10 more electrons, which occupy the lower orbitals, the energy of many covalent bonds is much lower. Furthermore, these lower orbitals strongly restrict the formation of π orbitals, so that neither silicon nor phosphorus can easily form double or triple bonds. As a result, silicon and phosphorus exist in the marine environment primarily in just one oxidation state.

In summary, the presence of a large number of stable oxidation states for nitrogen in the environment is a result of nitrogen having five valence electrons as well as it being small, giving its electron orbitals a lot of flexibility to rearrange themselves. It is interesting to point out that when comparing carbon, silicon, nitrogen, and phosphorus, the spatial flexibility of the electron orbitals in the smaller elements (carbon, nitrogen) is more important than the number of valence electrons. As a result, nitrogen behaves more like carbon, which has one valence electron less, and less like phosphorus that has the same number of valence electrons.

2.2. The major reactions

The many oxidation states of nitrogen in the marine environment and the resulting large number of nitrogen species give rise to many redox reactions that transform one species to another (Fig. 1.2). In today's ocean, all of the major reactions are mediated by biology, either in association with assimilatory or dissimilatory functions of the involved marine organisms. As discussed in more detail by Berman-Frank *et al.* (Chapter 35, this volume), this was not always the case in Earth's history. For example, biological N_2 fixation evolved much earlier on Earth than biologically driven denitrification (Falkowski 1997).

The most important biologically mediated reactions are summarized in Table 1.1 together with information about the redox environment these reactions take place in, the organisms that are usually conducting these processes, and what biochemical role these processes play for these organisms. I briefly discuss these reactions, but refer to the following chapters for details (see also Table 1.1). For a discussion of a series of additional reactions (e.g. Oxygen-Limited Autotrophic Nitrification-

Table 1.1 Summary of the most important biologically mediated processes transforming nitrogen in the ocean

Process	Organisms	Redox environment	Trophic status	Biochemical role	Chapter
NO_3^- assimilation	Phytoplankton	Aerob	Photo–autotrophic	Source of N	7
NO_2^- assimilation	Phytoplankton	Aerob	Photo–autotrophic	Source of N	7
NH_4^+ assimilation	Phytoplankton	Aerob	Photo–autotrophic	Source of N	7
	Bacteria	Aerob/anaerob	Heterotrophic	Source of N	7
Ammonification	Bacteria/zooplankton	Aerob/anaerob	Heterotrophic	Release of N	8
NH_4^+ oxidation	NH_4^+ oxidizers (e.g., *Nitrosomonas*)	Aerob	Chemo–autotrophic	Source of energy	5
NO_2^- oxidation	NO_2^- oxidizers (e.g., *Nitrobacter*)	Aerob	Chemo–autotrophic	Source of energy	5
Denitrification	Bacteria	Anaerob	Heterotrophic	Electron acceptor	6
Anammox	Bacteria	Anaerob	Chemo–autotrophic	Source of energy	6
N_2-fixation	Cyanobacteria, etc. (e.g., *Trichodesmium*)	Aerob	Photo–autotrophic	Source of N	4

Denitrification, OLAND), the interested reader is referred to the review by Brandes et al. (2007).

The *assimilation of nitrate or ammonium* into organic nitrogen by marine phytoplankton in order to satisfy their nitrogen demand for growth is the process that quantitatively dominates the marine nitrogen cycle. NH_4^+ is thought to be the preferred source of fixed nitrogen for phytoplankton, because its assimilation does not involve a redox reaction and therefore requires little energy (Zehr and Ward, 2002). It is therefore not surprising that all phytoplankton can grow on NH_4^+ as the only nitrogen source. By contrast, the assimilation of NO_3^- involves the reduction of nitrogen from an oxidation state of $+V$ to $-III$, i.e., the transfer of eight electrons, which requires the investment of a substantial amount of energy. Since NO_3^- in the ocean is generally much more abundant that NH_4^+ (see below), most phytoplankton have the enzymes necessary to undertake this reduction (nitrate reductase), with a few notable exceptions. The best studied organisms lacking the ability to use NO_3^- as a nitrogen source are Prochlorococcus and certain strains of Synechococcus (Moore *et al.*, 2002). All phytoplankton that can use NO_3^- can also use NO_2^- as a source of nitrogen. This is because NO_2^- is an intermediary product during the assimilation of NO_3^-. However, NO_2^- is usually only a very minor source of nitrogen for phytoplankton, as its concentration is often an order of magnitude lower than that of NO_3^- or NH_4^+ (see below).

The assimilation of nitrogen by phytoplankton is strongly linked to the photosynthetic fixation of carbon, because both elements are needed in order to build living organic tissue. The same applies to the assimilation of phosphate. Since most phytoplankton appear to have a relatively fixed cell quota for protein, lipids, carbohydrates, and DNA/RNA (see e.g., Hedges *et al.* (2002)), they tend to take up these different elements with a relatively fixed ratio, permitting us to write the synthesis of organic matter by the coupled assimilation of CO_2, NO_3^-, and PO_4^{3-} as:

$$106CO_2 + 16NO_3^- + HPO_4^{2-} + 78H_2O + 18H^+ \Rightarrow$$
$$C_{106}H_{175}O_{42}N_{16}P + 150O_2 \quad (1.1)$$
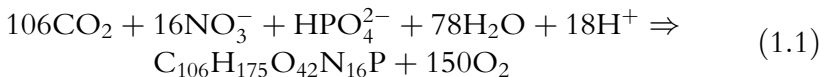
and in the case when NH_4^+ is used as nitrogen source:

$$106CO_2 + 16NH_4^+ + HPO_4^{2-} + 48H_2O + 14OH^- \Rightarrow$$
$$C_{106}H_{175}O_{42}N_{16}P + 118O_2 \quad (1.2)$$
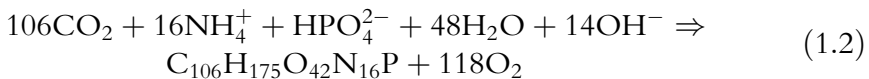
where $C_{106}H_{175}O_{42}N_{16}P$ denotes the average composition of organic matter in phytoplankton, and where I used the stoichiometric ratios of Anderson (1995). Note that these ratios differ from those originally proposed by Redfield *et al.* (1963), in particular with regard to the production of O_2. The uptake of NO_3^- and NH_4^+ has a notable impact on the photosynthetic quotient (PQ), i.e., the stoichiometric ratio between the uptake of CO_2 and the release of O_2, with the uptake of NO_3^- having a PQ of 1.4 and the uptake of NH_4^+ having a PQ of 1.1 (*cf.* Laws (1991)).

It is important to recognize that the stoichiometric ratios used in Eqs. (1.1) and (1.2) are averages, and that any given phytoplankton sample may deviate substantially

from these mean ratios (see e.g., Klausmeier *et al.* (2004) and discussion in Chapters 37 and 38). However, water column data suggest that when averaged sufficiently over space and time, these ratios hold up remarkably well (e.g., Takahashi *et al.* (1985)). As I will demonstrate below, the concept of constant stoichiometric ratios, often termed *Redfield ratio concept*, is very powerful to investigate and understand the large-scale distribution of nitrogen species in the ocean, and how their distribution is linked to that of other elements, in particular carbon and phosphorus.

Most of the fixed organic nitrogen in the ocean is returned back to nitrate by remineralization processes. This occurs in three distinct steps: *ammonification, ammonium oxidation, and nitrite oxidation*, where the latter two processes in combination are often referred to as *nitrification* (see Fig. 1.2 and Chapter 5 by Ward, this volume). Ammonification is the reverse process of the NH_4^+ assimilation reaction (1.2), i.e., the transformation of organic nitrogen to NH_4^+. Most of this process is done by heterotrophic bacteria, which use the oxidation of organic carbon to CO_2 as a source of energy, but release the organic nitrogen then as NH_4^+ as they lack the ability to oxidize it to NH_4^+ or NO_3^-. This latter step is undertaken by a specialized group of bacteria, which are using the oxidation of NH_4^+ to NO_3^- as a source of energy, i.e., they are chemo–autotrophic. The two steps of nitrification, the oxidation of NH_4^+ to NO_2^-, and the oxidation of NO_2^- to NO_3^- are most often done by two distinct groups of organisms. The best known are *Nitrosomonas spp*, an ammonium oxidizer, and *Nitrobacter spp*, a nitrite oxidizer. Nitrification requires the presence of O_2 and tends to be inhibited by light, which has important implications for the upper ocean nitrogen cycle, as I will discuss below. To summarize, the following three distinct reactions remineralize organic nitrogen to nitrate:

The heterotrophic process of ammonification, which is the return pathway of NH_4^+ assimilation (reaction 1.2):

$$C_{106}H_{175}O_{42}N_{16}P + 118\ O_2 \Rightarrow 106CO_2 + 16NH_4^+ \\ + HPO_4^{2-} + 48H_2O + 14OH^- \tag{1.3}$$
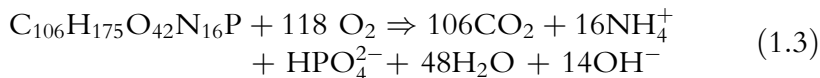
and the two processes of aerobic nitrification, i.e., ammonium oxidation, and nitrite oxidation:

$$2NH_4^+ + 3O_2 \Rightarrow 2NO_2^- + 4H^+ + 2H_2O \tag{1.4}$$

$$2NO_2^- + O_2 \Rightarrow 2NO_3^- \tag{1.5}$$

The vast majority of the oxidation of organic matter follows reaction (1.3) involving oxygen as the terminal electron acceptor. Most higher organisms are strict aerobes, i.e., they cannot switch to another oxidant, and therefore cannot live in the absence of free oxygen. In contrast, most bacteria are more flexible and can use a wide range of other electron acceptors, including sulfate, manganese, and iron. However, the first electron acceptor that is generally being used by bacteria in the ocean is NO_3^-, resulting in a process called *denitrification*. The preference of NO_3^- over other electron acceptors is because of the higher energy yield of

denitrification in comparison to the use of the alternative oxidants. Using organic matter with the stoichiometry shown in (1.1), heterotrophic denitrification can be written as:

$$C_{106}H_{175}O_{42}N_{16}P + 104NO_3^- \Rightarrow 106CO_2 + 60N_2 + H_3PO_4 + 138H_2O$$

$$(1.6)$$

Another anaerobic process that removes bio-available nitrogen from the water is the anaerobic oxidation of ammonium (*Anammox*), in which ammonium and nitrite are combined to form N_2, thus

$$NO_2^- + NH_4^+ \Rightarrow 2N_2 + 2H_2O \qquad (1.7)$$
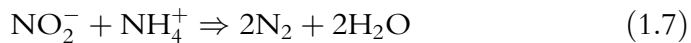
In contrast to denitrification, the Anammox reaction is used as a source of energy, i.e., the Anammox bacteria are chemo-autotrophic (Strous *et al.*, 1999). This process, first uncovered in wastewater bioreactors, has been demonstrated to occur in marine environments only very recently (see e.g., Kuypers *et al.* (2003) and Dalsgaard *et al.* (2003)). Its quantitative significance is not known yet on a global scale, but a recent study suggested that anammox rather than canonical denitrification is the primary process causing the fixed nitrogen loss in the oxygen minimum zone of the Benguela upwelling system (Kuypers *et al.*, 2005). It is unlikely that this conclusion can be easily extrapolated, since the requirement for NO_2^- in an anoxic environment means that another process must supply this reactant. The most likely candidate is the partial denitrification of NO_3^-, which means that the anammox bacteria tend to have to compete with denitrifying bacteria for the same resources. But given how little we know about this process and the associated organisms, there is ample room for surprises.

From a geochemical perspective, denitrification and anammox have the same implication, i.e., they both lead to a loss of fixed nitrogen from the ocean, albeit with a somewhat different stoichiometry. This loss requires a counterbalancing source elsewhere if fixed nitrogen in the ocean is to be maintained at roughly constant levels through time.

The most important source for fixed nitrogen in the ocean is *biological N_2 fixation*, which refers to the conversion of N_2 into organic nitrogen. It is undertaken in the ocean primarily by photoautotrophic organisms in order to obtain nitrogen in environments where fixed nitrogen is usually exhausted. The most conspicuous and best studied N_2 fixing organism (diazotroph) is *Trichodesmium* (Capone *et al.*, 1997), although there are many other organisms known to fix nitrogen, including uni-cellular bacteria (Zehr *et al.*, 2001), and cyanobacteria that live endosymbionti-cally within marine diatoms (e.g., Carpenter *et al.* (1999)). The magnitude of marine N_2 fixation has been a topic of intense research and discussion in the last two decades (see e.g., Capone (2001)). In particular, the extent to which the oceanic fixed nitrogen budget is actually in balance is controversial at present, as I will discuss below (e.g., Codispoti (2006); Codispoti *et al.* (2001); Codispoti and Christensen (1985); Gruber (2004); Gruber and Sarmiento (1997); McElroy (1983)).

Organic nitrogen is seldom completely converted to NO_3^- or N_2 during either nitrification or denitrification. Some small fraction ends up as nitrous oxide, N_2O

(Fig. 1.2), most of which escapes to the atmosphere (see Fig. 1.1 as well as Chapter 2 by Bange, this volume). Nitrous oxide acts as a greenhouse gas that is more than 200 times more potent than CO_2 (Ramaswamy *et al.*, 2001). Therefore variations of this gas in the atmosphere can lead to changes in Earth's temperature and climate. Since the oceanic emission of N_2O constitutes a substantial fraction to the total emission of N_2O into the atmosphere, N_2O provides for a direct potential link between the ocean nitrogen cycle and Earth's climate.

In the case of aerobic remineralization, the formation of N_2O is associated with the oxidation of ammonium during nitrification (see Fig. 1.2). Nitrous oxide is also formed during denitrification, as it represents an intermediary product during the reduction of NO_3^- to N_2 (Fig. 1.2). If some of this N_2O escapes the further reduction to N_2, denitrification can act as a source of N_2O. At the same time, denitrification can act as a sink for N_2O in cases where N_2O produced elsewhere is transported into a region of active denitrification. The overall balance generally is believed to be positive, i.e., denitrification is thought to act as a net source of N_2O (Suntharalingam *et al.*, 2000). The relative importance of the two production pathways is still debated, but it appears as if the majority of the N_2O in the ocean is formed in association with nitrification (Jin and Gruber, 2003; Nevison *et al.*, 2003; Suntharalingam and Sarmiento, 2000). However, given the much higher yield of N_2O at low oxygen concentrations, the production is biased toward the low oxygen regions of the world ocean.

2.3. Inventories and residence times

Table 1.2 shows a summary of the oceanic inventories of the major forms of nitrogen in the ocean and their estimated residence times with regard to key processes or the ocean fixed nitrogen inventory as a whole.

By far the largest amount of nitrogen in the ocean (about 1×10^7 Tg N, or about 94%) exists in the form of biounavailable N_2. The majority of the remaining fixed forms of nitrogen occurs as NO_3^- (about 88%), followed by *DON*, which makes up nearly all of the remaining 12%. The other forms, *PON*, NO_2^-, NH_4^+, and N_2O have similar oceanic inventories, but together represent less than 0.3% of the total fixed nitrogen pool. The dominance of N_2 in the ocean is entirely due to its inertness, as the thermodynamically most stable form would be NO_3^-. This explains its high abundance relative to NO_2^- or NH_4^+, but the high inventory of *DON* is remarkable, since one would expect that organic nitrogen is remineralized relatively rapidly. However, as will be discussed by *Aluwihare and Meador* in Chapter 3, certain constituents of *DON* are very refractory.

The different nitrogen species differ by more than 6 orders of magnitude in terms of their turnover times. Due to its large inventory and its inertness, the turnover time of dissolved N_2 with respect to N_2 fixation and/or denitrification is by far the longest, i.e., more than 50,000 years. By contrast, NO_3^- in the ocean gets turned over two orders of magnitude faster, i.e., once every 400 years. Due to their much smaller inventories and even larger turnover rates, NH_4^+ and *PON* have an even shorter turnover time, amounting to one to two weeks only. The estimated turnover time for *DON* is about 20 years, but this represents a mean turnover time for bulk *DON*. In

Table 1.2 Oceanie Inventory, Turnover Rates, and Residence Times for the Major Fixed Nitrogen Species in the Ocean

Species	Mean conc. euphotic zone (mmol m^{-3})	Mean conc. aphotic zone (mmol m^{-3})	Oceanic inventory (Tg N)	Turnover rate[i] (Tg N yr^{-1})	Turnover time (years)
Nitrate, NO_3^- [a]	7	31	5.8×10^5	1,570	370
Nitrite, NO_2^- [b]	0.1	0.006	160		
Ammonium, NH_4^+ [c]	0.3	0.01	340	7,000	0.05
Dissolved Organic N, DON [d]	6	4	7.7×10^4	3,400	20
Particulate Organic N, PON [e]	0.4	0.01	400	8,580	0.05
Nitrous oxide, N_2O [f]	0.01	0.04	750	6	125
Fixed Nitrogen[g]			6.6×10^5	200	3,300
Nitrogen gas, N_2 [h]	450	575	1×10^7	200	54,000

[a] Based on World Ocean Atlas, 2001 (WOA01) (Conkright *et al.*, 2002). Estimated uncertainty less than ±5%.
[b] Based on data from the World Ocean Circulation Experiment, average profile. Estimated uncertainty about ±20%.
[c] Average profile from 4 process studies of the Joint Global Ocean Flux Study data, see also Fig. 1.3. Estimated uncertainty about ±20%.
[d] Based on Bronk (2002) with an uncertainty of about ±30%. Turnover rate based on her estimate that about 40% of N uptake by phytoplankton ends up as DON.
[e] Based on Sharp (1983) with an uncertainty of about ±50%.
[f] Based on Nevison *et al.* (2003) with an uncertainty of about ±20%.
[g] Sum of NO_3^-, NO_2^-, NH_4^+, *DON*, *PON*, and N_2O.
[h] Computed from global temperature and salinity distribution (WOA01) assuming 100% saturation. Estimated uncertainty about ±10%.
[i] See Fig. 1.14 for sources of rate estimates.

reality, the different components of *DON* have been estimated to have turnover times from a few minutes to many hundreds of years (Bronk, 2002). N_2O has a remarkably long residence time of about 125 years. This primarily reflects the absence of strong oceanic sinks for N_2O, as only the loss to the atmosphere and reduction to N_2 in anoxic regions can remove N_2O from the ocean once it has been produced.

3. DISTRIBUTIONS AND PROCESSES

3.1. Global mean profiles

Sufficient data for computing global mean profiles for nitrogen species exist only for NO_3^- and NO_2^-. An approximation for a global mean profile for NH_4^+ can be obtained by simply averaging all available data, which are somewhat unevenly
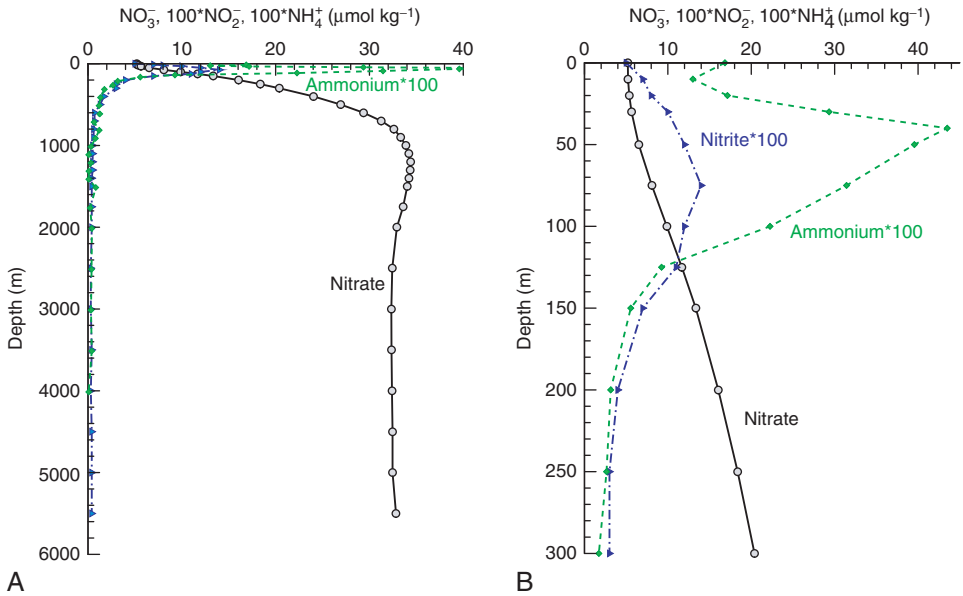
Figure 1.3 Global mean profiles of NO_3^-, NO_2^-, and NH_4^+ (A) for the entire water column (0–6000 m), and (B) for the upper 300 m only. Note that the NH_4^+ and NO_2^- concentrations were multiplied by a factor of 100 to see their variations. The NO_3^- profile is based on data from the World Ocean Atlas 2001 (Conkright *et al.*, 2002). The NO_2^- profile was computed by averaging all data from the World Ocean Circulation Experiment (data taken from whpo.ucsd.edu). The NH_4^+ profile is based primarily on data from the Arabian Sea, the Southern Ocean, the North Atlantic, and the Equatorial Pacific (using data from the Joint Global Ocean Flux Study (JGOFS) available at usjgofs.whoi.edu) and augmented by data from a few sites provided by the GLOBEC program (www.usglobec.org).

distributed, but cover the most important regions of the world's ocean. N_2O will not be discussed here, as it is the main focus of Chapter 2 by Bange, this volume.

The mean vertical profiles of NO_3^-, and NO_2^- show a very different behavior (Fig. 1.3). Nitrate exhibits the expected near-surface depletion and enrichment at depth driven by the biogeochemical loop (biological pump). In contrast, NO_2^- and NH_4^+ show a maximum around 50 to 80 m, i.e., in the lower parts of the euphotic zone, and rapidly decreasing concentrations below that depth. This distribution as well as their much lower mean concentrations reflect their status as intermediary nitrogen species, i.e., species that are both rapidly produced and consumed through-out the water column. These two labile species accumulate only to an appreciable level when their generation rate overwhelms their consumption. For NH_4^+ this occurs generally somewhere in the deeper parts of the euphotic zone, where organic nitrogen is rapidly remineralized, liberating large amounts of NH_4^+, but where NH_4^+ uptake by phytoplankton may be limited already by light. Near the bottom of the euphotic zone or below, where light levels are low enough for nitrification to escape light inhibition, part of this NH_4^+ is oxidized to NO_3^-, creating NO_2^- alongside. NO_2^- is also created as an intermediary product higher up in the euphotic

zone during the assimilation of NO_3^-. In the aphotic zone, neither NO_2^- nor NH_4^+ occur at any appreciable level, as they tend to be rapidly converted to NO_3^- by nitrification. An exception are the low oxygen regions, as discussed below.

3.2. Nitrate

3.2.1. Surface distribution

Over most of the surface ocean, the concentration of NO_3^- is below detection level (Fig. 1.4a). The only exceptions are the low-latitude upwelling regions, and the high latitudes. The coastal upwelling regions are accompanied with high levels of chlorophyll, as expected. In contrast, the high surface nitrate concentrations in the Southern Ocean, the equatorial Pacific, and the North Pacific have long been an enigma for marine biologists (see e.g., Cullen (1991)) as these regions exhibit uncharacteristically low chlorophyll levels. In response, they are often referred to as *High-Nutrient Low-Chlorophyll (HNLC)* regions.

In order to explain the HNLC regions, we first have to consider the processes that control surface nitrate. To first order the surface NO_3^- concentration reflects the balance between vertical NO_3^- supply and biological consumption and export. As a consequence, the residual NO_3^- at the surface is a poor indicator of the production and export of an ocean ecosystem, i.e., the surface NO_3^- concentration is poorly related to the strength of the biological pump (Sarmiento and Gruber, 2006). The residual surface NO_3^- concentration is by definition, however, a good indicator of the efficiency of the biological pump, i.e., a measure of how successful biology is in taking up nutrients and exporting them to depth against the physical resupply of new nutrients. This means that the question of what maintains the HNLC regions is equivalent to asking why the biological pump is so inefficient there.

Physiologically, phytoplankton are usually extremely efficient in taking up NO_3^- and the enzymes operate well down to very low concentrations (see discussion by Mulholland and Lomas in Chapter 7, this volume). Therefore, something else must be limiting phytoplankton growth and/or its biomass. Light-limitation, grazing, and low temperatures were proposed as an explanation (Cullen, 1991), but the current consensus is that iron limitation is the primary reason for the HNLC regions, as impressively demonstrated by a series of open-ocean iron fertilization experiments (Boyd *et al.*, 2000, 2004; Coale *et al.*, 1996, 2004; Martin *et al.*, 1994; Tsuda *et al.*, 2003).

The discussion of the distribution of surface NO_3^- in terms of biological pump efficiency is particularly useful for understanding the impact of the biological pump on the ocean carbon cycle and atmospheric CO_2. This is because surface nutrients are an excellent indicator for the direction of the biologically induced air-sea flux of CO_2. In a case where the biological pump is 100% efficient, all remineralized inorganic carbon that is brought to the surface together with the nutrients is taken up by phytoplankton and mostly exported back down again. As a result, little or no CO_2 can escape to the atmosphere. In contrast, regions where the biological pump is inefficient, much of the inorganic carbon transported upward to the surface remains there along with the unused nutrients, so that a significant fraction of it can escape into the atmosphere. In steady-state, this biologically induced outgassing of CO_2 has to be compensated by uptake in other regions. This occurs in regions of high
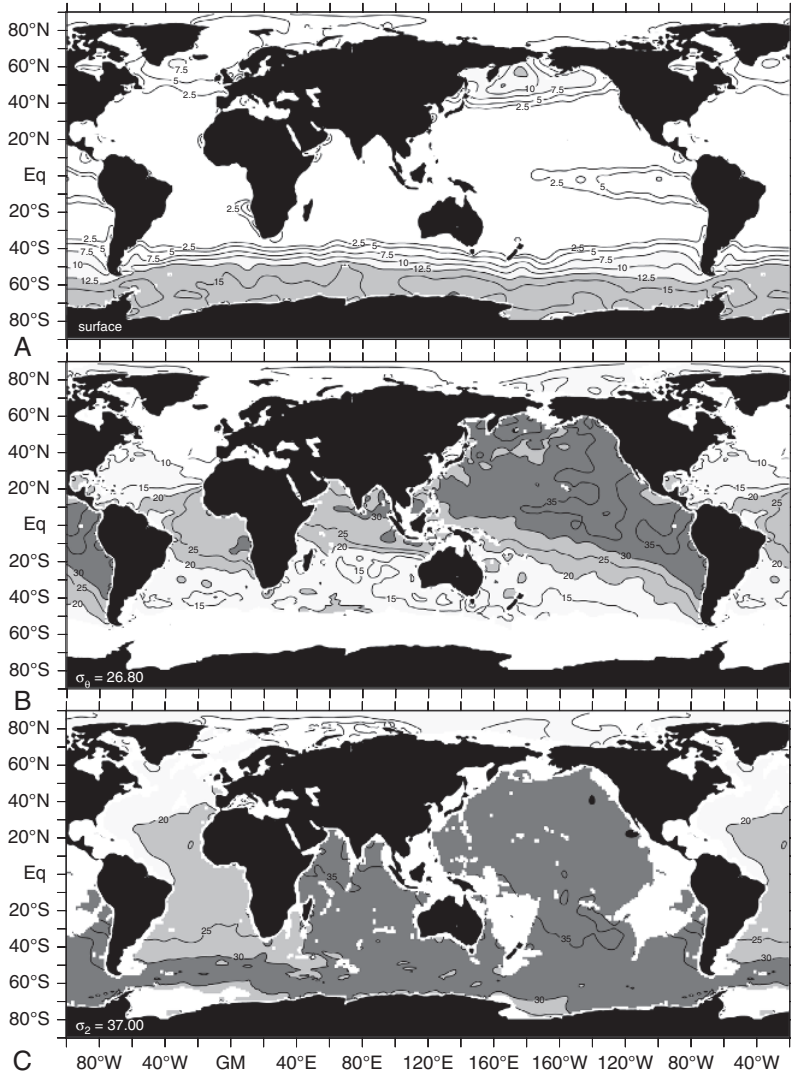
Figure 1.4 Maps of annual mean nitrate concentrations at (a) surface, (b) on the isopycnal surface $\sigma_\theta = 26.80$ representing Subantarctic Mode Water in the southern hemisphere, and subpolar mode waters in the northern hemisphere, and (c) on the isopycnal surface $\sigma_2 = 37.00$ representing North Atlantic Deep Water. Based on data from the World Ocean Atlas 2001 (Conkright *et al.*, 2002).

biological efficiency, where part of the fixed and exported organic carbon stems from inorganic carbon taken up from the atmosphere.

In summary, regions with low biological efficiency have high residual NO_3^- and tend to be biologically induced sources of CO_2 to the atmosphere. Regions with high biological efficiency have very low residual NO_3^- and tend to be biologically

induced sinks for atmospheric CO_2. Thus, in order to change atmospheric CO_2, one needs to make the biological pump primarily more efficient and not necessarily stronger. I will come back to this issue in the section on "nitrogen challenges."

3.2.2. Interior ocean distribution

Nitrate at depth exhibits much more spatial variation than revealed in the smooth global mean profile shown in Fig. 1.3. This is illustrated in Fig. 1.5a, which depicts the NO_3^- concentration along a global-scale vertical section that starts in the North Atlantic, goes south along approximately 20°W into the Southern Ocean, wraps around Antarctica at about 60°S, turns north at about 150°W, and ends up in the North Pacific (see map inset). NO_3^- increases very rapidly with depth in the upper 500 m of the tropics, while the vertical gradients are much smaller in the upper 500 m of the mid-latitudes. Below a 1000 m, the NO_3^- distribution follows an entirely different pattern, as the main gradients are horizontal, going from low concentrations in the deep Atlantic to high concentrations in the deep Pacific.

These variations in NO_3^- are highly correlated with variations in PO_4^{3-} and dissolved inorganic carbon (DIC) (Fig. 1.6), with a slope close to the stoichiometric ratio of photosynthesis and respiration, implying that the distribution of these constituents are primarily controlled by the biogeochemical loop. But how are the two main processes of this loop, i.e., biology and ocean circulation interacting with each other to create these spatial variations? For example, are the stronger vertical gradients in the tropics primarily a result of higher export of organic nitrogen and subsequent higher rates of remineralization at depth, or are there other mechanisms at work?

Let us explore the thermocline distribution first. As large-scale oceanic transport occurs primarily along surfaces of equal potential density, it is instructive to inspect variations along such surfaces. Figure 1.4b shows the NO_3^- distribution along the potential density surface $\sigma_\theta = 26.80$, which represents Sub-Polar Mode Water (SPMW) in the northern hemisphere and Sub-Antarctic Mode Water (SAMW) in the southern hemisphere (Hanawa and Talley, 2001). Nitrate concentrations near the outcrops, i.e., where the isopycnal surface intersects the surface of the ocean are near zero, but concentrations increase rapidly as one moves away from the outcrops into the ocean's interior.

The large-scale circulation on this isopycnal surface consists of a series of anticyclonic gyres that are confined to each hemisphere. Along the anticyclonic path of these gyres, the waters flow near the surface at the poleward outcrops, and descend to depth along the eastern side of the basin, bringing young, recently ventilated water from the surface into the interior. The gyre flow stays generally poleward of 20° on the equatorward side, and then becomes part of the western boundary current along the gyre's western flank. The interaction of this flow pattern with the continuous addition of NO_3^- from remineralization (nitrification) is best seen in the North Atlantic, where the younger waters in the northeastern part of the gyre have the lowest NO_3^- concentrations, and where concentrations gradually increase along the path of the gyre. The time it takes for an average water parcel to make one journey around this basin-scale gyre is on the order of years to decades (Follows *et al.*, 2002; Sarmiento *et al.*, 1982), resulting in a moderate increase in the NO_3^-
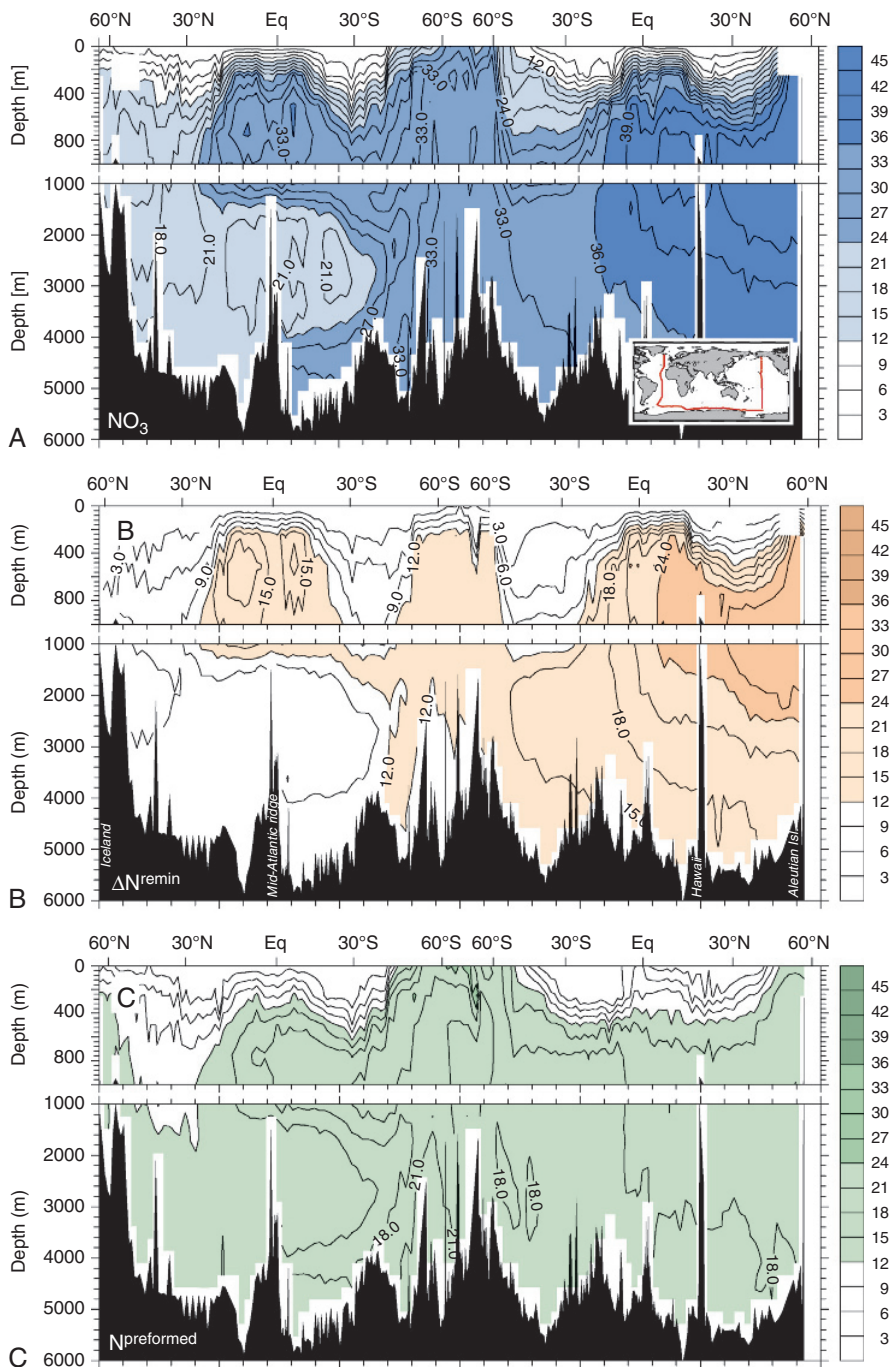
Figure 1.5 Vertical sections of (A) nitrate, (B) remineralized nitrate, and (C) preformed nitrate (all in $\mu mol\ kg^{-1}$) along a section that follows the global conveyor belt circulation (see map inset in (A)). Based on WOCE data from the A16, S4, and P16 cruises.
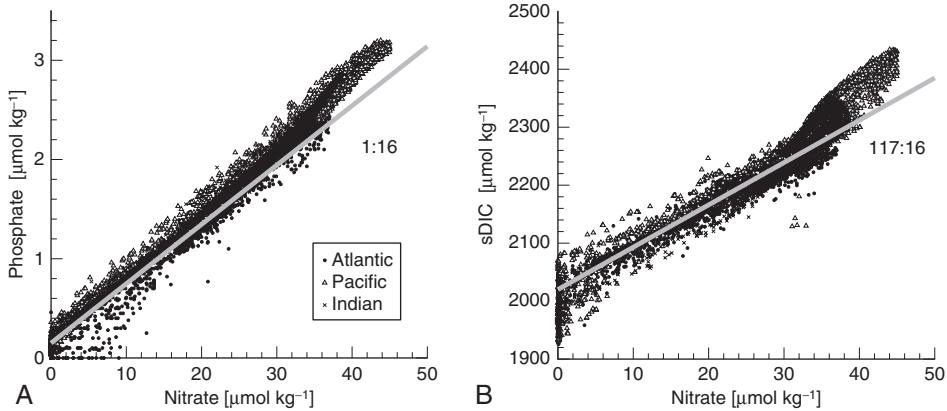
Figure 1.6 Plots of co-variances of (A) phosphate, and (B) dissolved inorganic carbon (*DIC*) with nitrate. DIC has been normalized to a constant salinity of 35 in order to remove the effect of fresh-water fluxes. Based on data from all depth and from selected cruises of the World Ocean Circulation Experiment, i.e., A16, 18NI9S, P16. The lines indicate the expected biologically induced trends.

concentration despite high rates of organic matter remineralization and hence high rates of NO_3^- addition from nitrification.

These basin-scale gyres do not reach the low-latitude regions of these isopycnals, however, resulting in a very poor water renewal in these regions. Consequently, NO_3^- and other nutrients tend to accumulate, while oxygen tends to be consumed. In certain regions, particularly in the eastern parts of the tropical thermocline, this consumption proceeds until all oxygen is consumed, creating conditions conducive for water–column denitrification.

The distribution of NO_3^- on the deep-ocean isopycnal $\sigma_2 = 37.00$ (Fig. 1.4c), representing North Atlantic Deep Water (NADW), illustrates the large-scale increase of NO_3^- from the Atlantic to the Pacific in more detail than the vertical section (Fig. 1.5a). Two observations on this deep isopycnal surface contrast with the NO_3^- distribution on the thermocline surface. First, concentrations near the outcrop regions are not near-zero. In the North Atlantic, the NO_3^- concentration at the outcrop is about 15 µmol kg^{-1}, and in the Southern Ocean, the outcrop concentration of NO_3^- exceeds 30 µmol kg^{-1}. Second, the horizontal gradients are generally smoother, showing no distinction between the tropics and the mid-latitudes. The latter observation reflects the very different flow pattern of the deep ocean, which is dominated by the lower branch of the global overturning circulation (Broecker, 1991). This circulation supplies newly ventilated waters to the deep ocean at two locations only: the high-latitude North Atlantic and the high-latitude Southern Ocean. From the North Atlantic, newly formed NADW is transported southward into the Southern Ocean, where it mixes with waters that are ventilated around Antarctica. This mixture provides then the source waters for the deep Indian and Pacific Oceans. Along this global-scale journey, which starts in the North Atlantic and ends in the North Pacific, water parcels slowly accumulate NO_3^- stemming from the remineralization of organic nitrogen falling through the water column.

Not all of the variations of nitrate on this isopycnal surface are due to remineralization processes, however. We need to consider also the contribution that stems from the mixing of surface source waters that already contain a significant amount of NO_3^-. This *preformed* nitrate is evident in the non-zero outcrop values noted above in Fig. 1.4a. In order to isolate the impact of remineralization on the interior ocean NO_3^- distribution, one therefore needs to separate the observed NO_3^- into a preformed and a *remineralized* component, where the latter refers to the amount of NO_3^- that has been added to a water parcel since it was last in contact with the atmosphere, thus:

$$[NO_3^-]_{observed} = [NO_3^-]_{preformed} + \Delta[NO_3^-]_{remineralized}. \qquad (1.8)$$

We can accomplish this separation for NO_3^- by taking advantage of this separation being relatively straightforward for O_2, since surface O_2 concentrations are often close to saturation. Setting preformed oxygen equal to the O_2 saturation concentration, $[O_2]_{sat}$, and assuming that the stoichiometry of O_2 consumption and NO_3^- release during remineralization is constant with a ratio, $r_{N:O_2}$, we can estimate the remineralized component:

$$\Delta[NO_3^-]_{remineralized} = r_{N:O_2} \cdot \left([O_2]_{sat} - [O_2]_{observed}\right). \qquad (1.9)$$

The preformed component is estimated by difference. This separation is only an approximation, however, as preformed oxygen sometimes deviates substantially from its saturation value (see discussion by Ito *et al.* (2004)). In such cases, one would need to employ an estimate of the true preformed oxygen. For our illustrative purposes here, we neglect this potential limitation.

Figure 1.5c shows that preformed NO_3^- represents a very substantial fraction of the total NO_3^- in the thermocline and deep ocean, and that it is responsible for about half of the surface to deep gradient in NO_3^-. It also shows that much of the NO_3^- increase in the deep Atlantic along the path of NADW is actually caused by mixing of a high preformed NO_3^- end-member from the Southern Ocean while the NO_3^- increase from remineralization is relatively small (Fig. 1.5b). The highest concentrations of remineralized NO_3^- are found in the thermocline, as expected from the fact that about 90% of the organic nitrogen exported from the upper ocean is remineralized above 1000 m (e.g., Martin *et al.* (1987)).

The existence of a significant amount of preformed NO_3^- in the ocean is a direct consequence of the low biological pump efficiency in the surface regions that ventilate the interior ocean. Since the Southern Ocean is a source region for most of the deep waters in the Ocean, the inability of biology to draw down the nutrients there leaves a global-scale imprint on the ocean interior distribution of NO_3^-. This gives biology in the Southern Ocean a uniquely large leverage on global-scale biogeochemistry (Sarmiento *et al.*, 2004). Increasing the biological pump efficiency in this region would not only draw down the (preformed) nutrients locally, but it would also prevent these nutrients from being exported laterally to low-latitude regions by SAMW and Antarctic Intermediate Waters (see elevated preformed NO_3^- concentrations in the mid thermocline of the Southern hemisphere in Fig. 1.5c). These preformed nutrients

eventually end up at the surface of the lower latitudes, fueling productivity there. Therefore, any increase in the biological pump efficiency in the Southern Ocean, such as induced by iron fertilization or Southern Ocean stratification, causes a drop in low-latitude productivity after some delay (Jin and Gruber, 2003; Sarmiento and Orr, 1991; Sarmiento et al., 2004). In a model experiment, Sarmiento et al. (2004) showed that making the biological pump 100% effective in the Southern Ocean would cause, in steady state, low-latitude productivity north of 30°S to decrease 4-fold. Therefore, consideration of such downstream effects is very important when considering past or future changes in the oceanic nitrogen cycle.

3.3. Ammonium

The inventory of NH_4^+ in the ocean is approximately three orders of magnitude smaller than that of NO_3^-. Nevertheless, the cycling of NH_4^+ in the ocean is a crucial component of the upper ocean nitrogen cycle. In fact, the chemical distinction of NO_3^- versus NH_4^+ permits oceanographers to investigate the sources and fate of nitrogen in the upper ocean in a much more detailed way than it would be possible if nitrogen existed just in one major chemical form, such as is the case for PO_4^{3-}, for example.

3.3.1. Distribution

Relative to NO_3^- and NO_2^-, the concentration of NH_4^+ is seldom measured in the open ocean, resulting in a much more patchy understanding of its distribution. This is because standard techniques do not work well at the very low concentrations usually encountered, requiring special high-sensitivity methods (see e.g., Brzezinski (1988), Jones (1991), and Clark et al. (2006)). An exception are the four major process studies undertaken as part of the U.S. component of the Joint Global Ocean Flux Study (JGOFS), i.e., the AESOPS study in the Southern Ocean, the Arabian Sea study, the EqPac program in the Equatorial Pacific, and the NABE experiment in the North Atlantic, as well as a few individual efforts (e.g., Varela et al. (2005)). Average profiles of NH_4^+ for each of the four JGOFS process studies are shown in Fig. 1.7, together with those for NO_3^-, NO_2^-, and PON.

   Several observations stand out. Although the euphotic mean concentration of NH_4^+ averaged from all regions is about 0.3 μmol kg^{-1} (see Fig. 1.3), the concentration varies by almost an order of magnitude between the Southern Ocean and the Arabian Sea. Since the NH_4^+ concentration reflects the balance between production by ammonification and consumption by NH_4^+ assimilation or nitrification, differences in any of these processes could explain this large range. The positive correlation between high concentrations of PON and NH_4^+ (Fig. 1.7C and D) suggests that higher rates of ammonification are the primary cause, but the much colder temperatures in the Southern Ocean and the resulting lower rates of nitrification, as well as strongly differing values of NO_3^-, may also contribute.

   During these four process studies, the average concentration of NH_4^+ was generally much lower than that of NO_3^-. It is not clear whether this applies globally, but the few available data from the oligotrophic regions suggest that NH_4^+ is extremely low there, and likely lower than NO_3^- (Brzezinski, 1988).
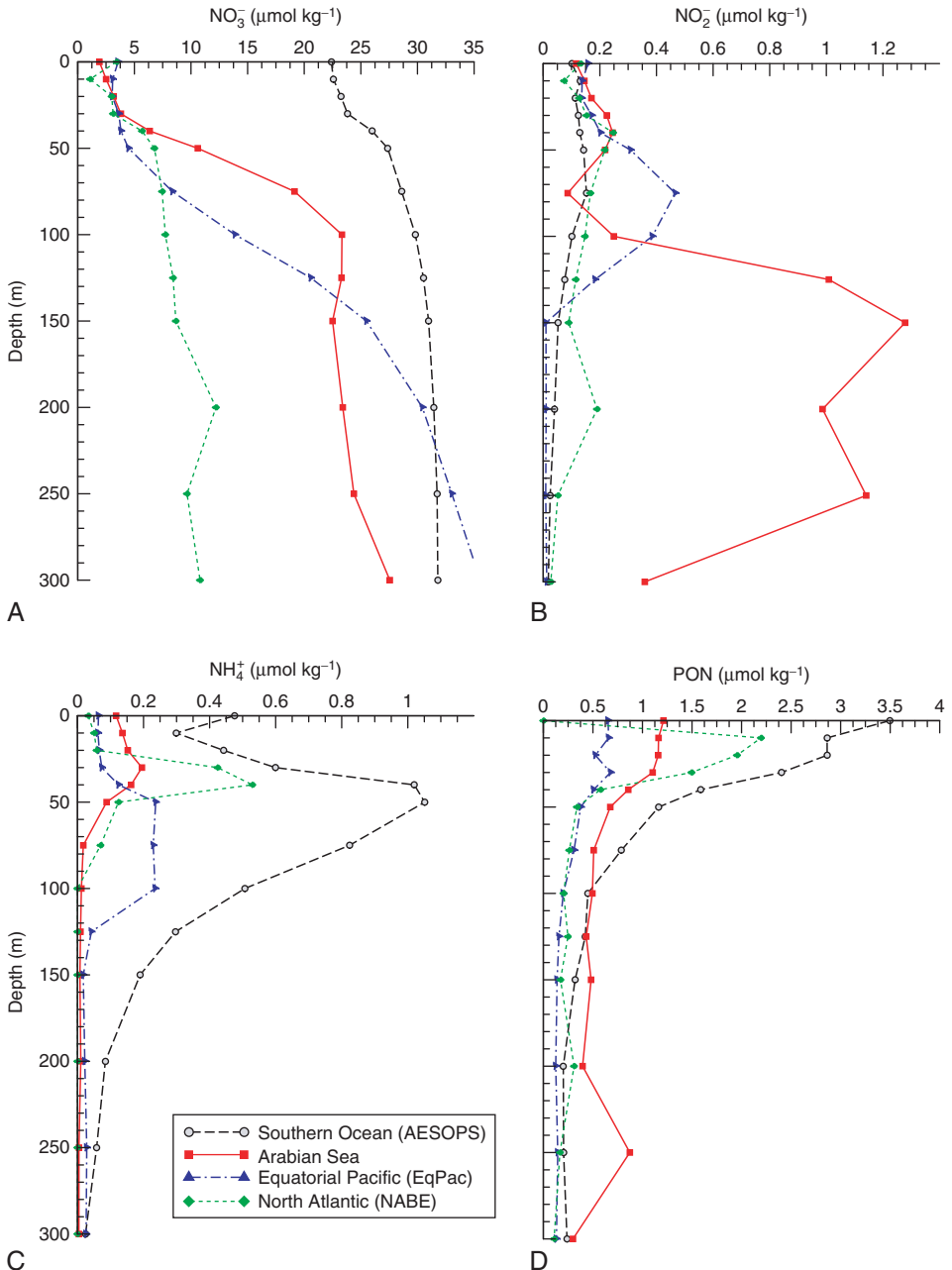
Figure 1.7 Average vertical profiles of (A) NO_3^-, (B) NO_2^-, (C) NH_4^+, and (d) *PON* from the four JGOFS process studies sites, i.e., Southern Ocean in 1996–1998 (80°S–51°S; 160°E–161°W), Arabian Sea in 1995 (8°N–26°N; 54°E–71°E), Equatorial Pacific in 1992 (146°W–135°W; 13°S–12°N), and North Atlantic in 1989 (41°N–59°N; 25°W–17°W). Data were obtained from usjgofs.whoi.edu and were averaged for each study without consideration of exact location or season.

The mid-depth maximum of NH_4^+ observed in the mean profile (Fig. 1.3) exists in all four regions, but occurs with different strengths and at different depths. As was the case above, small differences in production or consumption, such as arising from differences in productivity, depth of the euphotic zone, light, and *PON* availability likely determine the exact shape of the profile.

3.3.2. New versus regenerated production

Arguably, the concept of *new versus regenerated production* has been among the most important paradigms guiding biological and biogeochemical oceanography in the last two decades (Ducklow, 1995; Ducklow *et al.*, 2001; Falkowski *et al.*, 2003; Karl *et al.*, in Chapter 16, this volume). As introduced by Dugdale and Goering (1967), new production refers to that part of primary production that is fueled by nutrients inputs from outside the euphotic zone. Regenerated production is the remainder, i.e., that part of production that is based on inorganic nutrients that are recycled from organic matter within the euphotic zone.

There are two reasons why this paradigm has become so important: First, in steady-state, and if averaged over sufficiently long spatial and temporal scales (see e.g., Williams *et al.* (1989) and Plattner *et al.* (2005)), new production can be equated with export production, i.e., the flux of organic carbon and nutrient elements out of the euphotic zone (Fig. 1.8) (Eppley and Peterson, 1979). Since it is the magnitude
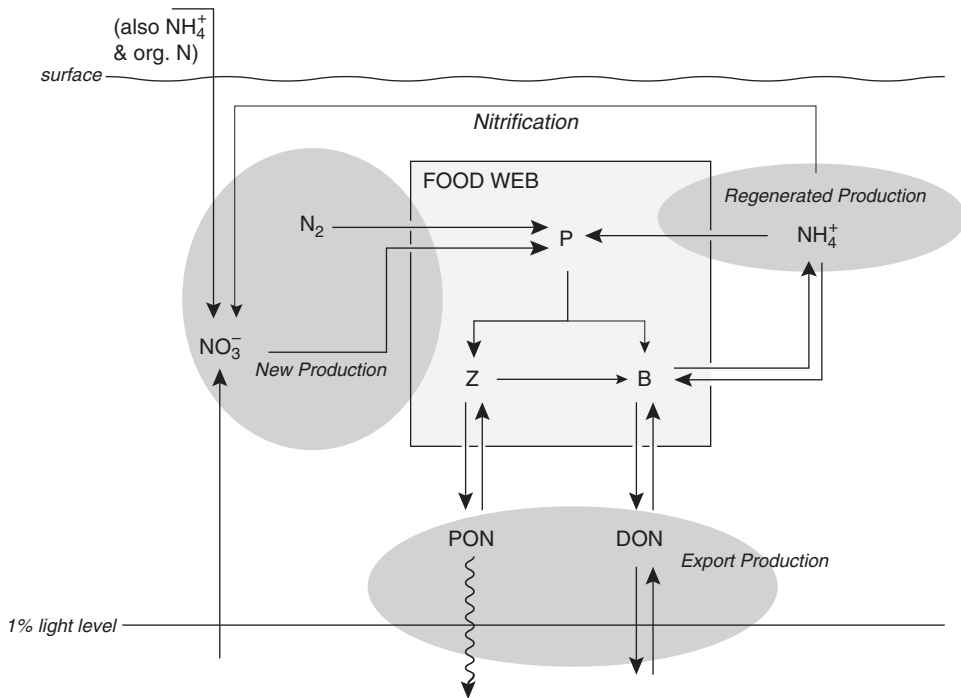
Figure 1.8 Schematic illustration of the nitrogen cycling paradigm in the euphotic zone. Adapted from Sarmiento and Gruber (2006).

of export production and not primary production that determines the efficiency of the biological pump, and it is the latter that determines the impact of biology on atmospheric CO_2, estimating export production has been a primary focus of bio-geochemical research (Ducklow *et al.*, 2001). However, export production is notoriously difficult to measure (see e.g., Buesseler *et al.* (2007) for a discussion of sediment traps), so using estimates of new production as a proxy for export production is attractive (Brix *et al.*, 2006; Neuer *et al.*, 2002).

The second reason for the success of the new production paradigm is that new production is relatively easy to measure. This is because new production in the open ocean is usually associated with the uptake of NO_3^-, while regenerated production is associated with the uptake of NH_4^+. This remarkable fact is a direct consequence of nitrification being inhibited by light. As a result, NH_4^+ that is created by ammonification in the euphotic zone is usually taken up again by phytoplankton rather than nitrified to NO_3^-. In contrast, nearly all NH_4^+ created in the aphotic zone is nitrified to NO_3^-, so that fixed nitrogen that is supplied back to the euphotic zone from the ocean's interior is almost exclusively in the form of NO_3^-. Recognizing the chemical distinction between nitrogen following either the new or the regenerated production pathways, Dugdale and Goering (1967) demonstrated that one can use ^{15}N labeled NO_3^- and NH_4^+ in incubation experiments together with ^{14}C labeled CO_2 to determine the fraction of primary production that is caused by either new or regenerated production.

This method has been widely adopted and has not changed much conceptually since its inception. Due to need to spike the phytoplankton sample with NO_3^-, the method is problematic to use in oligotrophic gyres (Falkowski *et al.*, 2003). As a result, it has been applied primarily in HNLC regions (e.g., Aufdenkampe *et al.* (2001)) and in more productive coastal or high-latitude regions (e.g., Wilkerson *et al.* (1987)). Many insights have been gained from these measurements, permitting to develop models of what controls the ratio of new or export production to primary production (see e.g., Laws *et al.* (2000) for a summary of results and a model of this ratio).

Although recognized from the very beginning that equating NO_3^- uptake with new production is only correct if other processes such as N_2 fixation, atmospheric deposition, or river input are negligible (Dugdale and Goering, 1967), this caveat has been ignored often. This was done because of either experimental limitations or lacking evidence that the other nitrogen supply pathways were substantial. However, research over the last two decades has revealed that atmospheric deposition, river fluxes, or N_2 fixation supplies in many regions a substantial amount of fixed nitrogen to the upper ocean, in some cases equaling or exceeding the classical vertical transport pathway (Capone *et al.*, 2005). Phytoplankton have been identified that can migrate vertically over substantial distances in order to take up excess fixed nitrogen at depth and then float to the surface to undertake photosynthesis (Villareal *et al.*, 1993), providing a novel pathway of introducing new nitrogen into the system. In addition, increasing evidence exists that nitrification occurs within the euphotic zone (Ward *et al.*, 1989; Zehr and Ward, 2002), and that organic nitrogen can be released and taken up by phytoplankton (Bronk *et al.*, 1994), further blurring the association of NH_4^+ uptake with regenerated production and the uptake of NO_3^- with new production. Nevertheless, the paradigm of new versus regenerated production remains a powerful concept to understand upper ocean biogeochemical cycles.

3.4. Nitrite

Since the commonly used colorimetric method to measure NO_3^- involves the reduction to NO_2^-, the latter is usually measured separately as well, so that the $NO_3^- + NO_2^-$ measurements can be corrected for NO_2^-. As a result, there are nearly as many measurements of NO_2^- as there are of NO_3^-. However, many of these samples have NO_2^- concentrations below the relatively high detection level. In addition, the colorimetric method is not very accurate at low concentrations, so that special low-level methods are required to study nitrite dynamics (Brzezinski, 1988; Dore and Karl, 1996; Lipschultz et al., 1996; Zafiriou et al., 1992).

The turnover time of NO_2^- is not well known, but has been estimated to be about 3–7 days (Lipschultz et al., 1996). This makes NO_2^- the shortest lived among the nitrogen species considered here. The main reason for this fast turnover is that NO_2^-, even more so than NH_4^+, represents an intermediary species (Figure 1.2). It is produced during NO_3^- assimilation, nitrification, and denitrification, and then immediately consumed again.

The interaction of these processes explains the ocean mean distribution shown in Fig. 1.3, as well as the distribution observed during the four U.S. led JGOFS process studies (Fig. 1.7). In general, the NO_2^- distribution in the euphotic zone appears to follow roughly that of NH_4^+, except that its mean concentration is slightly smaller, on average. While the correspondence of NH_4^+ and NO_2^- in the euphotic zone suggests that NO_2^- there is mostly controlled by nitrification (Ward et al., 1982), some of the deeper NO_2^- may reflect also its generation during the assimilation of NO_3^-. This is supported by the observation that elevated concentrations of NO_2^- are often associated with the nitracline, i.e., the first appearance of elevated NO_3^- concentrations in the water column (Dore and Karl, 1996; Herbland and Voituriez, 1979) (see e.g., Fig. 1.9).

The correlation of NO_2^- and NH_4^+ concentrations often breaks apart below the euphotic zone. This is particularly the case in oxygen minimum zones, where a secondary NO_2^- peak is frequently observed (see Fig. 1.7 and Codispoti and Christensen (1985)). In the Arabian Sea, NO_2^- concentrations exceed 1 µmol kg^{-1} between about 100 m and 500 m (Fig. 1.7). A similar secondary NO_2^- peak can be observed between 250 m and 500 m in the eastern Tropical Pacific (Fig. 1.9b) (Codispoti, 1983; Codispoti and Christensen, 1985; Codispoti and Packard, 1980). This secondary NO_2^- peak can be attributed to denitrification, as it occurs in association with a well developed anoxic region at mid-depths in the eastern tropical North Pacific (see Fig. 1.9c). A closer inspection shows that the NO_2^- maximum often occurs outside the region of strongest anoxia, i.e., at O_2 concentrations between 1 and 5 µmol kg^{-1} (see e.g., Codispoti et al. (1986) and Codispoti and Christensen (1985)). This location of the secondary NO_2^- maximum is usually interpreted as reflecting the superposition of NO_2^- formation by (aerobic) nitrification, and NO_2^- formation by (anaerobic) denitrification (e.g., Yakushev and Neretin (1997)) both of which can co-occur at these very low, but non-zero O_2 concentrations (Morrison et al., 1999). In contrast, inside the oxygen minimum zone, NO_2^- is very efficiently reduced further to N_2, preventing it from accumulating there. There exist more subtle reasons, involving, for example, trace metal limitations of the nitrite oxidizers at these very low oxygen concentrations (Granger and Ward, 2003).
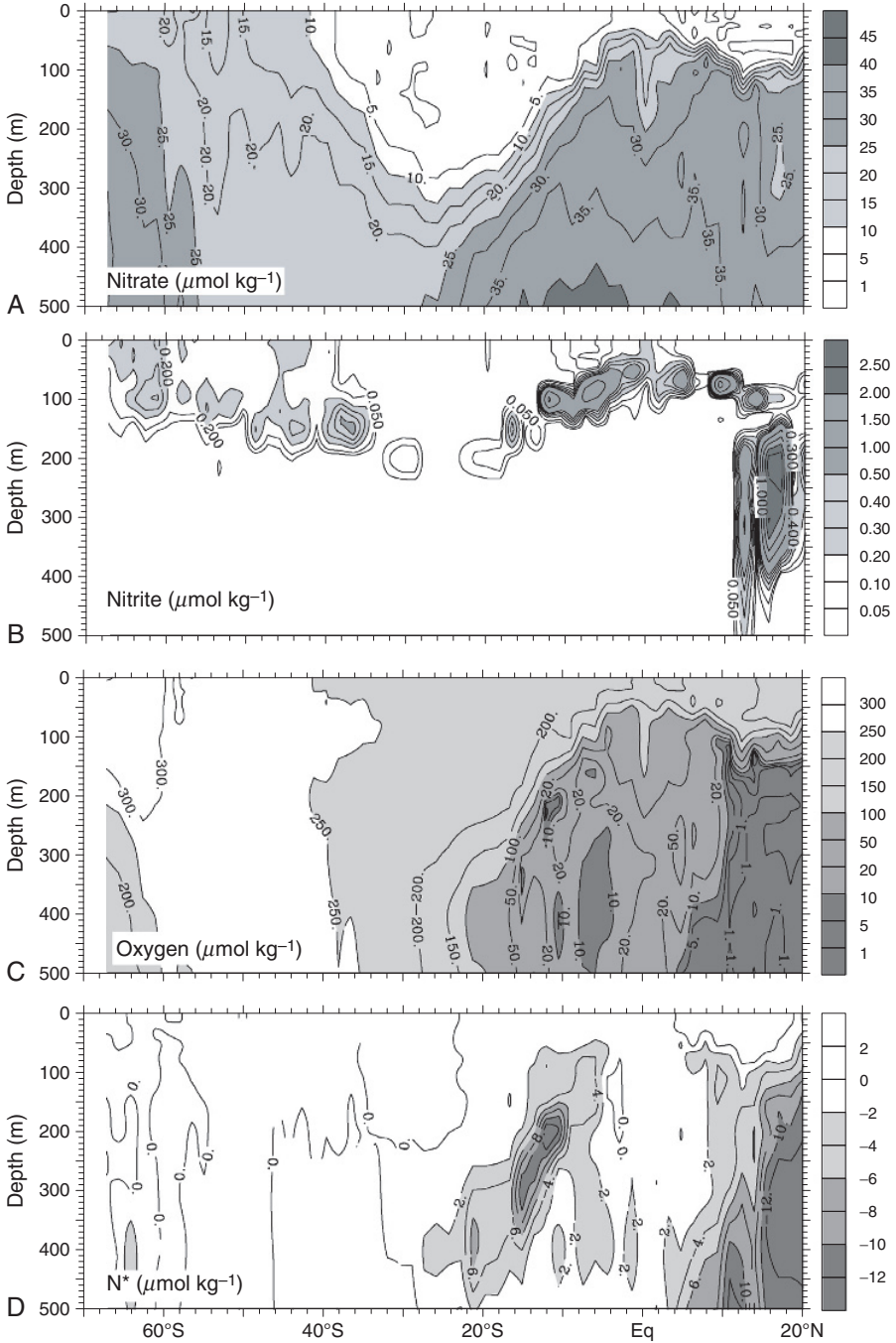
Figure 1.9 Meridional sections of (A) NO_3^-, (B) NO_2^-, (C) oxygen, and (D) N^* along about 100°W in the eastern Pacific. Data are from the WOCE cruise P18 conducted in 1994 (whpo.ucsd. edu).

3.5. Organic nitrogen

More than 99% of the organic nitrogen in the ocean exists in the form of dissolved organic nitrogen, *DON* (see Table 1.2). Only a small fraction of it is chemically characterized, reflecting the large number of organic molecules that contain nitrogen groups. I will not elaborate further on *DON* as it is discussed in detail by *Aluwihare and Meador* in Chapter 3, this volume (see also Bronk (2002)). I therefore limit my discussion to particulate organic nitrogen, *PON*.

   PON represents only about 0.5% of the total organic nitrogen pool, but since some of the *PON* is heavy enough to sink, it represents a crucial component of the marine biological pump. Its mean turnover time is only about 2 weeks (see Table 1.2), reflecting the rapid synthesis and degradation of *PON*. In the euphotic zone, most of the POC is believed to be living matter, although the composition of PON is not well characterized. *PON* exists in size across several orders of magnitude, ranging from very fine suspended particles to large aggregates, such as marine snow or fecal pellets.

   Figure 1.7d shows a summary of *PON* measurements from the four JGOFS process studies. In most cases, *PON* is highest near the surface and decreases quasi-exponentially with depth. There exist large differences in the mean concentrations between the different JGOFS process studies, with *PON* concentrations during the Southern Ocean experiment reaching above 3 μmol kg^{-1}, on average, while *PON* barely exceeds 0.5 μmol kg^{-1} in the Equatorial Pacific. The depth distribution within the euphotic zone as well as the spatial differences largely reflect variations in living biomass (mostly phytoplankton). For example, the Southern Ocean JGOFS program targeted spring blooms in the Southern Ocean, during which very high concentrations of phytoplankton can be reached (Smith *et al.*, 2000).

   Below about 200 m, *PON* concentrations tend to asymptote to similar levels of about 0.1 to 0.3 μmol kg^{-1}, irrespective of the large differences in *PON* in the euphotic zone. Overall, *PON* dynamics in the aphotic zone tends to follow that of particulate organic carbon (*POC*), with a stoichiometric C:N ratio of about 7.1 ± 0.1 (equivalent to 16:114 $\pm$ 2) for the particles sinking out of the euphotic zone (Schneider *et al.*, 2003) (see also Hebel and Karl (2001) for long-term observations of the *POC* and *PON* dynamics in an oligotrophic environment). In their analysis of sediment trap samples and particles collected by *in situ* pumps, Schneider *et al.* (2003) also noticed a slight increase of this ratio with depth of about 0.2 per 1000 m, so that particles sinking across 1000 m have a C:N ratio very close to the 117:16 ratio proposed by Anderson and Sarmiento (1994).

3.6. Nitrogen*: The imprint of N$_2$-fixation and denitrification

A remarkable observation from Fig. 1.6a is not only the strong co-variation of NO_3^- with PO_4^{3-} with a slope of about 16:1, but also the near-zero intercept of the mean trendline. While the existence of a well defined slope is not surprising since it reflects the mean stoichiometry of organic matter synthesis and remineralization (reactions 1.1 through 1.5), the reasons for the existence of a near-zero intercept are less obvious. Generally, this near-zero intercept is interpreted as evidence for the

existence of a stabilizing feedback between N_2 fixation and denitrification, which is controlled by the oceanic ratio of NO_3^- to PO_4^{3-} (Gruber, 2004; Redfield, 1958; Tyrrell, 1999) (see discussion below).

A convenient way to analyze the impact of N_2 fixation and denitrification on the oceanic NO_3^- distribution is to remove the photosynthesis/remineralization trend of 16:1, and focus instead on the anomalies from this trend. This is achieved by defining a linear combination of the two nutrients, i.e.,

$$N^* = \left[NO_3^-\right] - 16\left[PO_4^{3-}\right] + 2.9\mu\text{mol kg}^{-1} \qquad (1.10)$$

where the constant of 2.9 µmol kg^{-1} was added to obtain a global mean $N^\star$ of zero (see Gruber and Sarmiento, (1997, 2002) for an in depth derivation and a discussion of caveats). More recently, Hansell *et al.* (2004) introduced the tracer ''excess DIN'' (DIN_{xs}) based on the same concept. In fact, DIN_{xs} is identical to $N,^\star$ except that Hansell *et al.* (2004) set the constant to zero, i.e,. $DIN_{xs} = N^\star$ - 2.9 µmol kg^{-1}.

As illustrated in Fig. 1.10a, $N^\star$ is constant for any line with a slope of 16:1 and represents a measure of the distance between any NO_3^- and PO_4^{3-} pair and the mean oceanic trend line.
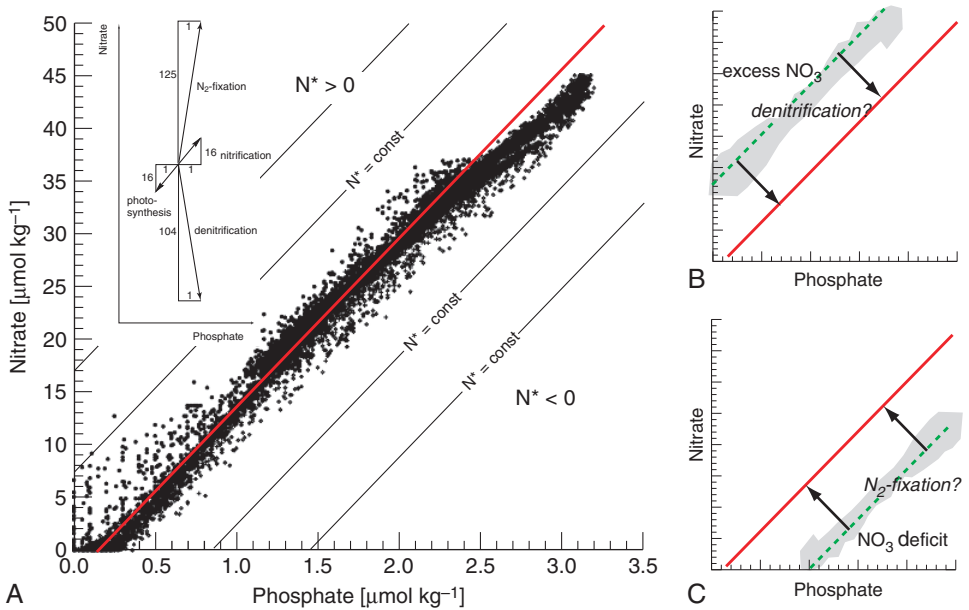
Figure 1.10 (A) Plot of NO_3^- versus PO_4^{3-} from all depths and from selected WOCE cruises in all ocean basins (A16,P16,I8NI9S). The inset shows how the various processes influence the NO_3^- versus PO_4^{3-} distribution. The solid line represents the mean ocean trend with a slope of 16:1, while the thinner lines show trends of constant $N^\star$ (see text for definition). (B) Hypothetical distribution of NO_3^- versus PO_4^{3-} in a situation of excess NO_3^-. (C) as (B), except for a situation of a NO_3^- deficit. It is unclear why the intercept of the mean oceanic trendline in (A) is so close to zero. From Gruber (2004).

The strength of the $N^\star$ concept is that it represents the time and space integrated impact of N_2 fixation and denitrification on the oceanic nutrient fields, which is attractive given the fact that direct rate measurements of N_2 fixation and denitrification are difficult, time-consuming, and generally very sparse (see Chapter 4 by *Carpenter and Capone* and Chapter 6 by *Devol*). A drawback is that $N^\star$ cannot distinguish between N_2 fixation and denitrification as it represents the sum of these two processes. However, the interpretation is greatly aided by the fact that these two processes are often spatially and temporally separated. In addition, concurrent analyses of the isotopic $^{14}N/^{15}N$ ratio can also help to disentangle the various processes that lead to variations in $N^\star$ (Gruber, 2004; Sigman *et al.*, 2003, 2005). Figure 1.11 shows the distribution of $N^\star$ along the same global section used before for NO_3^- (cf Fig. 1.5). $N^\star$ concentrations are highest in the North Atlantic and tend to decrease gradually toward the Pacific. This implies a net transport of fixed nitrogen from the Atlantic into the Pacific, suggesting that, from a global perspective, the North Atlantic acts as a net source of fixed nitrogen for the world ocean, while the Pacific acts as a net sink. This does not imply, however, that N_2 fixation in the Atlantic is higher than in the Pacific. It just implies that the balance of N_2 fixation and denitrification is positive in the Atlantic and negative in the Pacific.

Global maps of $N^\star$ on two isopycnal surfaces ($\sigma_\theta = 26.50$, representing subtropical mode waters (mean depth of about 300 m) and $\sigma_\theta = 27.10$, representing subpolar mode waters) reveal that the $N^\star$ minima seen in the thermocline of the Pacific (Figures 1.9 and 1.11) are part of a large-scale pattern extending over much of the eastern tropical Pacific (Figure 1.12). On the $\sigma_\theta = 26.50$ surface, the $N^\star$ minimum is well separated into an Eastern Tropical South Pacific (ETSP) and into an Eastern Tropical North Pacific (ETNP) part with a local $N^\star$ maximum in between (seen also in the P18 section (Fig. 1.9d)). On the deeper surface ($\sigma_\theta = 27.10$), the $N^\star$ minimum
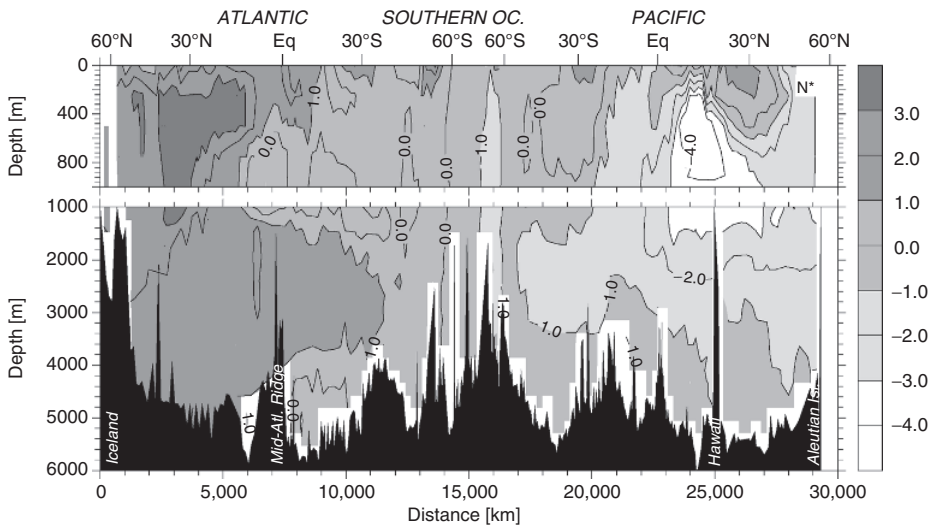
Figure 1.11 As Fig. 1.5, except for $N^\star$ (μmol kg^{-1}).

in the ETSP has lost much of its characteristics, while the minimum in the ETNP remains well defined. Finally, the maps reveal an additional minimum of $N^\star$ in the Arabian Sea.

A more detailed inspection of the $N^\star$ maximum in the subtropical North Atlantic (Fig. 1.12) reveals that the concentration of $N^\star$ increases as waters move
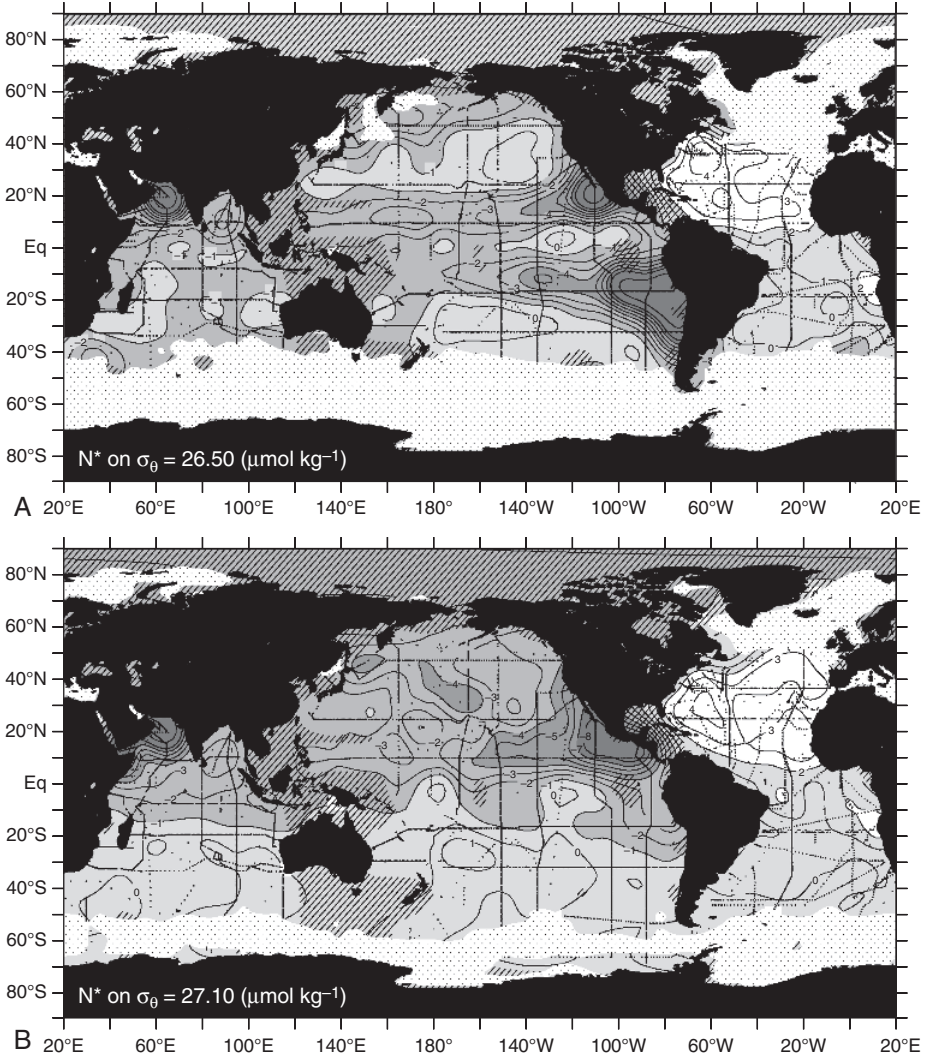
Figure 1.12 Global plots of objectively analyzed $N^\star$ (μmol kg^{-1}) on isopycnal surfaces. (A) $N^\star$ on the $\sigma_\theta = 26.50$ surface. This surface is characteristic of subtropical mode waters, such as the 18° Water in the North Atlantic, (B) $N^\star$ on the $\sigma_\theta = 27.10$ surface representing Subpolar Mode Waters. Solid circles denote the stations; stippling denotes areas where the waters of this potential density are not present in winter time. Cross-hatched areas are regions where the estimated error in objectively analyzed $N^\star$ is greater than 0.7 μmol kg^{-1}. Data are from the WOCE program augmented by selected pre-WOCE cruises and were adjusted by the GLODAP project.

from the outcrop region in the northeastern part of the gyre toward the southwest. This pattern of ingrowth of $N^\star$ was combined with information about the ventilation rate of different water masses to arrive at geochemically-based estimates of N_2 fixation (Gruber and Sarmiento, 1997; Hansell et al., 2004; Michaels et al., 1996). These studies inferred rather divergent N_2 fixation estimates for the entire North Atlantic (see discussion by Hansell and Follows, Chapter 13, this volume), although a new analysis using nitrogen-15 abundance in organic nitrogen by Capone et al. (2005) suggests an intermediate value close to that proposed by Gruber and Sarmiento (1997). This suggestion is also supported by the uncovering of a much larger abundance of Trichodesmium in the subtropical North Atlantic than previously observed (Davis and McGillicuddy, 2006).

Interpretation of the $N^\star$ distribution in the Pacific and Indian oceans is more difficult because of the existence of strong denitrification sites adjacent to the places where one expects to find the N_2 fixation signals. In the subtropical North Pacific, where N_2 fixation is well documented (Karl et al., 1997), the $N^\star$ maximum exists just near the surface and does not extend into the thermocline as is the case in the subtropical North Atlantic. This is likely a result of the eroding effect by waters that obtain extremely low $N^\star$ values in the eastern Pacific and are then advected westwards in the main thermocline (Fig. 1.12).

The strong $N^\star$ minima in the ETNP and ETSP (Figures 1.11 and 1.12) reflect mostly the intense water column denitrification that occurs in these two oxygen minimum regions (Fig. 1.13) (Codispoti and Packard, 1980; Codispoti and Richards, 1976; Hattori, 1983), although benthic denitrification may contribute as well. The two $N^\star$ minima have long tails that reach far westward into the central Pacific. The shape and position of these tails, together with their O_2 concentrations well above the critical O_2 concentration for the onset of denitrification (Fig. 1.13) suggests that these features are the result of westward advection of the $N^\star$ signals in the North and South Equatorial currents and do not reflect in situ water column denitrification. These waters slowly lose their signature as they mix with eastward moving waters in the equatorial undercurrent system and as they entrain subtropical waters, both having elevated $N^\star$ concentrations. A region of reduced $N^\star$ concentrations extends from the ETNP also northward along the American western margin. While most of this signal is thought to reflect the northward advection of low $N^\star$ waters from the ETNP in the California Undercurrent (Castro et al., 2001), these waters likely incorporate $N^\star$ signals from benthic denitrification (Christensen et al., 1987; Devol and Christensen, 1993) or Anammox (Dalsgaard et al., 2003) along the margin as well. These benthic processes explain also many of the low $N^\star$ values found in the North Pacific and elsewhere along the continental margins.

In the Indian Ocean, near surface values of $N^\star$ are slightly elevated (Gruber and Sarmiento, 1997). As these elevated values are located just above some of the lowest $N^\star$ values found anywhere in the world oceans, they suggest rather strong N_2 fixation. Observations of Trichodesmium abundance (Carpenter, 1983) and a few direct rate measurements (reported in Capone et al. (1997)) support this conclusion. The low $N^\star$ concentrations in the thermocline of the Arabian Sea (Fig. 1.12)
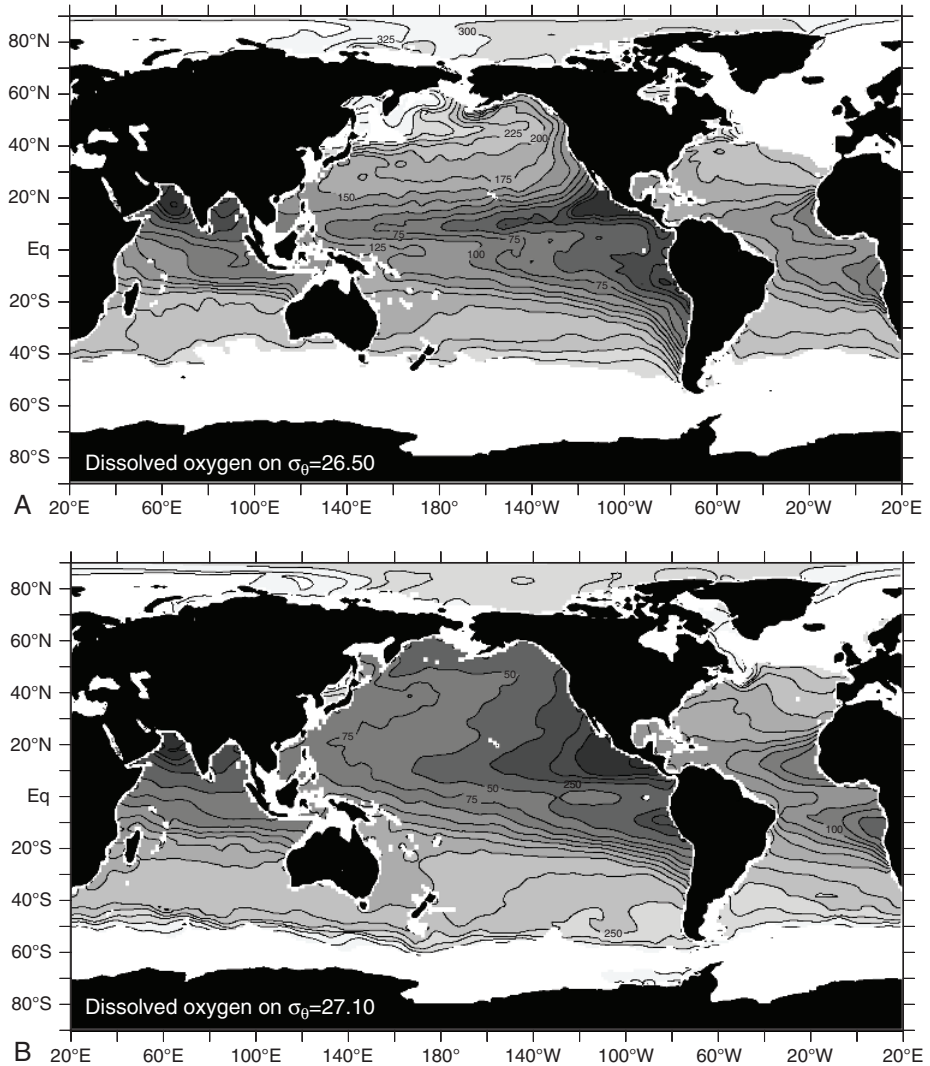
Figure 1.13 Global plots of oxygen (μmol kg^{-1}) on (A) the $\sigma_\theta = 26.50$ surface and on (B) the $\sigma_\theta = 27.10$ surface. Based on data from the World Ocean Atlas (Conkright *et al.*, 2002).

coincide with another well known oxygen minimum zone (Fig. 1.13) with high rates of water column denitrification (Mantoura *et al.*, 1993; Naqvi, 1987; Naqvi *et al.*, 1982; Naqvi, Chapter 14, this volume). Low concentrations of $N^\star$ are also found in the Bay of Bengal, but they likely reflect an advective signal from the Arabian Sea and from the shelf sediments rather than water–column denitrification within the Bay of Bengal itself (Gruber and Sarmiento, 1997).

4. BUDGETS

4.1. Oceanic Nitrogen-budget

Figure 1.14 depicts a summary of the current oceanic nitrogen budget, obtained by combining the fixed nitrogen budget of Gruber (2004) with an organic nitrogen budget derived from an organic carbon budget by Sarmiento and Gruber (2006) assuming a constant C:N ratio in organic matter. The budget is shown separately for the coastal/margin and the open ocean environments, where the former is defined as all regions shallower than 1000 m. The numbers shown are central estimates and in most cases contain uncertainties of at least ±30%.

From a quantitative perspective, the most important process of the fixed nitrogen cycle in the ocean is the assimilation of NO_3^- and NH_4^+ into organic nitrogen in the
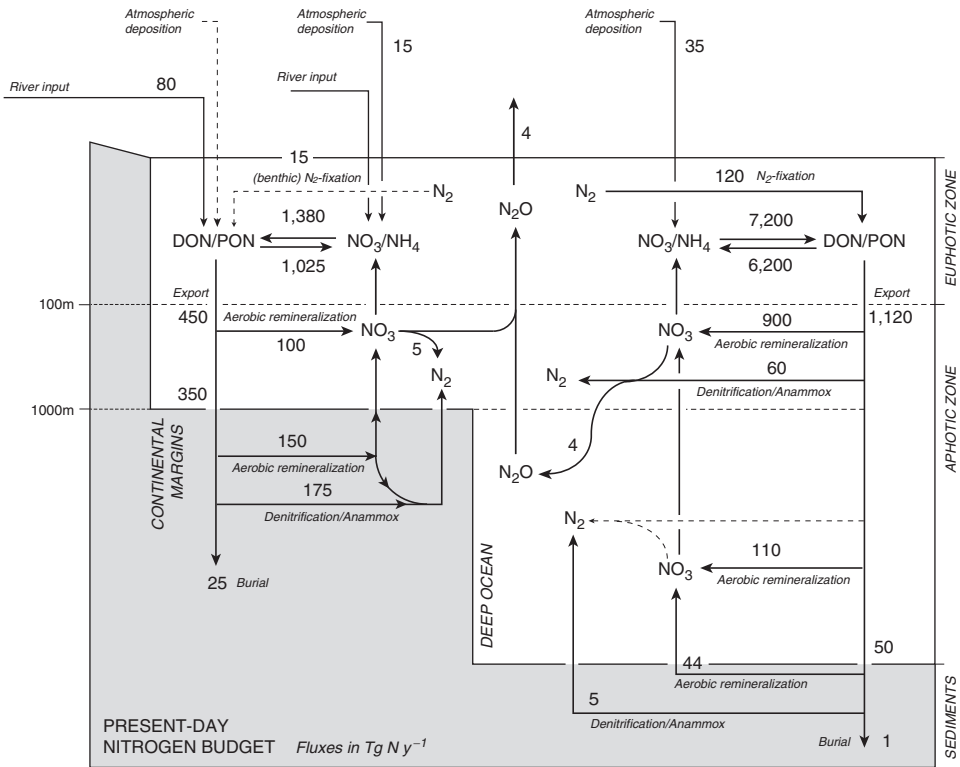
Figure 1.14 Schematic budget of the present-day (*ca.* AD 1990) marine nitrogen cycle. All fluxes are in units of Tg N year^{-1}. The budget is based on the marine organic carbon budget of Sarmiento and Gruber (2006) (their Fig. 6.5.2) and the fixed marine nitrogen budget of Gruber (2004) given in Table 1.3. A stoichiometric C:N ratio of 7.3 (Anderson and Sarmiento, 1994; Schneider *et al.*, 2003) was assumed when converting the organic carbon fluxes to organic nitrogen fluxes. Ill constrained fluxes were computed by difference, assuming a balanced budget. The level of uncertainty varies strongly between the individual fluxes, but is usually of the order of ±30% (see Table 1.3).

euphotic zone, resulting in the fixation of over $\sim$7000 Tg N year^{-1} in the open ocean, and another $\sim$1400 Tg N year^{-1} in the coastal/margin environments. As is the case for organic carbon, of the order of 20% of the organic nitrogen formed in the euphotic zone is lost to the ocean's interior, resulting in export fluxes of organic nitrogen of the order of $\sim$1100 Tg N year^{-1} in the open ocean, and about $\sim$450 Tg N year^{-1} in the coastal/margin environments. In the open ocean, nearly all of this exported organic nitrogen is nitrified back to NO_3^- in the water column, leading to a very small deposition flux of organic nitrogen to the seafloor (about 50 Tg N year^{-1}). In contrast, the majority of the exported organic nitrogen reaches the seafloor in the margin/coastal environments, leading to a deposition flux of $\sim$350 Tg N year^{-1}.

In the open ocean, the low fluxes of organic matter to the seafloor seldom cause anoxic conditions in the near-surface sediments, so that the majority of the 50 Tg N year^{-1} deposited on the seafloor is nitrified, and only a very small fraction is denitrified ($\sim$5 Tg N year^{-1}). This contrasts dramatically with the coastal/margin environment, where the high flux of organic carbon drives much of the sediments in this region anoxic, favoring denitrification and/or anammox over aerobic reminer-alization of the organic nitrogen. The exact magnitude of benthic denitrification is not well established with estimates reaching values as high as 300 Tg N year^{-1} (Codispoti et al., 2001) (Table 1.3). I adopt here the estimate of Gruber (2004) of 180 Tg N year^{-1}, which is similar to that used by Galloway et al. (2004) and is supported by the recent estimate of 190 Tg N year^{-1} by Deutsch et al. (2004).

Table 1.3 Present-day (ca 1990) Global marine nitrogen budgets of Codispoti et al. (2001), Gruber (2004), and Galloway et al. (2004)

Process	Codispoti et al.[a]	Galloway et al.[a,b]	Gruber[a]
	Sources (Tg N yr^{-1})		
Pelagic N$_2$ fixation	117	106	120 $\pm$ 50
Benthic N$_2$ fixation	15	15	15 $\pm$ 10
River input (DON)	34	18[c]	35 $\pm$ 10
River input (PON)	42	30[c]	45 $\pm$ 10
Atmospheric deposition	86	33	50 $\pm$ 20
Total sources	294	202	265 $\pm$ 55
	Sinks (Tg N yr^{-1})		
Organic N export	1		1
Benthic denitrification	300	206	180 $\pm$ 50
Water column denitrification	150	116	65 $\pm$ 20
Sediment Burial	25	16	25 $\pm$ 10
N$_2$O loss to atmosphere	6	4	4 $\pm$ 2
Total sinks	482	342	275 $\pm$ 55

[a] See the original publications for details, i.e. Galloway et al. (2004), Codispoti et al. (2001), and Gruber (2004).
[b] Listed are the central values reported by Galloway et al. (2004) (see Table 1 and Fig. 1 of their publication).
[c] Galloway et al. (2004) lists only the total river flux. I assumed that about two thirds of the total is PON, and one third is DON.

Subtracting the small amount of benthic denitrification in the open ocean ($\sim$5 Tg N year^{-1}), leads to a coastal/margin benthic denitrification flux estimate of $\sim$175 Tg N year^{-1}. Assuming that the influx of NO_3^- into the sediments from the overlying bottom water is small, this means that about 50% of the organic nitrogen being deposited on the seafloor in the margin/coastal environments is denitrified. 25 Tg N year^{-1} is buried, which implies that about 150 Tg N year^{-1} or about 43% of the deposited organic nitrogen is undergoing aerobic oxidation to nitrate (nitrification). The benthic denitrification estimate of Codispoti *et al.* (2001) of 300 Tg N year^{-1} would mean that nearly 90% of the nitrogen deposited on the seafloor was denitrified, leaving only 25 Tg N year^{-1} for nitrification. Considering that a substantial fraction of the bottom waters overlying the sediments in the margin/coastal environments are oxygenated, such a small role for nitrification is difficult to imagine. However, the presently available data are insufficient to reject either estimate.

Water column denitrification represents the second most important loss process for fixed nitrogen. Current rate estimates diverge quite substantially (see Table 1.3), with estimates as high as 150 Tg N year^{-1}. I adopted here the estimate by Gruber (2004) of 65 Tg N year^{-1}, which is at the low end of the current range of estimates, but satisfies the nitrogen–15 mass balance constraint of an approximately 3:1 ratio between benthic and water-column denitrification (Brandes and Devol, 2002). It is also in good agreement with the recent estimate of 70 Tg N year^{-1} by Deutsch *et al.* (2004). I assume that the majority of the water column denitrification occurs in the open ocean, such as the eastern Tropical Pacific and the Arabian Sea. The formation and subsequent loss of N_2O represents another loss term. Although its magnitude is small (about 4 Tg N year^{-1}), this flux is important when considering the atmospheric N_2O budget, as it represents about a third to half of the estimated pre-industrial N_2O sources to the atmosphere (Prather *et al.*, 2001; see also Chapter 2 by Bange, this volume).

These fixed nitrogen losses add up to more than 300 Tg N year^{-1} (Table 1.3). Without processes supplying fixed nitrogen to the ocean, these losses would lead to a complete exhaustion of all fixed nitrogen in the ocean within about 2000 years. Atmospheric deposition of fixed nitrogen is estimated to be about 50 Tg N year^{-1} (Duce *et al.*, 2008) and rivers add another 80 Tg N year^{-1} the ocean (see Seitzinger and Harrison in Chapter 9, this volume, for a more thorough discussion of nitrogen fluxes by rivers). The largest source of fixed nitrogen to the ocean is N_2 fixation, however. Over the last two decades, estimates of the magnitude of global pelagic N_2 fixation have increased dramatically, but most recent estimates con-verge around 100 to 150 Tg N year^{-1} (Table 1.3) (Gruber, 2004; Gruber and Sarmiento, 1997; Galloway *et al.*, 2004; Deutsch *et al.*, 2007). It is presently not well established how much of this global N_2 fixation occurs in the coastal/margin environment, although the canonical view is that most of it occurs in the open ocean, where conditions are usually more conducive for *Trichodesmium*, i.e., high temperatures, high vertical stability, and low NO_3^- concentrations (Karl *et al.*, 2002). However, recent observations in the Southern California Bight suggest also substantial rates of N_2 fixation in a coastal setting, particularly during the nutrient depleted, well stratified summer period (A. Leinweber and D. Capone, personal communication, 2006). Benthic environments add another $\sim$15 Tg N year^{-1} to the ocean, primarily in very nearshore environments (Capone, 1983).

Adding these numbers together, a nearly balanced marine nitrogen budget emerges, albeit with very large uncertainties. A strongly differing view was recently taken by Codispoti *et al.* (2001), who suggested that the losses of fixed nitrogen were underestimated in the past by nearly a factor of two, leading to a marine nitrogen budget that may be out of balance by nearly 200 Tg N year^{-1}. These authors suggested that this may reflect the substantial alteration of the global nitrogen cycle by humans, and therefore may be a very recent phenomenon. Galloway *et al.* (2004) recently reviewed the global nitrogen budget and arrived at an oceanic fixed nitrogen budget that is in between that of Codispoti *et al.* (2001) and that of Gruber (2004) (see Table 1.3). Galloway *et al.* (2004) also provided direct estimates of the human perturbation fluxes, and identified that these account for about $\sim$45 Tg N year^{-1}. Even when considering the substantial uncertainties with this estimate, this perturbation flux is much smaller than the imbalances in the marine nitrogen budgets of Codispoti *et al.* (2001) and Galloway *et al.* (2004). Therefore the human perturbation is unlikely the cause for the imbalance in the budget. The much more likely cause are uncertainties in the different terms of the budget, in particular the magnitude of benthic and water column denitrification. Therefore, a renewed focus on these two processes with the aim to reduce their uncertainties would provide currently the most efficient way to reduce uncertainties in the oceanic fixed nitrogen budget. However, regardless of these uncertainties, all budgets agree that the marine nitrogen cycle is very dynamic, with a residence time for fixed nitrogen of about 3000 years only (see Table 1.2). This contrasts with the residence time of PO_4^{3-} in the ocean, which is about 30,000 to 50,000 years (Delaney, 1998).

4.2. Global Nitrogen-budget

Figure 1.15 shows the marine nitrogen cycle in the context of the global nitrogen cycle in preindustrial times as well as how it has changed since then. A first remarkable observation is that the ocean is the largest reservoir of fixed nitrogen on Earth, containing about 5 times more fixed nitrogen than the land biosphere, where most of it is stored in soils. The amount of fixed nitrogen in the atmosphere is tiny, reflecting the high reactivity of these nitrogen species in the atmosphere and their quick removal. A second remarkable observation is that biological N_2 fixation on land and in the ocean is of similar magnitude. Interestingly, the same is the case for primary production, where photosynthetic organisms in both the ocean and on land fix about the same amount of carbon each year (Field *et al.*, 1998). However, due the lower fixed nitrogen inventory of the land biosphere, the turnover times for fixed nitrogen on land is faster than that in the ocean. Contrasting the land and ocean further, the land acted in preindustrial times as a net source of fixed nitrogen, while the ocean acted as a net sink. On land, preindustrial N_2 fixation exceeds denitrification so that a certain fraction of the fixed nitrogen can be exported laterally by rivers to the ocean, where it permits oceanic denitrification and burial to exceed N_2 fixation. The ocean also receives fixed nitrogen from the atmosphere, some of which has been produced there from N_2 by lightning, and some of which is exported from the land through the atmosphere.
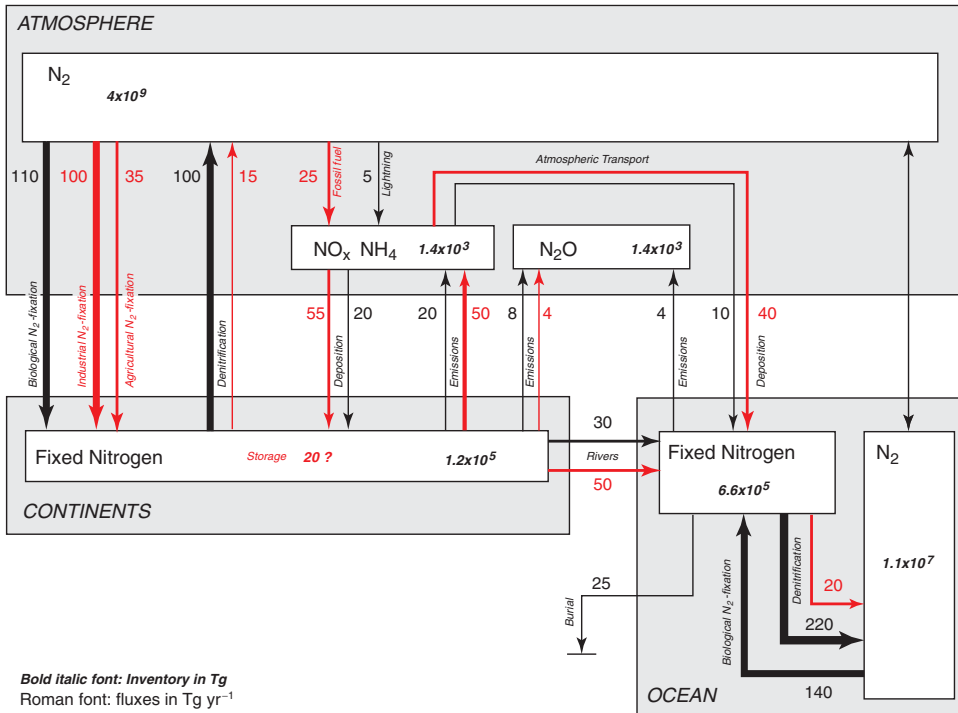
Figure 1.15 Schematic budget of the global nitrogen cycle in preindustrial times (black) and how it changed as a result of human intervention (red). Fluxes are in units of Tg N year^{-1} and inventories (bold italics) in Tg N. The flux estimates are based on Gruber and Galloway (2008).

Over the last 250 years, humans have caused a massive perturbation of the global nitrogen cycle (see Galloway (1998) and below for more discussion), with most impact in terrestrial ecosystems. Industrial N_2 fixation by the Haber–Bosch process now rivals biological N_2 fixation by the land biosphere. Furthermore, the planting of legumes and other plants with endosymbiontic N_2 fixers has led to an additional input of fixed nitrogen to the land. Finally, the burning of fossil fuels leads to the generation of NO_x, most of which gets oxidized in the atmosphere to NO_3^- before being deposited on land. The combined anthropogenic inputs of fixed nitrogen into land ecosystems now exceeds the preindustrial net input by nearly 50%. Most of this anthropogenic nitrogen input into the land ecosystems is either stored there or is denitrified, but a substantial fraction gets exported to the ocean via rivers and atmosphere, leading to more than a doubling of the input of fixed nitrogen into the ocean. The fate of this anthropogenic nitrogen in the ocean is not well understood. It appears as if most of the anthropogenic nitrogen transported by rivers is denitrified in the lower parts of the estuaries and in the coastal ocean (Seitzinger and Kroeze, 1998; Galloway *et al.*, 2004). By contrast, much of the increased atmospheric deposition likely makes it further out into the ocean, possibly stimulat-ing productivity there (Duce, 1986).

5. Nitrogen Challenges

5.1. Controls on the marine fixed Nitrogen-content

On some timescale approaching the turnover time of fixed nitrogen in the ocean (i.e., about 2000 to 3000 years), nitrogen fixation and denitrification in the ocean must be coupled to each other, otherwise the marine fixed nitrogen content of the ocean would have undergone massive swings in the past, with large implications for marine productivity. There is little evidence in the geological record that such massive variations occurred (Sigman and Haug, 2003), suggesting that the coupling must be reasonably tight. This requires the existence of well developed negative feedbacks that prevent the growth of initial perturbations, such as induced, for example, by the large climate variations associated with the glacial–interglacial transitions in the last million years.

Two negative feedback processes have been proposed to stabilize the oceanic nitrogen cycle (see Fig. 1.16 and discussions by e.g., Tyrrell (1999), Deutsch et al. (2004), and Gruber (2004), see also Galbraith et al. in Chapter 34, this volume). The first feedback process is based on denitrification (Broecker and Peng, 1982; Codispoti, 1989). Suppose that denitrification became stronger in response to some environmental change. This would cause the fixed nitrogen content of the ocean to decrease relative to the PO_4^{3-} content, thereby tending to reduce the supply of fixed nitrogen to the surface ocean as well. This would result in a smaller export production, leading to a smaller oxygen demand in the thermocline and a smaller organic nitrogen deposition onto the marine sediments. Since these two processes are thought to control denitrification, their decrease would cause both column and benthic denitrification do decrease as well. This would reduce the initial perturbation, i.e., close the negative feedback loop.

The second feedback builds on N_2 fixation and the assumption that diazotrophic organisms can outcompete "normal" phytoplankton in situations of severe NO_3^- depletion but where PO_4^{3-} is still present (Karl et al., 2002), but that diazotrophic organisms are otherwise at a disadvantage because of their slow growth. Therefore, if fixed nitrogen became scarce relative to PO_4^{3-} in the ocean, these surface regions characterized by residual PO_4^{3-} would expand, increasing the ecological niche for diazotrophic organisms. As a consequence, N_2 fixation would increase and add newly fixed nitrogen to the ocean. After a while, the resulting increase in fixed nitrogen would reduce the regions of PO_4^{3-} residuals, removing the competitive advantage of diazotrophs, and hence lowering N_2 fixation again. An equilibrium is reached when N_2 fixation is back in balance with denitrification. Haug et al. (1998) provided evidence that such a feedback might have operated across glacial–interglacial cycles in the Cariaco Basin.

In both proposed feedback mechanisms, the dominant controlling factor is the surface ocean NO_3^- to PO_4^{3-} ratio (see Fig. 1.16). Both feedbacks are expected to operate in situations of excess NO_3^- (i.e., high NO_3^- to PO_4^{3-} ratio, shown at the bottom of the feedback loop in Fig. 1.16), as well as in situations of NO_3^- deficiency (i.e., low NO_3^- to PO_4^{3-} ratio, shown at the top of the feedback loop). If this view is correct, these two feedbacks would provide a rather tight control of the marine nitrogen cycle, creating a well balanced homeostasis. A further consequence is that
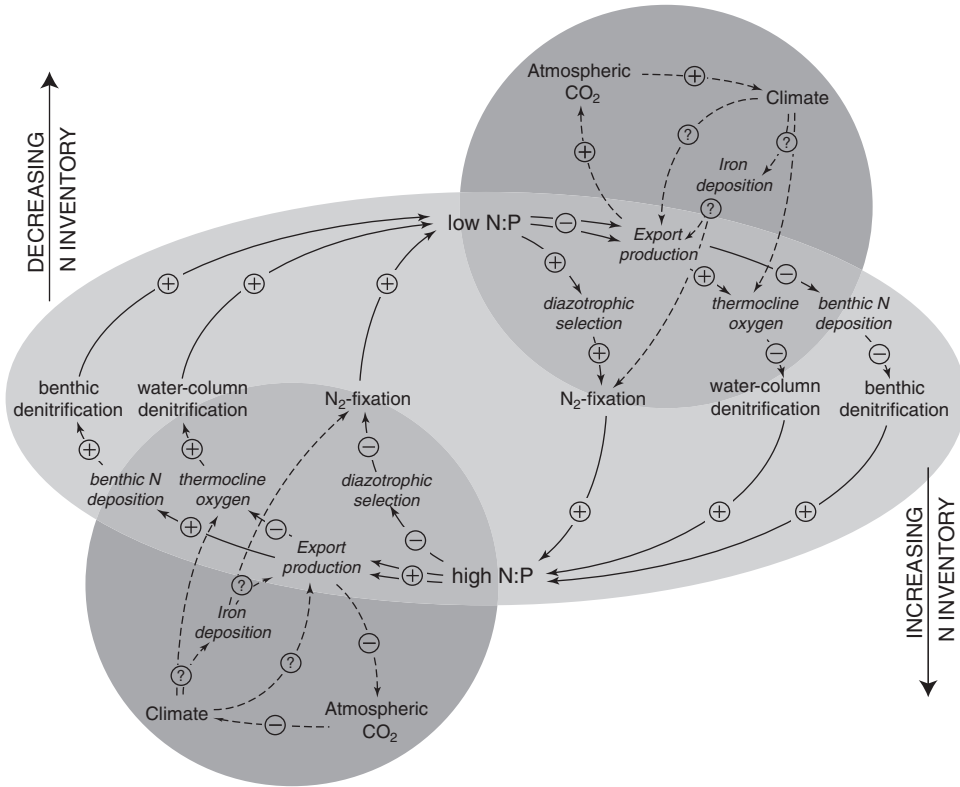
Figure 1.16 Schematic representation of feedbacks within the marine nitrogen cycle. The inner light grey ellipse shows the two nitrogen cycle "internal" feedback loops that tend to be stabilizing. These two feedback are proposed to be mainly controlled by the NO_3^- to PO_4^{3-} (N:P) ratio in surface waters. If the surface N:P ratio is low, denitrification tends to get reduced because of lower surface productivity, while N_2 fixation tends to increase because of favorable conditions for diazotrophic organisms. Both processes lead to an increase in the oceanic fixed nitrogen inventory. In contrast, when the surface N:P ratio is high, N_2 fixation tends to decrease, while denitrification increases, leading to a reduction of the oceanic fixed nitrogen inventory. These nitrogen internal feedbacks can be altered significantly by their interaction with the carbon cycle, climate and other processes (dark grey ellipses). The sign of these feedbacks are currently not understood and can be either positive or negative, leading to stabilizing or destabilizing feedbacks. From Gruber (2004).

on time–scales longer than the residence time of phosphorus in the ocean (i.e., about 30,000 to 50,000 years (Delaney, 1998)), the marine nitrogen cycle would be completely slaved to the marine phosphorus cycle, making PO_4^{3-} the ultimate limiting nutrient (Tyrrell, 1999). This view can be traced back to Redfield (1958) and is shared by many geochemists (e.g., Broecker and Peng (1982), see discussion by Codispoti (1989)). This also would move processes that control the marine phosphorus content, such as erosion on land, and burial in marine sediments to the center of attention if one wants to understand past oceanic productivity (Lenton

and Watson, 2000). However, upon closer inspection, the processes and factors controlling marine N_2 fixation and denitrification are more complex than simply the NO_3^- to PO_4^{3-} ratio of surface waters.

A first challenge to the PO_4^{3-} control hypothesis emerges from the observation that the tropical and subtropical North Atlantic are sites of high N_2 fixation (Capone *et al.*, 2005; Carpenter and Romans, 1991), yet these waters have extremely low PO_4^{3-} concentrations (Wu *et al.*, 2000). By contrast, there appear to be vast regions in the South Pacific that have substantial residual PO_4^{3-} concentrations, yet there is little evidence for enhanced N_2 fixation (Carpenter, 1983). Clearly, other factors besides the NO_3^- to PO_4^{3-} ratio must play a role in controlling N_2 fixation. Karl *et al.* (2002) suggested temperature, light, and the ambient concentrations of O_2, NO_3^-, NH_4^+, and essential trace metals. In particular, the availability of iron has been proposed as a major factor controlling N_2 fixation, based on the argument that N_2 fixers have a particularly high iron demand (Falkowski, 1997). If this was correct, there would exist substantial potential for the marine nitrogen cycle to escape the proposed negative feedbacks, and cause major changes in marine productivity and atmospheric CO_2 (Broecker and Henderson, 1998; Falkowski, 1997; Lenton and Watson, 2000; Michaels *et al.*, 2001).

The observed pattern of N_2 fixation, the distribution of $N^\star$ and the fact that the North Atlantic and to some degree the North Pacific receive much more iron from the atmosphere than the South Pacific appear to be consistent with the posit of iron playing an important role for controlling N_2 fixation. However, the evidence for large-scale iron limitation of N_2 fixation is largely circumstantial. Results from iron addition experiments are inconclusive (Hood *et al.*, 2000), or show that the stimulation is small unless PO_4^{3-} is added as well (Mills *et al.*, 2004). Recent analyses of the iron content in natural populations of *Trichodesmium* in the central Atlantic reveal neither a significantly higher iron requirement, nor a geographical pattern in cellular iron content consistent with patterns of iron deposition (Sanudo-Wilhelmy *et al.*, 2001). Rather, these authors assign PO_4^{3-} a key role in explaining the observed variability. More recently, Deutsch *et al.* (2007) pointed out that the global distribution of upper ocean NO_3^- and PO_4^{3-}, when interpreted diagnostically with a simple coupled ecological/physical model, suggest that N_2 fixation is mainly located in the Pacific Ocean and tends to be associated with regions of NO_3^- deficiency, i.e., PO_4^{3-} excess. They interpret these results as an indication that the NO_3^- to PO_4^{3-} ratio re-emerges as the primary factor controlling marine N_2 fixation, lending support to the N_2-fixation-based negative feedback loop in Fig. 1.16.

What about the denitrification-based feedback loops? The primary factor controlling benthic denitrification is thought to be the supply of organic nitrogen to the sediments (Middelburg *et al.*, 1996). As the flux of organic nitrogen to the seafloor is primarily a function of surface export production and bottom depth, these two factors ultimately control benthic denitrification. The former is connected to the surface ocean NO_3^- content, providing support for the negative feedback loop. However, bottom topography can be changed independently, leading to the potential for decoupling benthic denitrification from N_2 fixation. Such a scenario was proposed by McElroy (1983), who suggested that the lowering of sea-level during the last glaciation caused a substantial decrease of benthic denitrification, leading to a higher marine fixed nitrogen inventory, and ultimately lower atmospheric CO_2.

The primary factor controlling water column denitrification is the oxygen concentration. In the interior of the ocean, the condition for the generation of an oxygen depletion zone is given when the supply of O_2 by ocean transport from the surface ocean is smaller than the oxygen demand from the remineralization of organic matter. Therefore both low ventilation of the water column or high rates of remineralization of organic matter can cause oxygen depleted waters and hence water column denitrification. It appears that both processes are important for generating the oxygen depleted conditions in the three regions, where we currently observe such conditions i.e., the ETSP, the ETNP and the Arabian Sea. Therefore, the dependence of water column denitrification on surface ocean productivity and export provides the needed link for creating the proposed negative feedback loop. In contrast, the possibility of altering water column denitrification by changes in ocean circulation provides a potential avenue for undoing the tight control of the marine nitrogen content by the denitrification feedback loop. There is ample evidence in the geological record that water column denitrification indeed varied greatly in the past (Altabet *et al.*, 1995, 2002; Ganeshram *et al.*, 1995, 2000). However, evidence for large changes in glacial productivity are scarce, and in summary suggesting a rather similar biological productivity during those times, but with a different spatial distribution (Kohfeld *et al.*, 2005).

Despite substantial uncertainty, the view that emerges is an oceanic nitrogen cycle that is relatively well stabilized by the two proposed negative feedback loops. Both feedbacks operate on the time-scale of whole ocean overturning, i.e., decades to a few hundred years, thus keeping the system only in an approximate dynamic homeostasis, as on time-scales shorter than this, imbalances between N_2 fixation and denitrification can exist and significantly alter the fixed nitrogen content of the ocean. The internal feedbacks can be perturbed by outside factors, including changes in climate, iron deposition, and sea-level, some of which can form closed feedback loops (Fig. 1.16, see also Michaels *et al.* (2001)). While the basic structure of these controls on the marine nitrogen cycle seem relatively well established, there remain many open questions. For example, what is the role of iron limitation for marine N_2 fixation? For example, Lenton and Watson (2000) demonstrated on the basis of model simulations that the proposed negative feedback loops keep the marine nitrogen cycle indeed closely slaved to the marine phosphorus cycle under iron replete conditions. However, when they reduced the iron availability, N_2 fixation became decoupled from denitrification, leading to a substantial deficit in the marine nitrogen content relative to that of phosphorus.

5.2. The marine Nitrogen-cycle, Carbon-cycle, and climate

Given the importance of the marine nitrogen cycle in controlling marine productivity and the close connection between the marine carbon and nitrogen cycles (see Fig. 1.1), it is not surprising that changes in the marine nitrogen cycle were suggested very early on as a possible explanation of past variations in the global carbon cycle and particularly atmospheric CO_2 (e.g., McElroy (1983), and more recently Altabet *et al.* (1995), Ganeshram *et al.* (1995), Falkowski (1997), Broecker and Henderson (1998), and Michaels *et al.* (2001)) (see Chapter 34 by Galbraith *et al.* and Chapter 35

by Berman-Frank *et al.* for more in depth discussions). Although they vary in their details, the basic premise of all hypotheses is that it is possible to decouple marine N_2 fixation and denitrification enough to cause a long-term change in the marine inventory of fixed nitrogen. This change would then alter marine productivity, and consequently the ocean–atmosphere partition of CO_2. However, as discussed above, it appears to be relatively difficult to keep the marine nitrogen cycle out of balance for a long time, as the negative feedbacks seem rather strong. This prevents sizeable variations in marine productivity to occur in response to alterations in the marine nitrogen cycle, limiting the strength of the nitrogen cycle-based hypotheses to explain past changes in atmospheric CO_2.

A second argument against marine nitrogen cycle-based hypotheses is the observation that low- and mid-latitude alterations of the surface ocean CO_2 chemistry are generally much less efficient in changing atmospheric CO_2 than high–latitude changes (Broecker and Peng, 1998; Broecker *et al.*, 1999). This is primarily a result of the high-latitudes providing the "window" to the deep ocean, where most of the combined atmosphere-ocean carbon inventory resides. Since biological productivity in the high-latitudes, and particularly in the Southern Ocean are controlled by factors other than fixed nitrogen, the nitrogen–cycle based hypotheses primarily operate through changes in low- and mid-latitude productivity. This gives these hypotheses much less leverage on atmospheric CO_2 than iron based hypotheses that permit large changes in high–latitude productivity. In summary, despite a possibility for surprises given our limited understanding of the processes that lowered atmospheric CO_2 during the ice-ages, it appears as if changes in the marine nitrogen cycle have had relatively little influence on atmospheric CO_2 (Gruber, 2004).

Nevertheless, past changes in the marine nitrogen cycle were likely quite substantial. This is evidenced, for example, by the considerable changes in atmospheric N_2O associated with the glacial/interglacial transitions (Flückiger *et al.*, 1999), and the large changes in the extent of denitrification (Altabet *et al.*, 1995; Ganeshram *et al.*, 1995) and their possible connection with atmospheric CO_2 variations (Altabet *et al.*, 2002). However, our quantitative understanding of the past changes in the marine nitrogen cycle is only poorly developed, representing another major challenge of the marine nitrogen cycle. The development of a better quantitative understanding of these past changes is particularly important if we want to have some confidence in projecting the impact of future climate changes on the oceanic nitrogen cycle.

5.3. The anthropogenic perturbation

The direct anthropogenic perturbation of the global nitrogen cycle has been massive, leading to an enormous acceleration of this cycle (see Fig. 1.15 and Galloway *et al.* (1995), Vitousek *et al.* (1997), Galloway *et al.* (2002), and Gruber and Galloway (2008)). Although the widespread application of nitrogen-based fertilizers has been an important contributor to the ability of humans to grow enough despite a rapidly growing population, the negative environmental effects of this fertilizer use are widespread (Galloway *et al.*, 2002). In addition, the creation of reactive forms of nitrogen (mostly NO_x) during the combustion of fossil fuels has also increased dramatically over the last few decades, leading to problems as diverse as enhanced

tropospheric ozone formation, acid rain, and eutrophication in aquatic ecosystems. Galloway and Cowling (2002) introduced the term *nitrogen cascade* to emphasize that a fixed nitrogen molecule introduced into the environment by humans often leads to a whole series of impacts, some of which occur much later and far away from where the molecule was introduced first. For example, the application of nitrogen fertilizer on some agricultural patch may first lead to the loss of biodiversity. As some of this fertilizer gets washed into nearby streams, it leads to eutrophication there, and later, perhaps, in a lake downstream. If this fixed nitrogen escapes denitrification, it may end up in the coastal ocean, causing another episode of eutrophication.

On the global scale, there is little evidence so far that the acceleration of the global nitrogen cycle by humans has led to detectable changes in the marine nitrogen cycle (Duce *et al.*, 2008). This is not the case in many coastal regions, however, as there exist several well documented examples of coastal eutrophication in direct response to the input of anthropogenically derived nitrogen, mainly by rivers (see e.g., Beman *et al.* (2005), Rabalais (2002), and Chapter 11 by Paerl and Piehler, this volume for a discussion). The impact of these changes can be multi-faceted, ranging from the loss of habitat for fish due to reduced oxygen concentrations to the increased occurrence of blooms of harmful algae. Global creation of fixed nitrogen by humans is expected to increase severalfold in the next 100 years, perhaps going as high as 900 Tg N year^{-1} (Galloway *et al.*, 2002). We therefore have to expect a substantial worsening of the coastal eutrophication problem, as well as to consider the possibility of sizeable direct changes in the nitrogen cycle of the open ocean.

In addition, humans will affect the nitrogen cycle likely also indirectly through anthropogenically induced climate change. Climate projections for the 21st century are inherently uncertain due to uncertainties in the nature and magnitude of future energy use as well as uncertainties in the global carbon cycle and the physical climate system, but it is likely that Earth will warm another few degrees Celsius in this century (see Houghton *et al.* (2001)). This could cause a number of feedbacks within the Earth system involving the marine nitrogen cycle (see Chapter 35 by Berman-Frank *et al.* and Gruber and Galloway (2008)).

For example, nearly all climate projections for the 21st century suggest that oceanic stratification will increase (Sarmiento *et al.*, 1998), primarily as a result of the input of heat into the surface ocean, but in part also by a freshening of the upper ocean. This will likely cause a decrease in export production in the low latitudes, as it will restrict the upward transport of nutrients (Bopp *et al.*, 2001; Matear and Hirst, 1999; Sarmiento *et al.*, 1998;). On the other hand, export production in the mid-to high-latitudes may actually increase due to lower light stress in a shallower mixed layer (Bopp *et al.*, 2001).

The details of these productivity changes vary from model to model, but the future warming acting in concert with increased stratification and reduced ventilation of the thermocline will almost certainly decrease the oxygen content of the ocean's interior (Bopp *et al.*, 2002; Deutsch *et al.*, 2005; Matear *et al.*, 2000; Plattner *et al.*, 2002). This would enhance denitrification and the production of nitrous oxide. Unless this increased loss of nitrogen is compensated by increased N$_2$ fixation, the resulting decrease in the inventory of fixed nitrogen and biological productivity

would result in a net loss of CO_2 from the ocean, causing a positive feedback. The increased production of nitrous oxide would constitute a second positive feedback, leading to an overall acceleration of climate change due to the interaction of the climate system with the marine nitrogen cycle.

6. CONCLUSIONS

Although the marine nitrogen cycle occupies a central role within the biogeochemical cycles of the sea, we have just barely begun to understand its major processes and the factors that regulate them. This occurs at a time when human interventions in the Earth system has risen to unprecedented levels, with a particularly strong impact on the global nitrogen cycle. Although the nitrogen cycle in the open ocean appears to have remained largely unscathed from human influence, many coastal systems have already been seriously impacted. Projections of the future suggest that this may change soon, either directly by the continued addition of anthropogenically created fixed nitrogen to the Earth system and its cascading effects, or indirectly through anthropogenically induced climate change. Some of these alterations have the potential to cause positive feedbacks in the climate system, but they are neither well understood nor quantified. It behooves us well to address these difficult, but exciting challenges.

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Gaseous Nitrogen Compounds (NO, N$_2$O, N$_2$, NH$_3$) in the Ocean

Hermann W. Bange

Contents

1. INTRODUCTION

Nitric oxide (NO), nitrous oxide (N_2O), dinitrogen (N_2), and ammonia (NH_3) are constituents of the Earth's atmosphere. They play important roles in the chemistry and climate of the present-day Earth. Moreover, they are intermediates of the oceanic nitrogen cycle. In contrast to most of the other components of the oceanic nitrogen cycle, they exist as dissolved gaseous molecules. Being gases they can be transferred across the seasurface–troposphere interface.

In this chapter, I intent to give an overview about the current knowledge on the oceanic distribution and pathways of NO, N_2O, N_2, and NH_3, which has increased considerably since the publication of ''Gaseous nitrogen compounds in the sea'' by Scranton (1983).

Fundamental physical processes such as gas diffusivities in seawater and air–sea gas exchange as well as details of measurement techniques for dissolved gases in seawater are not discussed in the context of this chapter.

Because of the heavily increasing number of publications (especially for N_2O) it is not possible to give a comprehensive overview on all aspects of the gases discussed in this chapter. Therefore, it was necessary to focus the review on one particular topic—the water-column distribution. However, to cover emerging new developments, sedimentary and atmospheric measurements/processes are introduced when necessary. I tried to document the most actual developments, however, especially for N_2O, it was not possible to consider all relevant publications.

To have a high degree of transparency, I predominately used publications in international journals or books. ''Grey'' literature such as PhD thesis or submitted articles etc. is cited only when unavoidable.

In the literature, dissolved gases are generally expressed either in concentrations ($nmol\ L^{-1}$ or $nmol\ kg^{-1}$ or $mL\ mL^{-1}$) or are expressed as a saturation ratio, which is the ratio of the measured dissolved gas concentration to the expected equilibrium concentration. Saturation ratios <1 indicate undersaturation whereas saturation ratios >1 stand for supersaturation. Saturations are also expressed in percentage, which is equal to the saturation ratio times 100. For example, a supersaturation of 3% is equal to a total saturation of 103%, which in turn is equal to a saturation ratio of 1.03.

2. NITRIC OXIDE

2.1. Climatic and biogeochemical relevance

Nitric oxide or nitrogen monoxide (NO) is a colorless gas. It has a boiling point (bp) of $-151.8°C$ (at 1 atm) and molecular weight of $30.0\ g\ mol^{-1}$. Because it has an odd number of electrons, NO is a very reactive molecule (a so-called radical) (Greenwood and Earnshaw, 1984). It is rapidly oxidized within a few minutes to nitrogen dioxide (NO_2), thus NO and NO_2 are usually referred to as

NO_x (= $NO + NO_2$). NO is a short-lived intermediate in a variety of chemical reactions in both the troposphere and the stratosphere (Warneck, 2000). In the stratosphere NO is derived from the photochemical decomposition of nitrous oxide (N_2O, see Section 3) and is involved in one of the main catalytic ozone (O_3) reaction cycles (Crutzen, 1970, 1996). In the troposphere, NO is rapidly oxidized either to nitrous acid (HNO_2) by reacting with hydroxyl radicals (OH) or to nitrogen dioxide (NO_2) by reacting with O_3 or hydroperoxyl radicals (HO_2). NO_x is involved in the catalytic O_3 formation in the troposphere. The atmospheric lifetime of NO_x ranges from <1 day to several days. In regions with high NO emissions from fossil-fuel combustion (e.g., in urban areas), complex photochemical processes involving NO_x and hydrocarbons result in photochemical smog with enhanced O_3 concentrations. One of the final oxidation products of NO, nitric acid (HNO_3), forms NO_3^- aerosol particles or is washed out by rain. Enhanced NO emissions result in enhanced HNO_3 formation, which in turn lowers the pH of rain ("acid rain"). Photochemical smog and stratospheric O_3 depletion can only indirectly influence the marine nitrogen cycle, whereas acid rain and the dry and wet deposition of NO_3^- aerosol and NO_x can directly impact the marine nitrogen cycle and thus marine productivity in coastal areas (De Leeuw *et al.*, 2003; Jickells, 2006; Kouvarakis *et al.*, 2001) and to a lesser extent in remote open ocean areas (Bange *et al.* (2000); Jickells (2006)) (see Chapters 9 and 11).

NO_x atmospheric mixing ratios range from about 100 ppb (10^{-9}) in polluted urban areas to a few ppt (10^{-12}) in remote marine air masses, indicating that in remote marine locations pristine conditions prevail (Warneck, 2000). In past years, NO_x emissions from ship engines have been recognized as a significant pollution of the marine atmosphere with a profound impact on the atmospheric nitrogen chemistry of the marine boundary layer in ship plumes (Corbett and Fischbeck, 1997; Lawrence and Crutzen, 1999; Von Glasow *et al.*, 2003). However, the effects of NO_x emissions from ships (e.g., via deposition as HNO_3 and NO_3^- aerosols) on the marine nitrogen cycle are yet unknown.

2.2. NO in the ocean

The solubility of NO in water is low; Henry's law constants for NO in water are listed in the literature compilation by Sander (1999). Dissolved NO in seawater can be determined with a purge system, which is connected to a chemoluminescence detector for NO (Ward and Zafiriou, 1988; Zafiriou and McFarland, 1980, 1981).

In aquatic systems NO reacts rapidly with O_2, O_2^-, and NO_2 (Zafiriou *et al.*, 1990). Because of its reactivity, NO has been used as molecular probe to determine production rates of long-lived radicals in seawater (Zafiriou *et al.*, 1990). This method has been applied in various regions of the western North Atlantic Ocean (Dister and Zafiriou, 1993; Zafiriou and Dister, 1991).

In nitrite rich surface waters, NO is photochemically produced via the reduction of nitrite (NO_2^-) (Zafiriou and True, 1979):

$$NO_2^- + H_2O + hv \rightarrow NO + OH + OH^- (295 < \lambda < 410\,nm)$$

Indeed, Zafiriou and coworkers (Zafiriou et al., 1980; Zafiriou and McFarland, 1981) found a diurnal cycle of NO in the NO_2^--rich surface waters of the central equatorial Pacific Ocean. The photochemical induced build-up of NO during the day was found to be balanced by degradation during the night (Zafiriou and McFarland, 1981). During the same study, a mean daytime NO surface concentration of 46 pmol L^{-1} was calculated which translates into a 10,000-fold supersaturation when compared with the atmospheric NO concentration measured during the same cruise. Thus, the NO_2^- rich equatorial Pacific Ocean was a net source of NO to the atmosphere. NO surface concentrations determined during a cruise in the eastern tropical North Pacific (ETNP) were close to or below the detection limit (<1.5 pmol L^{-1}) (Ward and Zafiriou, 1988). This is in line with the assumption that detectable NO surface concentrations occur only in NO_2^--rich surface waters such as upwelling regions.In major parts of the ocean surface layer, NO_2^- concentrations are negligible, which implies that the global ocean is a net sink for atmospheric NO.

Five depth profiles of NO and the corresponding NO production rates have been measured in the ETNP (Ward and Zafiriou, 1988): NO concentrations were in the range from 0 up to 65 pmol L^{-1}. At four stations located in the open ocean, maximum NO concentrations were observed at the upper boundary of the oxygen minimum zone (OMZ, O_2<10 μmol L^{-1}), whereas one coastal station showed an increase of NO from 0 pmol L^{-1} at the surface to about 20 pmol L^{-1} at the bottom in about 250m. Maximum NO production rates were found at the upper boundary of the OMZ at the open ocean stations. However, Ward and Zafiriou (1988) could not unambiguously identify the NO formation process because NO production rates and nitrification rates (i.e., NH_4^+ oxidation rates) were not correlated. NO accumulation appeared when O_2 concentrations were lower than 100 μmol L^{-1}, whereas in the core of the OMZ with O_2 concentrations close to 0 μmol L^{-1}, denitrification seemed to cause a rapid turnover of NO. Highest ever-reported concentrations of dissolved NO were found off Peru ranging from 0 up to 400 pmol L^{-1} (Zafiriou, personal communication in Ward and Zafiriou (1988)).

2.3. The role of the ocean for the global budget of atmospheric NO_x

The major source of tropospheric NO_x is fossil–fuel combustion (33.0 Tg N yr^{-1}). Minor sources (values in parenthesis are given in Tg N yr^{-1}) are biomass burning (7.1), lightning (5.0), soil emissions (5.6), inputs from the stratosphere (<0.5), and exhausts from aircrafts (0.7) (Prather et al., 2001). As stated above, NO_x emissions from ships have gained increasing attention during the last years. The total annual NO_x emissions from ships was estimated to be 6.9 Tg N (Corbett and Koehler, 2003) and only recently, Eyring et al. (2005) estimated that the annual NO_x ships' emissions have increased from 2.2 Tg N in 1950 to 9.4 Tg N in 2001. These new assessments are especially important in view of the fact that NO_x has a very short lifetime and thus NO_x and/or its reaction products will be more-or-less immediately deposited to the ocean surface. This additional nitrogen source should be considered in future studies of the upper ocean nitrogen cycle.

The ocean as a source of atmospheric NO_x is negligible: Based on the data by Zafiriou and McFarland (1981), Logan (1983) estimated an annual source of 0.5 Tg N. Moreover, the ocean can act as a sink for tropospheric NO_x via wet and dry deposition. Duce *et al.* (1991) estimated that a total of 0.04 Tg N yr^{-1} is deposited, however, their estimate did not include ships' emissions (see above).

3. Nitrous Oxide

3.1. Climatic and biogeochemical relevance

Nitrous oxide (N_2O, dinitrogen monoxide, colloquial called laughing gas) is a colorless gas with an asymmetrical linear structure ($N = N = O$). It has a boiling point of $-88.5°C$ and a molecular weight of 44.01 g mol^{-1}. Its physical properties are comparable to the isoelectronic carbon dioxide (CO_2). For example, both gases show similar solubilities and diffusivities in water (King *et al.*, 1995; Wilhelm *et al.*, 1977). Like CO_2, N_2O is a radiatively active atmospheric trace gas, however, its global warming potential is, on a 100 years time horizon, about 296–340 times higher than that of CO_2 (Jain *et al.*, 2000; Ramaswamy *et al.*, 2001). Formation or decomposition reactions of N_2O in the troposphere are negligible resulting in an inert behavior of atmospheric N_2O. The atmospheric lifetime of N_2O is estimated to be about 114–120 years (Prather *et al.*, 2001). Due to its relatively long atmospheric lifetime, N_2O is mixed into the stratosphere where it is photochemically decomposed. Moreover, by reaction with $O(^1D)$ in the stratosphere, it forms nitric oxide (NO) radicals, which are involved in one of the major catalytic ozone (O_3) reaction cycles (Crutzen, 1970, 1996).

Atmospheric N_2O concentrations, depicted from Antarctic and Arctic ice cores as well as from Antarctic firn, show relatively uniform tropospheric N_2O mixing ratios in the range from 260 to 275 ppb (10^{-9}) in the period from 1000 to 1900 AD and an increase to the present values during the last 100 years (Khalil *et al.*, 2002; Prather *et al.*, 2001; Sowers, 2001). The global mean N_2O mole fraction in 1998 was about 313–314 ppb associated with a mean interhemispheric gradient of about 0.7–0.8 ppb (Khalil *et al.*, 2002; Prather *et al.*, 2001; Prinn *et al.*, 2000). Higher N_2O values are measured in the northern hemisphere because the majority of sources of atmospheric N_2O are located the northern hemisphere (Khalil *et al.*, 2002; Prather *et al.*, 2001; Prinn *et al.*, 2000). Due to the lack of significant atmospheric sink and source reactions, almost no vertical tropospheric N_2O gradient is observed, whereas stratospheric N_2O mixing ratios decrease due its photochemical decomposition (e.g. down to 120 ppb by 30 km in the mid-latitudes) (Prather *et al.*, 2001).

3.2. N₂O in the ocean

The Bunsen solubility of N_2O (C_{N_2O} in mol L^{-1}) in seawater in equilibrium with moist air at $P = 1$ atm can be calculated with the polynomial given by Weiss and Price (1980):

$$C_{N_2O} = Fx'P$$

and

$$\ln F = A_1 + A_2 \left(\frac{100}{T}\right) + A_3 \ln \left(\frac{T}{100}\right) + A_4 \left(\frac{T}{100}\right)^2$$
$$+ S \left[B_1 + B_2 \left(\frac{T}{100}\right) + B_3 \left(\frac{T}{100}\right)^2 \right]$$

with

$$A_1 = -165.8806, A_2 = 222.8743, A_3 = 92.0792, A_4 = -1.48425,$$
$$B_1 = -0.056235, B_2 = 0.031619, B_3 = -0.004847$$

F is in mol L^{-1} atm^{-1}. T stands for temperature in K, S stands for salinity, x' stands for the dry mole fraction of atmospheric N_2O, and P stands for the ambient pressure. The polynomial is valid in a temperature range from 273.15 to 311.15°K (0–40°C) and a salinity range from 0 to 40. A alternative equation for C_{N_2O} in gravimetric units (mol kg^{-1}) can be found as well in Weiss and Price (1980). The N_2O equilibrium concentration, $C_{N_2O, \text{equilibrium}}$, at $T = 298.15$ K (25°C), $S = 35$ and $x' = 318$ ppb is 6.38 nmol L^{-1}. It doubles (12.42 nmol L^{-1}), when the temperature is set to 278.15°K (5°C).

It was not until the advent of the electron capture detector (ECD) and the development an appropriate ECD calibration routine when precise and reliable N_2O measurements were made possible (Cohen, 1977; Elkins, 1980; Rasmussen *et al.*, 1976; Weiss, 1981). Up to now the use of an ECD in connection with equilibration or purge-and-trap techniques followed by gas chromatographic separation is state of the art for the determination of dissolved N_2O (Butler and Elkins, 1991).

3.3. Oceanic pathways

N_2O is in mainly produced by two microbial processes, namely denitrification and nitrification (see Fig. 2.1):

(i) Denitrification results in a loss of bioavailable (fixed) nitrogen in the form of gaseous products such as N_2O and N_2 (for details about denitrification see Chapter 6):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$
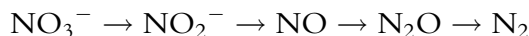
As can be seen from the denitrification reaction sequence, N_2O is a obligatory intermediate. Thus, N_2O originating from denitrification is resulting from the interplay of its formation and its consumption step to N_2. Denitrification is a
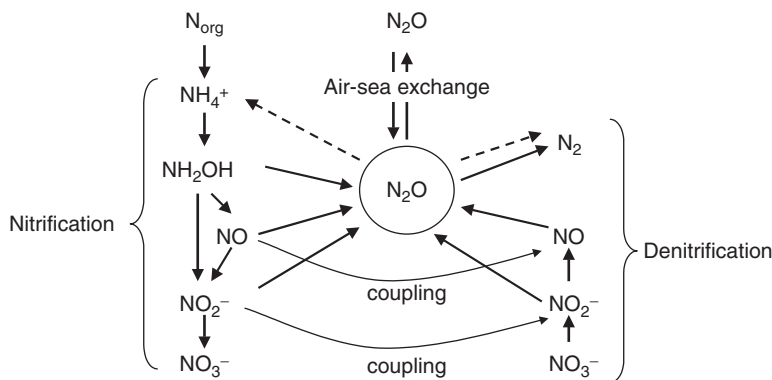
Figure 2.1 Overview of processes, which influence the N$_2$O distribution in the ocean. The dashed arrows indicate N$_2$O reduction during N$_2$ fixation (see e.g. Yamazaki *et al.* (1987)). Please note that NO is not an obligate intermdiate of the nitrification sequence.

well-known feature of many different bacteria species in terrestrial and oceanic environments (Bothe *et al.*, 2000; Tiedje, 1988; Zumft, 1997). Denitrifiers are facultative anaerobic bacteria, which can reduce NO$_3^-$ when oxygen becomes limiting. Thus the occurrence of denitrification is favored under suboxic ($0 < $ O$_2 < 2$–10 μmol L^{-1}, (Codispoti *et al.*, 2005)) conditions. Denitrification does not occur under anoxic conditions (O$_2 = 0$ μmol L^{-1}, hydrogen sulphide present).

(ii) Nitrification is the oxidation of ammonium (NH$_4^+$) to NO$_3^-$ via hydroxyl-amine (NH$_2$OH) and nitrite NO$_2^-$ (for details about nitrification see Chapter 5). Autotrophic nitrification represents the final step of the oceanic reminer-alization and is performed in two steps by ammonia-oxidizing bacteria (AOB, e.g. *Nitrosomonas* and *Nitrosospira*) and nitrite-oxidizing bacteria (NOB, e.g. *Nitrobacter* and *Nitrospira*), respectively (Arp and Stein, 2003; Bothe *et al.*, 2000; Kowalchuk and Stephen, 2001):

$$\text{AOB} : \text{NH}_4^+ \rightarrow \text{NH}_2\text{OH}(\rightarrow \text{NO}) \rightarrow \text{NO}_2^-$$

$$\text{NOB} : \text{NO}_2^- \rightarrow \text{NO}_3^-$$
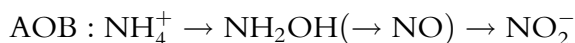
(NO is not known to be an obligate intermediate during ammonium oxidation. It can be produced by AOB but the mechanism is not well known, see also section ''NO in the ocean'' above). During autotrophic nitrification N$_2$O can be formed by AOB either via the pathways NH$_2$OH $\rightarrow$ N$_2$O and NO $\rightarrow$ N$_2$O (Arp and Stein, 2003; Stein and Yung, 2003) or via the pathway NO$_2^- \rightarrow$ NO $\rightarrow$ N$_2$O (the latter is part of the so-called nitrifier–denitrification process) (Stein and Yung, 2003; Wrage *et al.*, 2001). Nitrification is an aerobic process, however, under low-oxygen conditions, N$_2$O yields are enhanced (De Bie *et al.*, 2002; Goreau *et al.*, 1980). Alternatively, N$_2$O can be formed during heterotrophic nitrification (i.e. nitrification linked to aerobic denitrification) via the reaction NO$_2^- \rightarrow$ NO $\rightarrow$ N$_2$O as well, but, the

enzymes involved in the heterotrophic reaction sequence are different from those involved in the autotrophic pathway. Under oxic conditions, N_2O yields from heterotrophic nitrification are higher than those from autotrophic nitrification. However, the relevance of heterotrophic nitrification in the marine environment is not known yet (Stein and Yung, 2003; Wrage et al., 2001).

Both, nitrification and denitrification as sources and sinks of oceanic N_2O have been described in the water column, in the sediments and in association with suspended particles (e.g. Codispoti et al. (2005); Nevison et al. (2003); Schropp and Schwarz (1983); Seitzinger (1990)). N_2O yields from both water–column nitrification and water–column denitrification are listed in Table 2.1. N_2O yields from nitrification range from 0.004% to 0.4%, whereas the N_2O yield from the denitrifying suboxic zone in the Arabian Sea was estimated to be about 2%. (Please note that the yield from the Arabian Sea is a net yield because it includes both N_2O formation and consumption by denitrification). Culture studies with strains of nitrifiers revealed that the N_2O yield from nitrification is significantly enhanced (up to 10%) under suboxic conditions (Goreau et al., 1980). N_2O yields from sedimentary denitrification range from 0.1% to 0.5% with values up to 6% in nutrient-rich regions (see overview in Seitzinger and Kroeze (1998)).

N_2O depth profiles from regions with oxic water masses such as found in the major parts the Atlantic (Fig. 2.2), Pacific, and Indian Oceans are characterized by a subsurface N_2O maximum which coincidents with the minimum of dissolved O_2 and the maximum of NO_3^- (Butler et al., 1989; Cohen and Gordon, 1979; Oudot et al., 1990, 2002). The N_2O subsurface maximum is less pronounced or even absent

Table 2.1 N_2O yields of water-column nitrification and water-column denitrification

Region	N_2O yield(%)	Method[a]	References
	Nitrification 0.2–10[b]	A	Goreau et al. (1980) and references therein
Scheldt estuary	0.1–0.4	B	De Wilde and De Bie (2000)
Bedford Basin	0.01–0.11	B	Punshon and Moore (2004)
Western North Pacific	0.004–0.027	C	Yoshida et al. (1989)
Central Pacific	0.14	D	Elkins et al. (1978)
	Denitrification		
Arabian Sea	2	E	Bange et al. (2000)
Arabian Sea	2	E	Mantoura et al. (1993)

[a] A: Studies with cultures of Nitrosomonas sp. and Nitrosomonas oceanus of marine origin; N_2O yield=ratio of N_2O production to NO_2^- production. B: N_2O yield=ratio of N_2O production to NH_4^+ consumption; C: N_2O yield=ratio of N_2O production to NO_3^- production; D: N_2O yield=ratio of $\triangle N_2O$/AOU in combination with Redfield stoichiometry; E: N_2O yield=ratio of water-column integrated $\triangle N_2O$ to water-column integrated nitrogen deficit.
[b] 10% N_2O yield was observed under reduced O_2 concentration.
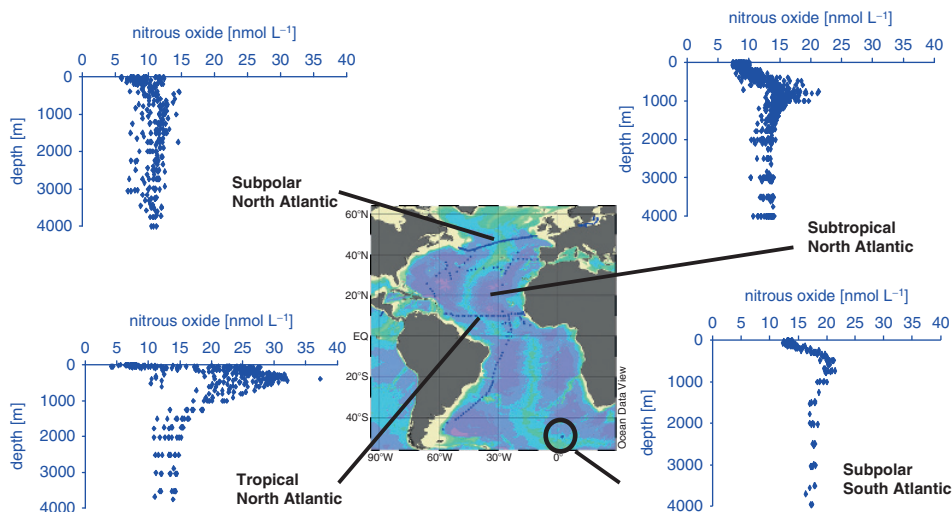
Figure 2.2 N_2O (in nmol L^{-1}) distribution in the Atlantic ocean (Walter *et al.*, 2005, 2006a).

in the northern North Atlantic Ocean in the regions of the deep-water formation (Fig. 2.2). N_2O concentrations in deep waters below 2000m show an increasing trend from the deep North Atlantic Ocean to the deep North Pacific Ocean. This trend correlates well with the water age distribution in the ocean and was attributed to nitrification (Bange and Andreae, 1999). N_2O input to deep waters by hydro-thermal vent systems seem to be of minor importance (Bange and Andreae, 1999; Lilley *et al.*, 1982).

N_2O profiles from oceanic regions with suboxic zones such as the Arabian Sea and the eastern tropical North Pacific Ocean, which are sites of intense denitrifica-tion activities, generally show a two-peak structure (Fig. 2.3): N_2O maxima are found at the upper and lower boundaries of the oxygen minimum zone (OMZ), whereas in the core of the suboxic zone, N_2O concentrations are considerably depleted (Bange *et al.*, 2001b; Cohen and Gordon, 1978). In anoxic water masses such as found in the central Baltic Sea, the Cariaco Basin, and Saanich Inlet, N_2O concentrations are close to the detection limit or not detectable (Brettar and Rheinheimer, 1991; Cohen, 1978; Hashimoto *et al.*, 1983; Rönner, 1983; Walter *et al.*, 2006b).

Depth profiles of N_2O (apart from of a exceptions found in suboxic and anoxic waters, see above) show positive concentration anomalies, expressed as $\Delta N_2O(= C_{N_2O, \text{ measured}} - C_{N_2O, \text{ equilibrium}})$. Positive ΔN_2O values are interpreted as an indicator for in-situ N_2O formation. This caused Yoshinari (1976) to coin the term "apparent N_2O production" for ΔN_2O. Because nitrification is directly linked to organic matter remineralization, plots of ΔN_2O vs. apparent oxygen utilization (AOU) or N_2O vs. O_2 have been used to identify prevailing formation and consumption processes of oceanic N_2O. AOU is usually used as a measure of the amount of O_2 consumed during organic matter oxidation in the ocean. Plots of
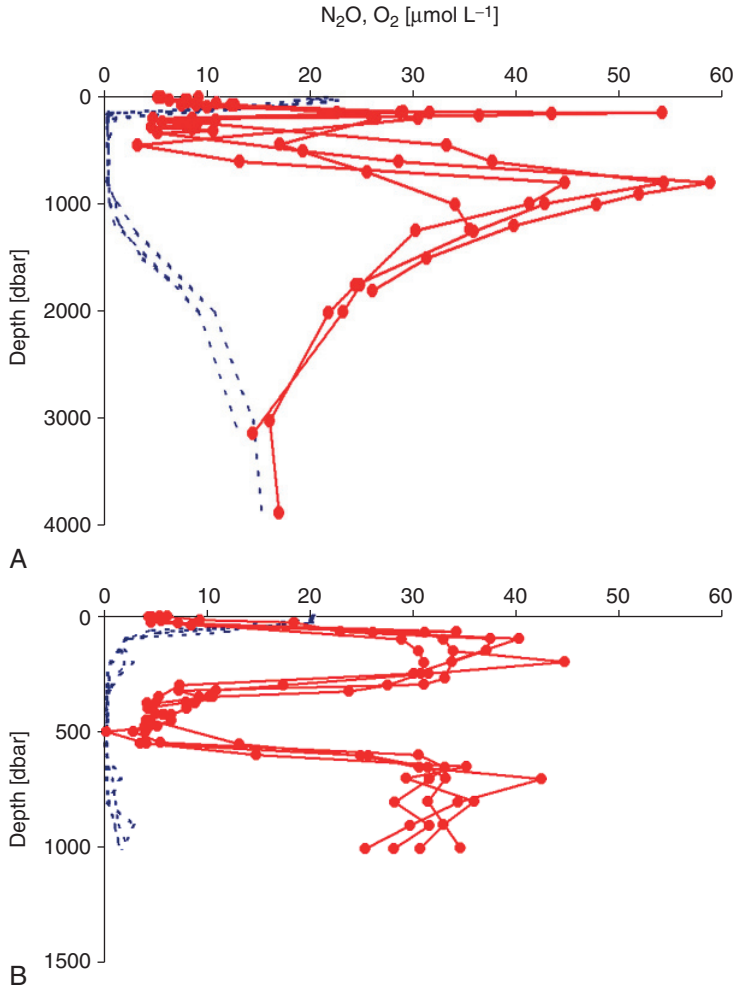
Figure 2.3 N_2O profiles (in nmol L^{-1}) from oceanic regions with denitrifying intermediate waters: (A) Central Arabian Sea (Bange *et al.*, 2001b) and (B) Eastern tropical North Pacific Ocean (Bange *et al.*, unpublished data). Dashed lines stand for dissolved O_2 (concentration in $\mu mol\,L^{-1}$ divided by 10).

$\triangle N_2O$ vs. AOU from the majority of the oceanic regions show clear positive linear relationships, suggesting that nitrification is the main N_2O formation process. This is supported by the fact that in the most oxic water columns N_2O is positively correlated with NO_3^{-}, the final product of nitrification. The slope of the linear $\triangle N_2O$–AOU relationship varies between 3×10^{-5} and 3×10^{-4} (Bange and Andreae, 1999; Bange *et al.*, 2001b; Nevison *et al.*, 1995; Oudot *et al.*, 2002; Suntharalingam and Sarmiento, 2000) and can be interpreted as the N_2O yield per O_2 molecule consumed indicating that it needs between 33,000 to 3000 molecules O_2 to produce 1 molecule N_2O. Beside the well-documented linear relationships,

non-linear $\triangle N_2O$–AOU 2nd order polynomial fits have been reported from the Arabian Sea (Upstill-Goddard *et al.*, 1999) and the shallow coastal Bedford Basin (Punshon and Moore, 2004). The non-linear relationship have been also attributed to N_2O formation by nitrification. The obvious considerable discrepancies of the $\triangle N_2O$–AOU slopes reported in the literature might result from a variety of reasons:

(i) *Water mass age and history of atmospheric N_2O:* $\triangle N_2O$ is also a function of the atmospheric N_2O mole fraction. This in turn implies that $\triangle N_2O$ should be computed with the atmospheric N_2O mole fraction at the time when a water mass had its last contact with the atmosphere. Using ''historical'' N_2O dry mole fractions would shift $\triangle N_2O$ to higher values. However, it requires the knowledge of the water mass ages and the associated atmospheric N_2O mole fractions, which are not available in most cases.

(ii) *Variability of the N_2O yield:* the N_2O yield from nitrification is not necessarily uniform in the ocean since it depends on the abundance and composition of the bacterial community (i.e., the biological diversity). The O_2-dependence of N_2O formation might be varying for different bacteria species. Moreover, Nevison *et al.* (2003) speculated that the remineralization of nitrogen-enriched organic material (which can be found in regions with high N_2 fixation activities) might lead to an enhancement of the N_2O yield. However, detailed studies on the N_2O yields from marine nitrifiers are lacking.

(iii) *N_2O from denitrification:* signals of N_2O produced in suboxic zones during denitrification can be advected to adjacent oxic, nitrifying zones (Bange *et al.*, 2001b). Moreover, N_2O formation in O_2-depleted microniches such as found in sinking particles, can release additional N_2O (Schropp and Schwarz, 1983).

(iv) *Applicability of AOU:* AOU in deep waters does not represent the true oxygen utilization because surface O_2 concentrations at sites of deep-water mass formation might not be in equilibrium with the atmosphere. This leads to overestimation or underestimation of deep water AOU and raises questions concerning the use of AOU as a measure of respiration (see e.g. Ito *et al.*, 2004). Moreover, uncertainties in the O_2 solubility equations are most pronounced at low temperatures ($<1°C$) and high salinities (i.e. in the typical features of deep ocean water masses of the thermohaline circulation) as pointed out by *Garcia and Gordon* (1992).

There are caveats against a straightforward interpretation of the linear $\triangle N_2O$–AOU relationship as an indicator for N_2O formation via nitrification. In other words, a linear $\triangle N_2O$–AOU relationship may not necessarily result from nitrification. Most recently, based on N_2O isotopomer data (see below), Yamagishi *et al.* (2005) argued that net N_2O formation in the oxygen minimum zone (OMZ) of the western North Pacific Ocean mainly results from denitrification with only a small contribution by nitrification. They showed that this N_2O, when diffusing into deep waters, produces a reasonable linear $\triangle N_2O$–AOU relationship. Moreover, by applying a two end-member mixing model, Nevison *et al.* (2003) showed that isopycnal mixing of water masses with different preformed N_2O and O_2 concentrations can result in a linear $\triangle N_2O$–AOU relationship, which can mask the ''true'' biological N_2O production.

In view of the listed uncertainties, the use of a simple $\triangle N_2O$–AOU relationship in model studies of the global oceanic N_2O distribution (Goldstein et al., 2003; Jin and Gruber, 2003; Suntharalingam and Sarmiento, 2000) might fail to simulate small–scale features of N_2O water–column distributions. A linear relationship between $\triangle N_2O$ (N_2O) and AOU (O_2 or NO_3^-) does not exist in suboxic water masses (eastern tropical Pacific Ocean and Arabian Sea) and in anoxic water masses (e.g., Baltic Sea, Cariaco Basin) indicating a more complex interplay between N_2O formation and consumption during denitrification: The characteristic N_2O double peak structures found in the central Arabian Sea were explained with a coupling of denitrification and nitrification in about 150 m at upper boundary of the OMZ, followed by N_2O consumption due to denitrification in the core of the OMZ in about 400 m. The second, broad N_2O peak in about 800–1000 m was attributed to nitrification (with a signal of denitrification still visible) (Bange et al., 2001b). Incubation experiments with water samples from the denitrifying oxygen minimum zone off northern Chile are in line with the observations from the Arabian Sea: Denitrification was the main N_2O formation process but also the main consumption process resulting in a net depletion of N_2O in the core of the suboxic zone (Castro-González and Fraías, 2004).

Global maps of N_2O in the upper 10 m of the world's oceans have been computed based on the extensive N_2O data set collected by Weiss et al. (1992) since 1977 (see also Nevison et al. (2004) with additional data from two campaigns by Butler et al. (1988) and Lobert et al. (1996). Based on the combined data sets by Weiss et al. (1992) and Butler et al. (1988), Nevison et al. (1995) calculated a global mean N_2O surface saturation of 103.5%. The global distribution of N_2O surface anomalies ($\triangle pN_2O = pN_2O_{measured} - pN_2O_{equilibrium}$, positive anomalies indicate the ocean as a source of N_2O to the atmosphere) is shown in Fig. 2.4. Common features of both maps in Fig. 2.4 are:

- enhanced N_2O anomalies in the equatorial upwelling regions of the eastern Pacific and Atlantic Oceans,
- enhanced N_2O anomalies along coastal upwelling regions such as along the west coasts of North and Central America, off Peru, off Northwest Africa and the northwestern Indian Ocean (Arabian Sea) and
- N_2O anomalies close to zero (i.e. near equilibrium) in the North and South Atlantic Ocean, the South Indian Ocean and the central gyres of the North and South Pacific Ocean.

Differences in the two maps (Fig. 2.4) result mainly from different extrapolation methods (Nevison et al., 1995; Suntharalingam and Sarmiento, 2000).

3.4. N_2O in coastal areas

During the last years coastal areas such as estuaries, intertidal areas, upwelling regions and mangrove ecosystems (to name just a few examples) have received increasing attention as sites of intense N_2O formation and release to the atmosphere (see also the sections on eutrophication and N_2O budget). Studies on the biogeochemical cycling of N_2O in coastal regions have been performed to a
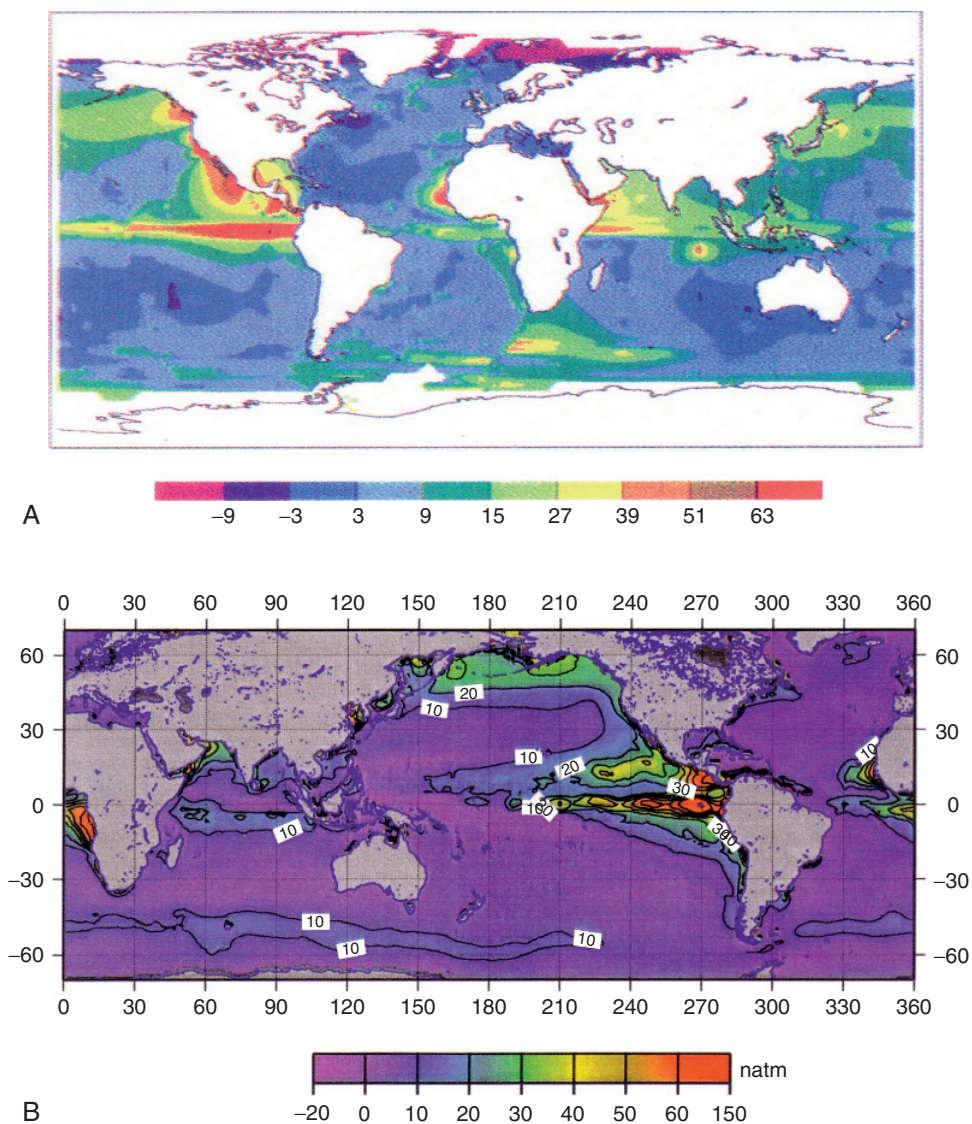
Figure 2.4 Maps of $\triangle p$N$_2$O (in natm) in surface layer of the world's oceans: (A) map by Nevison *et al.* (1995) and (B) map by Suntharalingam and Sarmiento (2000). Please note that the color coding is non-linear and different for both maps.

large extent in European and North American coastal regions. A compilation of coastal N$_2$O measurements worldwide is given in Bange *et al.* (1996). More recently, Bange (2006a) compiled and reviewed N$_2$O studies in European coastal regions (Baltic Sea, North Sea, NE Atlantic, Mediterranean Sea, Black Sea). His major conclusions are:

- Maximum N_2O saturations were usually observed in estuaries, whereas in open coastal waters (i.e. shelf waters not influenced by freshwater) N_2O saturations are close to the expected equilibrium saturation indicating that N_2O is exclusively formed in estuarine systems.
- It is obvious that sedimentary denitrification and water-column nitrification seem to be the major N_2O formation processes. However, the yield of N_2O from both processes strongly depends on the local O_2 concentrations, thus dissolved O_2 is the key factor regulating N_2O production (and its subsequent emissions to the atmosphere). Additionally, N_2O distributions in estuaries show a pronounced seasonal variability.
- In anoxic waters, such as the deep basin of the central Baltic Sea or parts of the shallow Po River delta, N_2O is consumed by water-column denitrification.

Bange (2006a) conclusions are in line with a recent seasonal study of N_2O in the water column of the Bedford Basin (Northwest Atlantic Ocean), which showed that water-column nitrification was the dominant N_2O formation pathway (Punshon and Moore, 2004). N_2O consumption was only temporarily observed when dissolved O_2 was considerably depleted (2.5 μmol L^{-1}) shifting the system from oxic to suboxic conditions (Punshon and Moore, 2004).

The narrow band of coastal upwelling such as found along the Arabian peninsula and the coast off Somalia have been identified as "hot spots" for N_2O emissions showing N_2O surface saturations of up to 330% (Bange *et al.*, 2001a; De Wilde and Helder, 1997). The high N_2O saturations result from the upwelling of subsurface water masses where N_2O formation is favored due to suboxic conditions (see e.g., Bange *et al.*, 2001b; Codispoti *et al.*, 1991). Because the coastal upwelling in the western Arabian Sea is triggered by the seasonal occurring monsoon winds, N_2O emissions also show a pronounced seasonality (Bange *et al.*, 2001a).

High precision time series of atmospheric N_2O have been successfully used to quantify N_2O emissions from temporal upwelling events along the coast off California (Lueker *et al.*, 2003) and from the Southern Ocean (Nevison *et al.*, 2005). This seems to be a promising approach, however, it depends on the existence of high-precision N_2O time series, which are at the moment only available at a few coastal sites (Prinn *et al.*, 2000).

The ecosystems of the mangrove forests have a high potential of N_2O formation and release to the atmosphere. N_2O in mangrove sediments from Puerto Rico is mainly produced by nitrification (Bauzá *et al.*, 2002) whereas the results of incubation experiments with mangrove soils from the east coast of Australia revealed that denitrification is the main N_2O formation pathway (Kreuzwieser *et al.*, 2003). The N_2O release from Puerto Rico mangrove sediments was found to be higher than comparable N_2O emissions from intertidal estuarine sediments (Corredor *et al.*, 1999; Middelburg *et al.*, 1995). Actual results from N_2O measurements at three sites in a mangrove forest on the east coast of central China (Jiulongjiang estuary) support this view (Alongi *et al.*, 2005). However, at these sites a pronounced seasonality was observed showing maximal N_2O emissions in summer, whereas in autumn they were close to zero. In a recently published seasonal study of N_2O emissions from a pristine mangrove creek on South Andaman Island (Gulf of

Bengal), Barnes *et al.* (2006) found no seasonality. However, N_2O emissions were negatively correlated with tidal height indicating that N_2O from sediment pore-water was released during hydrostatic pressure drop toward low water. A realistic estimate of the N_2O emissions from mangroves is not possible at the moment because of the very small number of available measurements.

3.5. The effect of coastal eutrophication and Fe fertilization on oceanic N_2O

Coastal eutrophication (i.e., enhanced nutrient input to the coastal areas) can result in a shift from oxic conditions to anoxic conditions (so-called hypoxia). This is especially important in view of the fact that oceanic N_2O formation strongly depends on dissolved O_2. And indeed, Naqvi *et al.* (2000) observed, during a hypoxic event along the West coast of India, N_2O concentrations up to 533 nmol L^{-1}. The accumulation of N_2O was attributed to the onset of denitrification at low O_2 concentrations with the assumption that the activity of the N_2O reductase (which catalyzes the reduction of N_2O to N_2) was not established ("stop and go" denitrification). Based on their results, Naqvi *et al.* (2000) cautioned that N_2O formation and thus its subsequent release to the atmosphere from shallow hypoxic systems might increase due to the fact that the numbers of coastal regions with hypoxia is increasing as well (UNEP, 2004). Eutrophication can also significantly stimulate the sedimentary N_2O formation by denitrification, which was demonstrated by Seitzinger and Nixon (1985) in microcosm experiments.

N_2O release from mangrove ecosystems appear to be very sensitive to eutrophication: N_2O release across the sediment/atmosphere interface was enhanced significantly (up to 2800 times) when adding NH_4^+ to mangrove sediments from Puerto Rico (Bauza *et al.*, 2002; Muñoz-Hincapié *et al.*, 2002). When NO_3^- was added the enhancement was less pronounced. Comparable results were obtained at a Australian mangrove site where N_2O emissions increased by a factor of 50–100 times when nitrogen was added (Kreuzwieser *et al.*, 2003). Therefore, it seems realistic to expect that the contribution of mangrove ecosystems to global coastal emissions of N_2O will increase due to increasing nutrient inputs caused by the ongoing industrialization and intensification of agricultural activities in tropical countries.

Fuhrman and Capone (1991) pointed out that stimulating open ocean productivity by iron (Fe) addition (Martin *et al.*, 1991) might result in an enhanced formation of N_2O. Thus, enhanced N_2O formation by Fe addition might counteract the climatic benefits of a drawdown of atmospheric CO_2. Fuhrman and Capone (1991) argued that enhanced productivity will lead to an enhanced nitrogen export from the euphotic zone, which in turn would result in additional N_2O formation via enhanced nitrification. The idea of a link between Fe fertilization and enhanced N_2O formation was supported by the study of Law and Ling (2001) who found a small but significant N_2O accumulation in the pycnocline during the Southern Ocean Iron Enrichment Experiment (SOIREE) in the Australasian sector of the Southern Ocean (61°S, 140°E) in February 1999. Recently, Jin and Gruber (2003) predicted the long-term effect of Fe fertilization on oceanic N_2O emissions on a global scale with a coupled physical–biogeochemical model. Based on their model

results they concluded that Fe fertilization-induced N_2O emissions could offset the radiative benefits of the CO_2 drawdown (Jin and Gruber, 2003). However, during EIFEX, the European Iron Fertilization experiment in the subpolar South Atlantic Ocean in February/March 2004, no N_2O accumulation was detected within 33 days (Walter *et al.*, 2005). It seems that Fe fertilization does not necessarily trigger additional N_2O formation, which might depend on the prevailing environmental conditions (e.g., the fate of the Fe-induced phytoplankton bloom). The link between Fe addition and enhancement of N_2O formation and the subsequent release of N_2O to the atmosphere remains to be experimentally demonstrated. However, regionally restricted, short-term fertilization experiments such as SOI-REE and EIFEX cannot account for the predicted enhancement of N_2O production due to expansion of suboxic regions as a result of large-scale and long-term (10 yr) Fe fertilization (Jin and Gruber, 2003).

3.6. Isotope studies

The stable isotopic ratio $^{15}N/^{14}N$ of N_2O is expressed as $\delta^{15}N_{atm}$ relative to atmospheric N_2 (for the exact definition equation see N_2 section). In the same way, the isotope ratio of $^{16}O/^{18}O$ of N_2O is usually expressed as $\delta^{18}O_{VSMOW}$ relative to Vienna standard mean ocean water (VSMOW). However, in some cases $\delta^{18}O_{atm}$ relative to O_2 in the atmosphere is reported. $\delta^{18}O_{VSMOW}$ can be converted to $\delta^{18}O_{atm}$ with the equation $\delta^{18}O_{atm} = -23.0 + \delta^{18}O_{VSMOW}/1.0235$ (Kim and Craig, 1990). Mean atmospheric $\delta^{15}N_{atm}$ and $\delta^{18}O_{VSMOW}$ of N_2O in tropospheric air are 6.72‰ $\pm$ 0.12‰ and 44.62‰ $\pm$ 0.21‰, respectively (Kaiser *et al.*, 2003).

Following the early studies of $\delta^{15}N$ and $\delta^{18}O$ of atmospheric N_2O by Moore (1974), Yoshida and Matsuo (1983), and Wahlen and Yoshinari (1985), measurements of the isotopic composition of oceanic N_2O have become a tool to decipher the various biogeochemical pathways of N_2O in the oceanic environment and to quantify the oceanic contribution to the atmospheric N_2O (Kim and Craig, 1993; Stein and Yung, 2003). The isotopic composition of oceanic N_2O is composed of the isotopic signals of biological sources and sinks, its atmospheric imprint, and mixing processes within the ocean. This, in turn, implies that there are characteristic signals of enrichment or depletion (so-called fractionation), which can be attributed to different biological processes as well as physical processes such as the gas exchange across the ocean/atmosphere interface.

The isotope composition of biologically derived N_2O depends on the isotope composition of the substrates such as NO_3^- (denitrification) and NH_4^+ (nitrification) and the isotopic depletion/enrichment during these processes: An overview of the isotopic depletion/enrichment of N_2O in culture experiments with different strains of denitrifying (*Pseudomonas flourescens*, *Pseudomonas aureofaciens*, *Paracoccus denitrificans*), nitrifier-denitrifying (*Nitrosomonas europaea*) and methane oxidizing bacteria (*Methylococcus capsulatus*) is shown in Fig. 2.5. It is obvious that the range of the resulting nitrogen depletion in N_2O during denitrification and nitrification is similar. The isotope signal of oxygen in N_2O produced during nitrification is introduced by the $\delta^{15}O$ value of dissolved O_2 ($\delta^{15}O = 22–37‰$) and
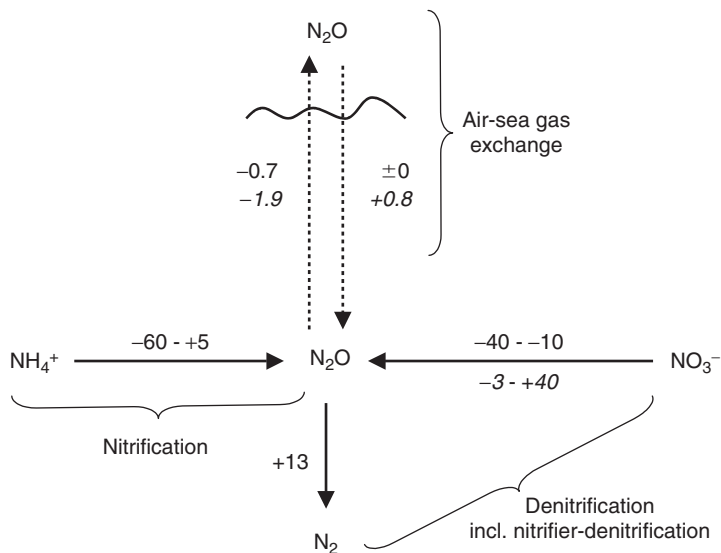
Figure 2.5 Isotopic depletion/enrichment for nitrogen and oxygen in N_2O relative to the substrates NO_3^- and NH_4^+, and the product N_2. Negative values depict isotopic depletion in N_2O and positive values depict isotopic enrichment in N_2O. Data for oxygen depletion/enrichment in N_2O are given in italics. Biological data were taken from Barford *et al.* (1999), Casciotti *et al.* (2003), Sutka *et al.* (2003), (2004), Toyoda *et al.* (2005) and Yoshida (1988). Air–sea gas exchange data are from Inoue and Mook (1994).

H_2O ($\delta^{15}O = \sim 0‰$) as oxidizing agents (Ostrom *et al.* (2000)). The isotopic signal resulting from air–sea exchange is small compared to the biological processes. Therefore, biological N_2O formation should yield a clear isotopic signature in oceanic N_2O. However, the identification of nitrification or denitrification as N_2O producing processes strongly depends on the knowledge of the isotope signatures of the substrates, which can vary temporarily and spatially. An overview of studies of the isotopic signature of oceanic N_2O is given in Table 2.2. The main results are discussed in detail in the following sections.

First measurements of $\delta^{15}N$ of dissolved N_2O in the eastern tropical North Pacific Ocean revealed that N_2O in the suboxic zone was enriched in ^{15}N relative to N_2O in the atmosphere whereas N_2O was slightly depleted in ^{15}N relative to atmospheric N_2O (Yoshida *et al.*, 1984). This in line with the argumentation that N_2O in suboxic waters is consumed during denitrification and that N_2O is formed during nitrification in oxic waters. Surprisingly, Yoshida *et al.* (1989), found ^{15}N-enriched N_2O in the oxic waters of the upper 2000 m of the western North Pacific and concluded that N_2O is mainly produced by denitrification. A conclusion which seems to be counterintuitive, because denitrification in oxic waters should be negligible.

The first N_2O dual isotope ($\delta^{15}N$ and $\delta^{18}O$) measurements were presented by Kim and Craig (1990). At three stations in the North and South Pacific Ocean, N_2O was found to be slightly depleted relative to atmospheric N_2O in ^{15}N and ^{18}O down

Table 2.2 Overview of the suggested N_2O formation pathways based on isotope measurements of N_2O

Oceanic region	Measured isotopic parameters[*]	Suggested main formation pathways	References
Eastern tropical N Pacific	$\delta^{15}N$	Nitrification in oxic waters, denitrification in suboxic waters	Yoshida et al. (1984)
Western N Pacific	$\delta^{15}N$	Denitrification in oxic waters	Yoshida et al. (1989)
N and S Pacific	$\delta^{15}N$, $\delta^{18}O$	Nitrification or coupled nitrification/ denitrification in deep waters	Kim and Craig (1990)
Subtrop. N Pacific, stat. ALOHA	$\delta^{15}N$, $\delta^{18}O$, SP_{N_2O}	Nitrification via two different pathways	Dore et al. (1998); Ostrom et al. (2000); Popp et al. (2002)
Arabian Sea	$\delta^{15}N$, $\delta^{18}O$	Coupled nitrification/ denitrification in oxic waters, denitrification in suboxic waters	Naqvi et al. (1998a); Naqvi et al. (1998b)
Eastern tropical N Pacific	$\delta^{15}N$, $\delta^{18}O$	Coupled nitrification/ denitrification in oxic waters, denitrification in suboxic waters	Yoshinari et al. (1997)
Western N Pacific, stat. KNOT	$\delta^{15}N$, $\delta^{18}O$, SP_{N_2O}	Nitrification	Toyoda et al. (2002)
Western N Pacific, stat. KNOT	$\delta^{15}N$, $\delta^{18}O$, SP_{N_2O}	Denitrification in oxic waters	Yamagishi et al. (2005)

[*] SP_{N_2O} stands for the site preference, for a definition see the section about isotopomers.

to about 600 m, whereas in deep and bottom waters N_2O was found to be enriched in ^{15}N and ^{18}O relative to atmospheric N_2O. The reason for the unexpected enrichment of deep water N_2O is unclear. Kim and Craig (1990) speculated about nitrification with subsequent reduction by denitrification (which might take place in the interior of sinking particles) or, alternatively, N_2O formation during nitrification from a ^{15}N enriched intermediate such as hydroxylamine.

With the application of the newly developed continuous–flow isotope–ratio monitoring mass spectrometers, the required seawater sample volume for N_2O dual isotope measurements have been drastically reduced and therefore facilitated the determination high resolution depth profiles of the N_2O dual isotope signature (Brand, 1996; Yoshinari et al., 1997): Repeated measurements of depth profiles at

the Hawaii ocean time series station ALOHA in the subtropical North Pacific Ocean revealed that $\delta^{15}N$ and $\delta^{18}O$ of N_2O were in equilibrium with atmospheric N_2O at the ocean surface and steadily decreased from the ocean surface to minimum values in about 100–300 m at the base of the euphotic zone, followed by an increase to maximum values in 800 m. The depletion of both ^{15}N and ^{18}O was attributed to result from nitrification (Dore *et al.*, 1998; Ostrom *et al.*, 2000; Popp *et al.*, 2002). A more detailed study which included measurements of $\delta^{18}O$ in dissolved O_2 and H_2O, revealed that N_2O might be formed by two different pathways: First, by nitrification via NH_2OH or NO in most depths or, second, by nitrifier-denitrification via reduction of NO_2^- (between 350–500m) (Ostrom *et al.*, 2000).

The situations in the central Arabian Sea and the eastern tropical North Pacific Ocean are complex. N_2O was found to be strongly enriched in both ^{15}N and ^{18}O in the denitrifying oxygen minimum zone, whereas N_2O in the surface layer was depleted in ^{15}N but slightly enriched in ^{18}O compared to atmospheric N_2O (Naqvi *et al.*, 1998a, 1998b; Yoshinari *et al.*, 1997). N_2O in the core of the oxygen minimum zone was obviously formed by denitrification since the final reduction step to N_2 should result in enriched N_2O. However, the ''light'' N_2O found above the OMZ might be explained with a coupled nitrification-denitrification pathway where NO is formed during nitrification which is then reduced to N_2O during denitrification (Naqvi *et al.*, 1998a, 1998b; Yoshinari *et al.*, 1997).

As mentioned in the introductory section, N_2O is an asymmetrical molecule and therefore it is possible to distinguish so-called isotopomers according to the position of ^{15}N within the N_2O molecule (the corresponding δ notation is given in parenthesis: $^{14}N^{15}NO$ ($\delta^{15}N^{\alpha}$) and $^{15}N^{14}NO$ ($\delta^{15}N^{\beta}$) (Toyoda and Yoshida, 1999). The ^{15}N site preference (SP_{N_2O}) in N_2O is given as $\delta^{15}N^{\alpha} - \delta^{15}N^{\beta}$. The mean tropospheric SP_{N_2O} is 18.7‰ $\pm$ 2.2‰ (Yoshida and Toyoda, 2000). Recently Kaiser *et al.* (2004) reported a mean tropospheric SP_{N_2O} of 46.3‰ $\pm$ 1.4‰. The reason for the large discrepancy is unknown, but it was speculated that it might result from the use different primary standards (Kaiser *et al.*, 2003, 2004). Measurements of SP_{N_2O} should allow to identify the mechanisms of N_2O formation according to the different microbial pathways (Sutka *et al.*, 2003, 2006; Toyoda *et al.*, 2005). The SP_{N_2O} strongly depends on both the bacterial strains used and the actual formation pathway. For example, *Nitrosomonas europaea* and *Nitrosospira multiformis*, both nitrifier-denitrifiers, can produce N_2O via both NH_2OH oxidation and NO_2^- reduction. Indeed, the SP_{N_2O} were different for the NH_2OH oxidation pathway (~33‰) and the NO_2^- reduction pathway (~ −0.4‰) (Sutka *et al.*, 2003, 2004). Most recently, based on a study with cultures of AOB, nitrifier-denitrifiers and denitrifiers, Sutka *et al.* (2006) concluded that the characteristic SP_{N_2O} of nitrification and denitrification (incl. nitrifier-denitrification) are generally ~33‰ and ~0‰, respectively. Thus, isotopomers might be used to distinguish between N_2O produced during oxidation (nitrification) and reduction (denitrification and nitrifier-denitrification) processes, however, it seems that isotopomers cannot be used to reveal subtle differences such as nitrifier-denitrification and denitrification (Sutka *et al.*, 2006). The range of SP_{N_2O} (−0.6‰ to −0.5‰) from the denitrifiers *Pseudomonas chlororaphis* and *Pseudomonas aureofaciens* measured by Sutka *et al.* (2006) are in contrast to the results by Toyoda *et al.* (2005) who found a much larger variability for the SP_{N_2O}

produced by two other denitrifiers which showed SP_{N_2O} of 23.3‰ ± 4.2‰ (*Pseudomonas fluorescens*) and −5.1‰ ± 1.8‰ (*Paracoccus denitrificans*) (Toyoda *et al.*, 2005). However, this discrepancy is in line with the theoretical considerations by Schmidt *et al.* (2004), who argued that the observed SP_{N_2O} and the associated $\delta^{18}O$ signatures during NO reduction to N_2O are reflecting the different types of NO reductases used by the different bacteria.

Up to now, the oceanic distribution N_2O isotopomers was determined at station ALOHA in the subtropical North Pacific (Popp *et al.*, 2002), at station KNOT (Kyodo North Pacific Ocean Time series) in the western North Pacific (Toyoda *et al.*, 2002), and in the eastern tropical North Pacific (ETNP) (Yamagishi *et al.*, 2005). The SP_{N_2O} profile at KNOT showed a steady increase from low values at the ocean surface (12‰) to a maximum (up to 36‰) in about 250–750 m (Toyoda *et al.*, 2002). A similar SP_{N_2O} profile was observed at ALOHA, however, the low SP_{N_2O} values were rather uniform in the depth range 0–500 m and then increased to about 25‰ in 600–900 m (Popp *et al.*, 2002). The shape of the SP_{N_2O} profiles at KNOT and ALOHA were explained with N_2O formation during nitrification, however, the exact reaction pathway remained unclear (Popp *et al.*, 2002; Toyoda *et al.*, 2002). In contrast, on the basis of additional isotopomer data from KNOT, Yamagishi *et al.* (2005) suggested net N_2O formation in the oxygen minimum zone which is mainly resulting from both formation and consumption during denitrification with only a minor contribution by nitrification. This revives the debate on whether nitrification or denitrification is the main N_2O formation process in the ocean. A debate, which began with the one of the first publications on oceanic N_2O isotope data (Yoshida *et al.*, 1989).

3.7. The role of the ocean for the global budget of atmospheric N_2O

The 2001 report of the Intergovernmental Panel on Climate Change (IPCC) of the sources and sinks of atmospheric N_2O suggests a reasonably balanced present-day budget of atmospheric N_2O (Prather *et al.*, 2001) (all fluxes in parenthesis are given in Tg N yr^{-1}): Major natural sources of atmospheric N_2O are emissions from the world's oceans (3.0) and soils (6.0). A minor natural source is the N_2O formation by atmospheric ammonia oxidation (0.6). Agricultural soils (4.2) and a bundle of smaller sources such as biomass burning (0.5), various industrial sources (1.3) and cattle farming/feedlots (2.1) have been identified. The only sink for atmospheric N_2O is its photochemical decomposition in the stratosphere (12.3). The IPCC 2001 report indicates an oceanic contribution of about 17% to the overall sources of N_2O and about 31% to the natural sources. Thus, the oceanic N_2O emissions play a major role in the atmospheric N_2O budget. However, the IPCC 2001 estimate does not take into account N_2O emissions from coastal areas such as continental shelves, estuarine and coastal upwelling areas: On the basis of a comprehensive compilation of N_2O measurements in coastal areas, Bange *et al.* (1996) calculated an overall oceanic flux of 7–11 Tg N yr^{-1} and concluded that coastal areas such as estuaries, shelf and coastal upwelling areas contribute significantly (approximately 61%, i.e., 4.2–6.6 Tg N yr^{-1}) to the global oceanic emissions. This is in line with predictions from the model studies by Capone (1991), Seitzinger and Kroeze (1998) and Nevison *et al.*

(2004) who estimated that coastal areas may contribute 7–49% to the overall global oceanic N_2O emissions. Beside different methodological approaches (empirical models vs. extrapolation of measurements) the considerable range of uncertainty is introduced by the fact that the applied classification of coastal areas is not uniform. For example, the overall areal contributions range from 3.3% (Nevison *et al.*, 2004) up to 18.6% (Bange *et al.*, 1996). The results of the global N_2O coastal emissions estimates is supported by the regional study along the West coast of India (Naqvi *et al.*, 2000) which revealed that N_2O emissions were considerably enhanced (up to 0.25 Tg N yr^{-1}) due to the temporarily occurring hypoxia (see above). Based on a compilation of literature data Bange (2006b) recently suggested that the oceanic emissions should be revised upwards by at a factor of two.

4. DINITROGEN

4.1. Climatic and biogeochemical relevance

Dinitrogen (N_2) is a colorless gas at room temperature. It has a boiling point of $-195.8°C$ (at 1 atm) and a molecular weight of 28.01 g mol^{-1}. Because of the very stable N-N triple bond and the symmetric electron distribution in the molecule, which prevents the development of polarity in the N-N bond, N_2 is comparably inert at room temperature (Greenwood and Earnshaw, 1984). The chemically inertness of N-N triple bond results from the high activation energy necessary to break it, thus, it is a kinetic effect and not a thermodynamical issue. Because the formation of NO_3^- is thermodynamically favored, all atmospheric N_2 would react with atmospheric O_2 and H_2O to the form dissolved NO_3^-, however, this is only true for a world without any biological processes (Sillén, 1966). N_2 is the most abundant nitrogen-containing compound in the Earth's atmosphere (78.1% by volume or 75.5% by mass). Nitrogen (N) has two stable isotopes, ^{14}N and ^{15}N, which contribute 99.63% and 0.37%, to its composition (Greenwood and Earnshaw, 1984). The stable isotopic ratio, $^{15}N/^{14}N$, of nitrogen containing compounds is expressed as $\delta^{15}N_{atm}$ relative to atmospheric N_2, thus, $\delta^{15}N_{atm}$ of N_2 in the atmosphere = 0‰:

$$\delta^{15}N_{atm}(sample)[‰] = ((^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{std} - 1) \times 1000$$

Isotopic studies of the oceanic nitrogen cycle are discussed in Chapter 31.

In the lower atmosphere (i.e. the troposphere and stratosphere) N_2 is not radiatively active, whereas in heights above 100 km (i.e. in thermosphere) N_2 absorbs at wavelengths less than 100 nm (Graedel and Crutzen, 1993).

Despite the inertness of N_2, the atmospheric N_2 pool is available for biological productivity by N_2 fixation ($N_2 \rightarrow NH_4^+$), which takes place in both the oceanic and the terrestrial environments. Further details about oceanic N_2 fixation can be found in Chapter 4. The release of nitrogen as N_2 from the fixed inorganic nitrogen pool (i.e. NO_3^-, NO_2^- and NH_4^+) is mediated by two microbial processes:

Denitrification ($NO_3^- \rightarrow N_2$) and anaerobic ammonium oxidation (anammox, $NO_2^- + NH_4^+ \rightarrow N_2$). Details about oceanic denitrification and anammox can be found in Chapter 6.

4.2. N_2 in the ocean

Because N_2 is a non-polar molecule, its solubility in seawater is low. Based on new measurements, Hamme and Emerson (2004) recently presented an improved equation for the Bunsen solubility of N_2 dissolved in seawater in equilibrium with a moist atmosphere at 1 atm (C_{N_2} in µmol kg^{-1}):

$$\ln C_{N_2} = A_0 + A_1 T_S + A_2 T_S^2 + A_3 T_S^3 + S(B_0 + B_1 T_S + B_2 T_S^2)$$

with

$$T_S = \ln\left(\frac{298.12 - t}{273.15 + t}\right)$$

and

$$A_0 = 6.42931, A_1 = 2.92704, A_2 = 4.32531, A_3 = 4.69149$$

and

$$B_0 = -7.44129 \times 10^{-3}, B_1 = -8.02566 \times 10^{-3}, B_2 = -1.46775 \times 10^{-2}$$

S stands for salinity and t stands for water temperature in the range from 1 to 30°C. With $t = 10°C$ and $S = 35$, the equilibrium concentration of N_2 is 500.9 µmol kg^{-1} (Hamme and Emerson, 2004). (The N_2 equilibrium concentration is much higher than the concentration of oceanic nitrate, the most abundant fixed nitrogen compound!) Because of the high equilibrium concentration of oceanic N_2, small deviations from the equilibrium, which can be caused by biological and physical processes (see below), are difficult to detect. The precise knowledge of the solubility function together with high precision measurements are, therefore, a prerequisite to investigate the oceanic distribution of N_2. Early measurements of the oceanic N_2 distribution partly suffer from inadequate analytical precision (for a detailed discussion of the early measurements see Scranton (1983)).

Dissolved N_2 can be determined by measuring the O_2/N_2 ratios by means of seawater/headspace equilibration followed by analysis via a mass spectrometer (Emerson *et al.*, 1999). Absolute N_2 concentrations are derived from O_2/N_2 ratios by using high precision O_2 measurements (e.g. via Winkler titration, see Hansen (1999)). Alternatively, N_2 can be determined with a purge method in combination with gas chromatographic separation and detection with a thermal conductivity detector (Nakayama *et al.*, 2002; Weiss and Craig, 1973). The latter method has the advantage of being suitable for onboard use. Recently, a membrane inlet mass

spectrometry method for shipboard measurement of N_2, among other gases, was introduced (Tortell, 2005). Automated systems for the use on moorings or during continuous underway surface measurements consist of a gas tension device which measures the total pressure of dissolved gases in seawater and sensors for O_2 and/or CO_2 (Anderson and Johnson, 1992; Carignan, 1998; Emerson et $al.$, 2002; McNeil et $al.$, 1995, 2005). The dissolved N_2 concentrations can be calculated as the difference of the total gas pressure and the known or estimated partial pressures of O_2, CO_2, Ar, and H_2O (Emerson et $al.$, 2002; McNeil et $al.$, 1995).

Because dissolved N_2 is assumed to be inert in seawater (at least under the most common condition, see discussion below), it can serve as a tracer of physical processes (Emerson et $al.$, 1995): Measurements of dissolved N_2 in combination with Ar and O_2 measurements were successfully used in mass balance models of the upper ocean in order to quantify the net biological O_2 production and the associated carbon production (Emerson et $al.$, 1991, 1995, 1997).

Recently, a 10 days continuous time series measurements of N_2 at 5 m depth off Massachusetts, provided the possibility to estimate the air–sea transfer coefficient of N_2 and the subsequent computation of the air–sea fluxes of the reactive gases CO_2 and O_2 (McNeil et $al.$, 2006).

The global oceanic distribution of N_2 is mainly controlled by physical processes such as water temperature variability and gas exchange via bubble entrainment and diffusion (Hamme and Emerson, 2002; Nakayama et $al.$, 2002; Schudlich and Emerson, 1996). Exceptions occur only in oceanic regions with denitrifying activities (see below), whereas the influence of N_2 fixation on N_2 surface concentrations is usually ignored. However, under certain conditions N_2 fixation rates are comparable to air–sea gas exchange rates and thus have to take into account when using N_2 as tracer for physical processes: At a wind speed of 4 m s^{-1}, a N_2 supersaturation of 0.5%, and a water temperature of 25°C (N_2 fixation is favored in subtropical and tropical regions with temperatures >20°C (LaRoche and Breitbarth, 2005)), N_2 fixation rates may equal up to 50% of the N_2 air–sea gas exchange (for details of the estimate see caption of Fig. 2.6).

The oceanic distribution of dissolved N_2 is outlined in the following paragraphs. During a transect in the western North Atlantic Ocean (from New York to Puerto Rico), N_2 surface concentrations were monitored continuously and ranged from slightly undersaturated (−1%) to slightly supersaturated (1%). Supersaturation was observed in waters north of 37°N, whereas undersaturations occurred in open ocean waters from 37°N to 18°N. The marked decrease in N_2 saturation at 37°N was associated with the crossing of the Gulf Stream (McNeil et $al.$, 2005). The observed N_2 undersatuation was attributed to rapid solubility changes due to the seasonal cooling of the seasurface layer in connection with low gas exchange rates (McNeil et $al.$, 2005). Based on a simple two-layer box model, McNeil (2006) recently proposed a diurnal thermal pumping mechanism to explain undersaturation of surface N_2 during periods low gas exchange rates (i.e. low wind speeds).

Concentrations of N_2 in non-denitrifying waters are generally close to the equilibrium concentrations. For example, dissolved N_2 concentrations in the upper 200m of the western North Pacific Ocean and eastern Okhotsk Sea were in the range from 500 to 650 µmol kg^{-1} (i.e. 1–4.3% supersaturated) (Nakayama et $al.$,
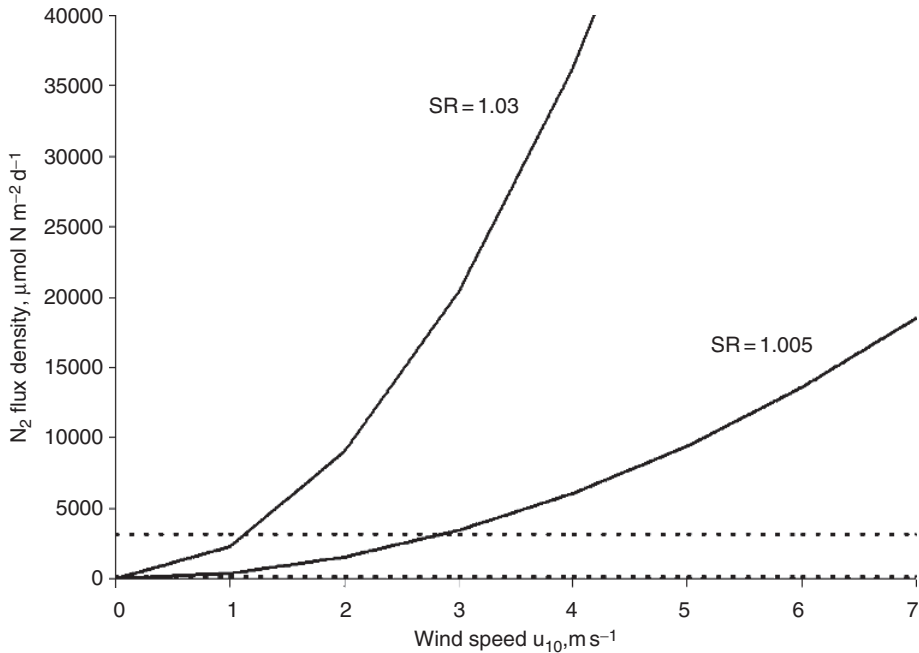
Figure 2.6 Air–sea flux densities of N_2 (bold lines) calculated as $F = (0.39u_{10}^2)\,(SRC_{air}-C_{air})$ $(Sc_{N2}/660)^{-0.5}$ (Wanninkhof, 1992). SR stands for the N_2 saturation ratio and u_{10} stands for the wind speed in a height of 10m. Water temperature and salinity were set to 25°C and 35, respectively. C_{air} is the equilibrium concentration of atmospheric N_2 and was calculated with the equation given by Hamme and Emerson (2004). The dashed lines represent maximum (3110 µmol N m^{-2} day^{-1}) and minimum (161 µmol N m^{-2} day^{-1}) mean N_2 fixation rates for the North Atlantic Ocean (Capone *et al.*, 2005).

2002). The observed supersaturation was attributed to bubble injection from the overlying atmosphere (Nakayama *et al.*, 2002).

An annual study of N_2 concentrations in the upper ocean at the US JGOFS time series station ALOHA in the suptropical North Pacific Ocean showed a steady increase from 390 µmol kg^{-1} at the surface to 450 µmol kg^{-1} in 200m (Emerson *et al.*, 1995). The corresponding N_2 supersaturations ranged from 0 to 5% but did not show the steady increase with depth (Emerson *et al.*, 1995). No seasonal signal was observed. Depth profiles from the subtropical and the subpolar North Pacific (stations HOT and KNOT, respectively) and subtropical North Atlantic (station BATS) revealed that N_2 was supersaturated by 1–3% in the upper 200 m and slightly undersaturated (−2–0%) in the depth range from 200–4500 m (Hamme and Emerson, 2002). Based on the results from a quasi-steady-state mixed-layer model, Hamme and Emerson (2002) concluded that the N_2 distribution in both surface and deep waters of the North Pacific and North Atlantic Oceans is resulting from only three processes, namely temperature variability, and diffusive and bubble mediated gas exchange.

N$_2$ in the suboxic deep waters (70–440 m) of the western part of the central Baltic Sea was supersaturated up to 8%, whereas in the surface layer N$_2$ was slightly undersaturated (Rönner and Sörensson, 1985). The accumulation of N$_2$ resulted from denitrification. In the suboxic intermediate water masses (150–1000 m) of the central Arabian Sea, N$_2$ concentrations were found to be significantly enhanced with a maximum in 250 m (Codispoti *et al.*, 2001, 2005). This corresponds to a minimum of $\delta^{15}N_{atm}$ of dissolved N$_2$ in the core of the suboxic zone, which drops from 0.6‰ to 0.20‰ (Brandes *et al.*, 1998; Naqvi *et al.*, 1998a). The minimum in $\delta^{15}N_{atm}$ of dissolved N$_2$ is an agreement with a biological formation process. However, the observed N$_2$ excess in the central Arabian Sea exceeds the expected nitrogen loss calculated from the classical denitrification routes. The reason of the extraordinary N$_2$ excess in the central Arabian Sea remains speculative (Codispoti *et al.*, 2001, 2005). See also Chapter 6 about denitrification.

5. AMMONIA

5.1. Climatic and biogeochemical relevance

Ammonia (NH$_3$) is a colorless gas. It has a boiling point of $-33.4°C$ (at 1 atm) and molecular weight of 17.03 g mol^{-1} (Greenwood and Earnshaw, 1984). Gaseous NH$_3$ in the atmosphere dissolves in rain droplets as dissolved ammonium (NH$_4^+$). Moreover, NH$_3$ can form NH$_4^+$ salts (NH$_4^+$ aerosols) by rapid gas-to-particle conversion (Warneck, 2000). Thus, NH$_3$ plays a unique role in the formation of tropospheric aerosols: Because of its basic character it neutralizes acidic sulfuric acid–water aggregates by forming ammonium sulfate aerosols which can act as cloud condensation nuclei (Warneck, 2000; Yu, 2006). Therefore, NH$_3$, as a precursor of aerosols and clouds, is of considerable importance for the Earth atmosphere's composition, chemistry and climate. Additionally, NH$_3$ is a greenhouse gas, but its contribution to the present-day greenhouse effect is negligible. However, during the Archean (4.8–2.2 Gyr ago) oceanic derived atmospheric NH$_3$ might have contributed significantly to the warming of the early Earth, however, this theory is controversially debated (Pavlov *et al.*, 2001; Sagan and Chyba, 1997).

Atmospheric NH$_3$ in the present day marine boundary layer has a short life time ranging from only a few hours to days (Norman and Leck, 2005; Quinn *et al.*, 1988). Atmospheric mixing ratios in pristine remote marine boundary layers can be less than 1 nmol m^{-3} (see e.g. Norman and Leck (2005) and Quinn *et al.* (1990)). Strong gradients of atmospheric NH$_3$ over the ocean are detectable when plumes of biomass burning are influencing the marine boundary layer (Norman and Leck, 2005). NH$_3$ and NH$_4^+$ in the marine boundary layer are mainly removed by dry and wet deposition to the ocean. Oxidation of atmospheric NH$_3$ by OH seems to be of minor importance (Warneck, 2000). Therefore, the distribution of NH$_3$/NH$_4^+$ in both the marine boundary and the oceanic mixed layer is tightly coupled (Fig. 2.7).
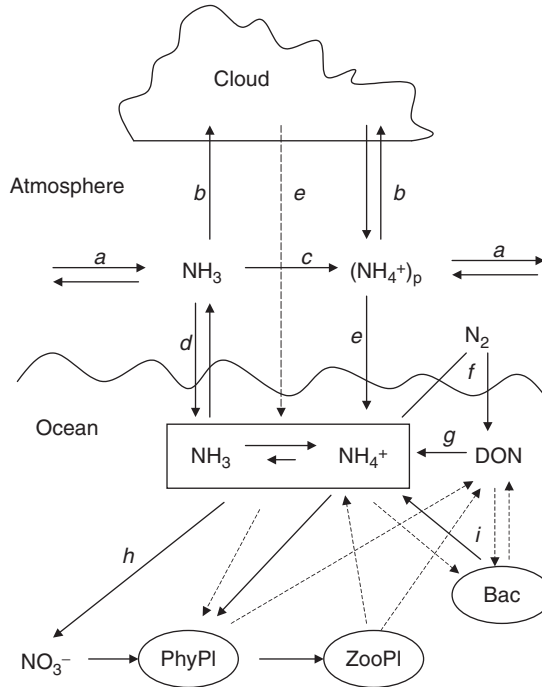
Figure 2.7 Simplified scheme of NH_3/NH_4^+ cycling in the marine boundary and ocean surface layers. Exchange processes of NH_4^+ across the ocean/sediment interface are not shown. $(NH_4^+)_p$ stands for particulate NH_4^+ (NH_4^+ aerosol), DON stands for dissolved organic nitrogen, PhyPl and ZooPl stand for Phytoplankton and Zooplankton respectively, and Bac stands for bacteria. Dashed arrows depict uptake/assimilation and release/excretion. Small letters in italics represent physical, chemical, and biological processes: *a*, atmospheric transport processes; *b*, uptake and release in clouds; *c*, gas-to-particle conversion; *d*, gas exchange; *e*, wet and/or dry deposition; *f*, nitrogen fixation; *g*, photochemical decomposition, *h*, nitrification; *j*, decomposition. The scheme is based on publications by Liss and Galloway (1993), Quinn *et al.* (1990), and Bronk *et al.* (1994).

5.2. The NH_3/NH_4^+ equilibrium in water*

When dissolved in water, NH_3 reacts as a base:

$$NH_3 + H_2O \overset{K_b}{\longleftrightarrow} NH_4^+ + OH^-$$
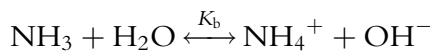
where K_b stands for the equilibrium constant. According to Van Neste and Duce (1987), dissolved NH_3 concentrations, $[NH_3]$, can be calculated as

$$[NH_3] = \frac{[NH_x][OH^-]}{K_b + [OH^-]}$$

where $[NH_x]$ stands for the sum of dissolved NH_3 and NH_4^+. K_b is usually given as pK_b ($= -\log_{10} K_b$, $pK_b = 14 - pK_a$). Gibb et al. (1999a) presented the following equations to calculate pK_b of NH_3:

* See also 'Note added to proof' at the end of the chapter.

$$pK_b = 14 - (pK_a + C)$$

with

$$C = (0.1552 - 0.003142T)I$$

and

$$I = 0.00147 + 0.01988S + 2.08357 \times 10^{-5}S^2$$

where C represents the seawater correction of pK_a ($= -\log_{10} K_a$, dissociation constant of $NH_4^+ \rightarrow NH_3 + H^+$) for temperature ($T$ in K) and salinity (S), I is the ionic strength of seawater. K_a can be calculated according to Bates and Pinching (1950):

$$K_a = 5.67 \times 10^{-10} \exp\left(-6286\left(\frac{1}{T} - \frac{1}{298.15}\right)\right)$$

For $T = 293.15$ K ($20°C$), K_a has the value 9.246.

Whitfield (1974) derived a formula for calculating K_a as a function of temperature, salinity and pressure (P):

$$pK_a = pK_a(T = 298, P = 1atm) + 0.0324(298 - T) + 0.0415P/T$$

with

$$pK_a(T = 298K, P = 1atm) = -0.21428I^2 + 0.40714I + 9.1511$$

for $0.4 < I < 0.8$.

Because the mean pH of the today's ocean surface layer is about 8.08 (with a range from 7.9–8.25) (Raven et al., 2005), oceanic NH_3 can exist as a dissolved, non-protonated gas and, thus, it is available for gas exchange across the ocean/atmosphere interface. For example, for a pH of 8.1, a water temperature of $25°C$, and a salinity of 35, about 6% is available as dissolved NH_3, $[NH_3]$, (Fig. 2.8). The NH_3/NH_4^+ equilibrium is very sensitive to changes of the pH and water temperature. Changes in salinity and pressure are comparably less important (Fig. 2.8).

The theoretical gaseous concentration of NH_3, $(NH_3)_g$ in g L^{-1}, which is in equilibrium with dissolved $[NH_x]$ can be calculated according to Asman et al. (1994) and references therein as

$$(NH_3)_g = \frac{M_{NH_3}[NH_x]}{RTH_{NH_3}\left(\dfrac{1}{\gamma_{NH_3}} + \dfrac{10^{-pH}}{\gamma_{NH_4^+}K_a}\right)}$$

where R is the gas constant ($8.2075 \ 10^{-5}$ atm m^3 mol^{-1} K^{-1}), γ is the activity coefficient of NH_3 and NH_4^+, respectively, M_{NH_3} is the molecular weight of NH_3,
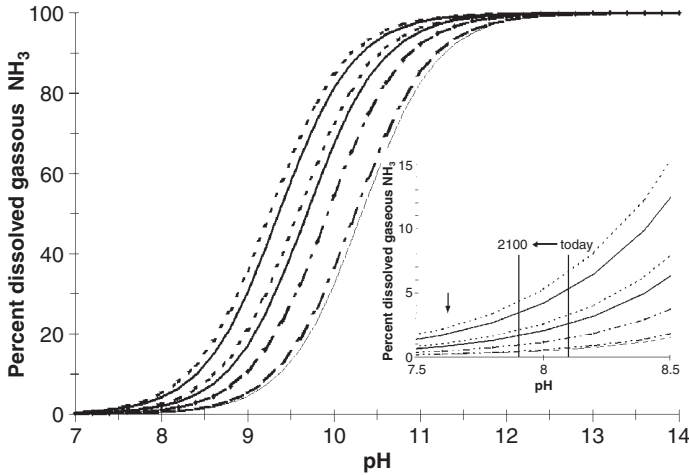
Figure 2.8 Percent dissolved gaseous NH_3 as a function of pH. Dashed and (bold and thin) solid lines were calculated with the equations from Whitfield (1974). Dashed-dotted lines were calculated with the equations from Gibb *et al.* (1999a). Dashed lines, salinity (S) = 0, temperature (T) = 25°C (upper curve) or 15°C (lower curve), pressure (P) = 1 atm; bold solid lines, S = 35, T = 25°C (upper curve) or 15°C (lower curve), P = 1 atm; dashed-dotted lines, salinity, (S) = 35, temperature (T) = 25°C (upper curve) or 15°C (lower curve); thin solid line, S = 35, T = 0°C, P = 1000 atm. The insert shows the possible effect of pH decrease from 8.1 (today) to a predicted 7.9 (for the year 2100), see text for details.

and N_{NH_3} is the Henry's law constant (in mol m^{-3} atm^{-1}). γ_{NH_3} and $\gamma_{NH_4^+}$ can be calculated as follows:

$$\gamma_{NH_3} = 1 + 0.0851 I$$

$$\gamma_{NH_4^+} = 0.883 - 0.0768 \ln(S), \text{ for } 5 < S < 40$$

A frequently used formula for H_{NH_3} is

$$H_{NH_3} = 56 \times 10^{-3} \exp\left(4029\left(\frac{1}{T} - \frac{1}{298.15}\right)\right)$$

(Dasgupta and Dong, 1986). The value for the dimensionless Henry's law constant ($H'_{NH_3} = RT\, H_{NH_3}$) at a temperature of 25°C is 1.37×10^{-3}. Alternative expressions for Henry's law constant of NH_3 in water are listed in the literature compilation by Sander (1999). Recently, a formula for Henry's law constant, corrected for seawater (H'_{sNH_3}), was derived by Gibb *et al.* (1999a):

$$H'_{sNH_3} = \frac{1}{28 \exp\left(\ln H_{298} - 4029\left(\frac{1}{T} - \frac{1}{298.15}\right)\right)}$$

where H_{298} is the value of $H_{\mathrm{NH_3}}$ for a temperature of 298 K. At a temperature of 25°C $H'_{s\mathrm{NH_3}}$ has a value of 0.64×10^{-3} which is considerable lower than the corresponding freshwater value (see above).

5.3. NH$_3$ in the ocean

Maybe not very surprisingly, the distribution of oceanic NH$_3$ is tightly coupled to the distribution of dissolved NH$_4^+$ via the NH$_3$/NH$_4^+$ equilibrium. NH$_4^+$ is one of the essential nutrients in the oceans (Dugdale and Goering, 1967). NH$_4^+$ is the substrate or final product of major biological transformation processes of the oceanic nitrogen cycle such as bacterial nitrification (NH$_4^+ \rightarrow$ NO$_3^-$), assimilation by phytoplankton (NH$_4^+$ $\rightarrow$ nitrogen–containing biomass), and excretion by zooplankton (see Fig. 2.7). See also Chapters 5 and 7. Moreover, NH$_4^+$ can be formed by photochemical decomposition of dissolved organic nitrogen (DON), but, the existing data are partially contradictory, showing also photo-induced losses of NH$_4^+$ (Grzybowski, 2003; Mopper and Kieber, 2002). See also Chapter 10. Because the biological uptake of NH$_4^+$ is rapid, NH$_4^+$ concentrations in the oceans are generally low (e.g., <0.03–2.7 µmol L^{-1} in the North Atlantic Ocean (Quinn *et al.* 1996)). Only in anoxic water masses (e.g., in the Black Sea and the deep basins of the Baltic Sea), NH$_4^+$ concentrations can rise to about 30 µmol L^{-1} (Kuypers *et al.*, 2003; Nausch *et al.*, 2003). Considering the fact that under equilibrium conditions, NH$_3$ contributes only up to 6% of [NH$_x$] (see above), oceanic NH$_3$ concentrations are expected to be low and, thus, difficult to determine: Methods applied in chemical oceanography measure the sum of NH$_3$ + NH$_4^+$ (Hansen and Koroleff, 1999). Dissolved gaseous NH$_3$ can be calculated from [NH$_x$] with the equations given above. Because oceanic NH$_4^+$ concentrations are low and, therefore, are close to or below the detection limit of conventional analytical methods, determination of dissolved NH$_3$ demands a high degree of analytical precision, a low detection limit, and an appropriate contaminant-free working conditions (Genfa *et al.*, 1998; Watson *et al.*, 2005).

Thus, there are only a few data of dissolved oceanic NH$_3$ available. (Reports on oceanic NH$_4^+$ measurements are not considered here because without the knowledge of pH, water temperature, and salinity, the ''true'' concentration of dissolved NH$_3$ remains speculative). Because the NH$_3$/NH$_4^+$ equilibrium strongly depends on the temperature, maximum NH$_3$ concentrations are expected to occur in surface waters and should rapidly decrease with depth in association with temperature profiles in the subsurface and deep ocean. (Exceptions should occur in the few cases where subsurface NH$_4^+$ maxima have been observed (Brzezinski, 1988; Gibb *et al.*, 1999b)). Oceanic NH$_3$ concentrations discussed in the following paragraph are exclusively from the oceanic mixed layer.

Quinn *et al.* (1988) report a mean concentration of 16 ± 7 nmol L^{-1} (ranging from 4.9 to 22 nmol L^{-1}) for NH$_3$ measurements in the coastal area of the northeastern Pacific Ocean. This is agreement with data reported by Genfa *et al.* (1998) from coastal waters of Coconut Island, Hawaii (17.4 ± 6.9 nmol L^{-1}). Comparable concentrations have been found on a transect along 170°W in the central Pacific Ocean: 10 ± 7 nmol L^{-1} (ranging from 2.8 to 21 nmol L^{-1}) (Quinn

et al., 1990). Mean NH_3 concentrations in the Northwest Indian Ocean (i.e., the Arabian Sea) ranged from 8.0 ± 1.9 to 11.9 ± 1.7 nmol L^{-1} (Gibb *et al.*, 1999a).

5.4. The ocean as a source or sink of atmospheric NH_3

The direction of the NH_3 flux cross the ocean/atmosphere interface depends on the concentration difference between NH_3 in the surface ocean $(NH_3)_{g,ocean}$ and $(NH_3)_{g,atmos}$ in the marine boundary layer. In the case of an ocean/atmosphere equilibrium the concentration difference is 0 and no gas exchange is possible. Ideally, both parameters should be measured simultaneously to allow a reasonable flux estimate. Disequilibria, $(NH_3)_{g,ocean} \neq (NH_3)_{g,atmos}$, can develop because the gas exchange across the ocean/atmosphere interface is slow compared to the life times of NH_3 in the marine boundary layer and in the surface ocean (Quinn *et al.*, 1992).

In the pristine marine boundary layer, atmospheric NH_3 concentrations are extremely low (see above) and in this case $(NH_3)_{g,ocean} > (NH_3)_{g,atmos}$ which implies that the ocean can release NH_3 to the atmosphere. In the reverse case of $(NH_3)_{g,ocean} < (NH_3)_{g,atmos}$, the ocean takes up NH_3. Indeed, both cases have been observed: In heavily anthropogenic influenced coastal areas such as the North and Baltic Seas, high atmospheric NH_3 concentrations can lead to a net flux of NH_3 from the atmosphere to the ocean (Asman *et al.*, 1994; Barrett, 1998; Lee *et al.*, 1998). However, in a recently published study, Sørenson *et al.* (2003) pointed out that even at low atmospheric NH_3 concentrations, the coastal North Sea is a sink for atmospheric NH_3. This in line with observations of NH_3 fluxes in the Chesapeake Bay, which varied seasonally. During winter the flux was from the atmosphere into the surface waters, whereas during summer the Chesapeake Bay acted as a source of NH_3 (Larsen *et al.*, 2001). The central Pacific Ocean,the Northwest Indian Ocean (Arabian Sea) and the Northeast Indian Ocean (Bay of Bengal) have been shown to be sources of NH_3 to the atmosphere (Gibb *et al.*, 1999a; Quinn *et al.*, 1990; Schäfer *et al.*, 1993), whereas the Sargasso and Caribbean Seas were found to be a sink for NH_3 (Quinn *et al.*, 1996)

Additionally, micrometeorological methods have been applied to measure NH_3 fluxes in coastal areas. For example, Sørensen Geernaert *et al.* (1998) measured the vertical NH_3 profile above the seasurface in the Kattegat Strait (western Baltic Sea) and found a flux of NH_3 to the seasurface. Recently, Biswas *et al.* (2005) applied a similar technique and could show that the Sundarban mangrove forest (northeastern Bay of Bengal) was a sink of atmospheric NH_3.

Several additional observations based on the distribution and composition of NH_4^+ aerosols over the oceans suggest that large parts of the ocean are, indeed, sources of NH_3 to the atmosphere: During a cruise in the equatorial Pacific Ocean, Clarke and Porter (1993) found a clear relationship between enhanced NH_4^+ aerosols concentrations and enhanced chlorophyll concentrations implying that NH_3 emissions from equatorial upwelling resulted in formation of NH_4^+ aerosols of marine origin. The estimated release of NH_3 from the ocean to the atmosphere (10 ± 10 μmol m^{-2} day^{-1}) needed to form aerosol NH_4HSO_4 was found to be in agreement with a flux estimate by Quinn *et al.* (1990) (1.8–15 μmol m^{-2} day^{-1}) for

the Pacific Ocean. In another study, the concentrations of NH_4^+ aerosols were monitored in an air mass of European origin passing over the North Atlantic Ocean near the Azores (Zhuang and Huebert, 1996). Despite the fact that the plume was diluted with NH_4^+ -free tropospheric air, NH_4^+ aerosol concentrations were relatively constant, thus, an additional, oceanic NH_3 source was suggested to maintain the observed NH_4^+ aerosol concentrations with a mean NH_3 flux of about 26 ± 20 μmol L^{-1} day^{-1}. This flux is in reasonable agreement with the range of sea-to-air fluxes calculated for the Pacific Ocean, the Arabian Sea and the Bay of Bengal by Quinn *et al.* (1990), Gibb *et al.* (1999a), and Schäfer *et al.* (1993) respectively. However, estimates of the NH_3 air–sea exchange for eastern basins of the central Atlantic Ocean (Caribbean and Sargasso Seas) suggested that these basins act as sink for NH_3. This obvious discrepancy might be caused by possible analytical artifacts of the measurements and the fact that the available measurements of dissolved $[NH_x]$ and $(NH_3)_{g,atmos}$ were not simultaneously measured (Quinn *et al.*, 1996). Because NH_3 ocean/atmosphere flux estimates are very sensitive with respect to $[NH_x]$ and $(NH_3)_{g,atmos}$, spatial and temporal differences of non–simultaneous NH_3 measurements in the ocean and in the atmosphere introduce a significant bias (Quinn *et al.*, 1996).

More recently, Jickells *et al.* (2003) measured the isotopic abundance of NH_4^+ aerosols from the North and South Atlantic Ocean. They found a positive correlation between NH_4^+ aerosol concentrations and $\delta^{15}N_{atm}$ of NH_4^+ and concluded that the light NH_4^+ must have originated from oceanic NH_3 emissions (Jickells *et al.*, 2003). Norman and Leck (2005) observed a pronounced diurnal cycle of atmospheric NH_3 in the pristine atmospheric boundary layer of the southeastern Atlantic and attributed this phenomenon to diurnal changes in the oceanic NH_3 source.

5.5. The role of oceanic emissions in the global budget of atmospheric NH$_3$

Based on the fact that only a limited amount of oceanic NH_3 measurements are available and, therefore, the spatial and seasonal variabilities of the NH_3 exchange across the ocean/atmosphere interface are largely unknown, only a few attempts have been made to estimate the global oceanic NH_3 emissions. Schlesinger and Hartley (1992) extrapolated the fluxes from the Pacific Ocean calculated by Quinn *et al.* (1990, 1988) to the global ocean and yielded a mean annual global flux of 13 Tg N with 8 and 18 Tg N as minimum and maximum values, respectively. Dentener and Crutzen (1994) scaled the oceanic NH_3 emissions to the oceanic emissions of dimethyl sulfide (DMS) by assuming equal molar emissions of DMS and NH_3. Their global estimate yielded an annual oceanic NH_3 release of 7.0 Tg N. This estimate is in agreement with the result of a model study, which resulted in a global oceanic NH_3 emission estimate of 8.2 Tg N yr^{-1} (3–16 Tg N yr^{-1}) (Bouwman *et al.*, 1997). Thus, oceanic emissions are a major source of atmospheric NH_3 and contribute about 15% of the total sources (Bouwman *et al.*, 1997). Other major sources are excreta from domesticated animals (21.6 Tg N yr^{-1}) and synthetic fertilizer use (9.0 Tg N yr^{-1}) (Bouwman *et al.*, 1997). Major sinks are dry/wet deposition on land

(40 Tg N yr^{-1}) (Schlesinger and Hartley, 1992) and to the oceans (16.7 Tg N yr^{-1}) (Duce *et al.*, 1991).

Because the NH$_3$/NH$_4^+$ equilibrium and, thus, the concentration of "free" dissolved gaseous NH$_3$ is strongly tightened to the oceanic pH, any changes in the oceanic pH will influence the magnitude and in the extreme case even the direction of the NH$_3$ flux across the ocean/atmosphere interface. This point is especially important since the ongoing increase of atmospheric carbon dioxide results in a significant acidification of the oceans (Caldeira and Wickett, 2005; Orr *et al.*, 2005; Raven *et al.*, 2005). The predicted shift of the pH in the surface ocean from the present-day mean of about 8.1–7.9 in the year 2100 might lead to a reduction of the oceanic NH$_3$ emissions since the fraction of dissolved gaseous NH$_3$ available for gas exchange is further reduced (see Fig. 2.8). This, in turn, implies that the uptake capacity of a more acidic ocean for the atmospheric base NH$_3$ might be enhanced by 7–40% (Jacobson, 2005).

6. Outlook

Despite the fact that our knowledge about the oceanic distribution of NO, N$_2$O, NH$_3$, and N$_2$ has considerably increased during the last decades, we are far from being able to draw a comprehensive picture. Of the four gases discussed above, N$_2$O is the most studied. Increased attention have been paid to NH$_3$ and N$_2$ during the last years. However, because of the analytical difficulties, we still have only a rough idea about the oceanic pathways of NO cycling. In the following section I sketch some major gaps of our knowledge about the gases discussed above:

6.1. Nitrous oxide (N$_2$O)

Future work on oceanic N$_2$O should pay more attention to N$_2$O pathways in coastal areas such as estuaries, mangroves, upwelling areas etc. since it is evident that a significant portion of the oceanic flux to the atmosphere comes from the coastal areas. We still do have only a rough idea how coastal N$_2$O emissions will be altered by ongoing environmental changes such as eutrophication and the development of coastal hypoxia. Another point to be considered for future formation of N$_2$O is that we might expect an expansion of suboxic zones in the world ocean. This, in turn, might lead to enhanced N$_2$O accumulation at the oxic/suboxic boundary layers.

Isotope measurements of N$_2$O have been introduced as a promising tool to decipher the formation pathways of oceanic N$_2$O. However, it turned out that isotopic measurements in some cases are difficult to explain (e.g. in the Arabian Sea) and that they even can lead to contradicting results: For example, from the interpretation of the N$_2$O isotope measurements at station KNOT in the western North Pacific Ocean it is not clear whether nitrification or denitrification is the main N$_2$O formation pathway (see Table 2.2). We need to learn more about the N$_2$O forming pathways and the resulting N$_2$O isotope signatures of the different types of N$_2$O producing organisms. The recently isolated NH$_4^+$ oxidizing archaeon (Könneke *et al.*, 2005) raises the question whether we have overlooked N$_2$O forming organisms other than bacteria. (Especially in view of the fact that NH$_4^+$-oxidizing Archaea have been found to dominate soil ecosystems (Leininger *et al.*, 2006),

which might also be true for the ocean ecosystems.) N_2O isotope studies might help to identify unknown N_2O formation pathways. Moreover, there is an obvious discrepancy in the measured mean tropospheric SP_{N_2O} (19‰ vs. 43‰, Kaiser *et al.* (2004)), which is not resolved yet. Thus, there seems to be an urgent need for interlaboratory calibration of the N_2O isotope methods.

6.2. Dinitrogen (N_2)

In order to quantify the loss of fixed nitrogen via denitrification and anammox we definitely need more N_2 concentration measurements and more formation rate measurements in suboxic zones such as found in the Arabian Sea and the eastern tropical Pacific Ocean.

6.3. Ammonia (NH_3)

More simultaneous measurements of NH_3 in the ocean and in the atmosphere are needed to reduce the considerable uncertainties of the ocean/atmosphere flux esti‐mates. The ongoing acidification of the ocean will shift the NH_3/NH_4^+ equilibrium to NH_4^+. On the one hand this might have implication for the atmospheric distribution of NH_3, since the uptake capacity of the ocean will be increased with unknown consequences for chemistry of the atmosphere (e.g. the aerosol formation) over the ocean. On the other hand this might have severe implications for the nitrification rates in seawater because they are influenced by the *pH*. When the pH drops from 8 to 7, nitrification rates can be reduced by 50% (Huesemann *et al.*, 2002). (One explanation for this is that the ammonia monooxygenase enzyme uses rather NH_3 than NH_4^+ as substrate.) Most recently it was suggested that atmospheric NH_3 serves as a foraging cue for seabirds such as the blue petrel (Nevitt *et al.*, 2006): NH_4^+ is an excretion product of seabird prey. It has been shown that krill swarms release significant amounts of NH_4^+ (Johnson *et al.*, 1984). Shifting the NH_3/NH_4^+ equilibrium to NH_4^+ due to acidifi‐cation reduces the release of NH_3 and might therefore directly influence the ability of seabirds to locate patches of prey in the surface ocean (Nevitt *et al.*, 2006).

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I am very grateful to Tom Bell (School of Environmental Sciences, University of East Anglia, Norwich, UK) to draw my attention to some critical points in the calculations of *[NH₃]* and *H'ₛNH₃* as discussed in detail in Bell *et al.*, (2007) and Johnson *et al.*, (2007), (2008). These publications have been published after the

submission of my manuscript and, therefore, were not available to be included in the main text.

According to Bell *et al.* (2007) there is an alternative way to calculate [NH$_3$]:

$$NH_4^+ \xleftrightarrow{k_a} NH_3 + H^+$$

$$[NH_3] = \frac{[NH_x]K_a}{(K_a + [H^+])}$$

As shown in Bell *et al.* (2007), the calculation proposed by Khoo *et al.* (1977) to correct K_a for salinity and temperature effects is incorrect. Alternative corrections for pK_a (= $-\log_{10}K_a$) are given in Bell *et al.* (2007) and Johansson and Wedborg (1980), respectively:

$$pK_a = 10.0423 - (0.0315536\ t) + (0.14737\ I)$$

$$pK_a = -0.467 + (0.00113S) + \left(\frac{2887.9}{T}\right)$$

where t is the temperature in °C, T is the temperature in K, S is the salinity and I is the ionic strength.

The dimensionless Henry's constant given in Gibb *et al.* (1999a) (see Section 5.2) is erroneous (Johnson *et al.*, 1997; 2008). The correct form is:

$$H'_{sNH_3} = \frac{1}{17.93\left(\frac{T}{273.15}\right)\ \exp\left(\frac{4029}{T} - 9.70\right)}$$

Please note that the two curves in Figure 2.8 (dashed-dotted lines) which were calculated with the equations given in Gibb *et al.* (1999a) are, therefore, erroneous as well.

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CHEMICAL COMPOSITION OF MARINE DISSOLVED ORGANIC NITROGEN

Lihini I. Aluwihare *and* Travis Meador

Contents

1. INTRODUCTION

As this collection of papers suggests, Nitrogen plays an important role in maintaining the biogeochemical balance of the marine environment. Many studies have focused on the role and distribution of inorganic forms of nitrogen—primarily, nitrate and ammonia and more recently, dinitrogen (N_2) gas—because of their demonstrated participation in primary production in the ocean and in biological controls on the oceanic distribution. In many parts of the surface ocean, dissolved organic nitrogen (DON) is the most abundant nitrogen species, excluding N_2 (Bates and Hansell, 1999; Cavender-Bares *et al.*, 2001; Wong *et al.*, 2002), and several studies have demonstrated the importance of DON as a currency for N exchange

within the marine ecosystem (see reviews by Antia *et al.*, 1991 and Bronk, 2002). However, the ecological role of marine DON and factors that control its reservoir size remain elusive. Some studies have examined the cycle of model N-containing compounds (urea and amino acids for example), and the presence of these same compounds in seawater has been demonstrated; but much of the DON reservoir remains poorly characterized, leaving its "molecular messages" (Hedges, 2002) unread.

In this chapter we attempt to summarize what is known about the chemical composition of marine DON with a special focus on the fraction that has been isolated by ultrafiltration through a spiral wound membrane of 1 nm pore size. This fraction is identified as high molecular weight (HMW) DON throughout this chapter and is identical to UDON (ultrafiltered DON) as discussed by some investigators (e.g., McCarthy *et al.*, 1998). This review is not meant to be an exhaustive survey of total DON composition but a focused report on more recent analytical advances in the study of complex DON compounds. The compositional information provided by these studies is further interpreted in the context of sources and sinks for particular organic N compounds in the marine environment. We would like to direct the readers to some recent reviews that provide extensive coverage of particular aspects of the DON story that may not have taken center stage in this chapter (Antia *et al.*, 1991; Benner, 2002; Bronk, 2002).

2. DEFINITIONS OF DISSOLVED ORGANIC NITROGEN

Like dissolved organic carbon (DOC), total DON is operationally defined and is differentiated from its particulate counterpart as the fraction of marine organic N passing through a filter having a pore size of either 0.7 μm (typically glass fiber) or 0.2 μm (polycarbonate, polysulfone, or aluminum filters). Note that all DON compounds also contain larger amounts of C, and that DON may be considered a subset of the DOC reservoir. Therefore, it can be useful to make observations of the DOC reservoir (e.g., percent recovery, isotopic composition, etc.) in order to provide a framework for studying the DON pool. Parts of this review employ this strategy.

Unlike DOC, which can be easily differentiated from dissolved inorganic C (DIC), DON is calculated by subtracting the concentrations of nitrate, nitrite, and ammonia from total dissolved N (TDN). This technique introduces several errors to estimates of DON concentration, and robust marine DON concentrations are a relatively recent phenomenon (Sharp *et al.*, 2004). Methods for quantifying each of these N pools are discussed in McCarthy and Bronk (this volume). Contemporary studies often omit the filtration step that differentiates dissolved organic matter (DOM) from particulate organic matter (POM) because DOM is far more abundant even in surface waters. Therefore, reported concentrations often represent *total* organic N (TON) rather than simply the *dissolved* species.

Several separation and isolation methods based primarily on solid phase extraction (SPE) or size fractionation (e.g., ultrafiltration) have been employed to

fractionate the complex DON reservoir (reviewed in McCarthy and Bronk, this volume). In most of these studies seawater was first filtered through a 0.2 μm filter (or at the very least, a 1 μm filter) prior to DON separation. This is important particularly for ultrafiltration, where large volumes of seawater are concentrated to compensate for naturally low concentrations of DOM compounds. Ultrafiltration typically isolates 20–25% of the total DOC (see below) in open ocean environments. This represents <30 μM C (<400 μg C/L). If organisms such as bacteria—whose surface water abundance is approximately 10^9 cells/L—are not removed, they will be included in the ensuing compositional studies. Assuming a C content of 15 fg C/ bacterial cell (Fukuda et al., 1998), one liter of surface water contains approximately 15 μg C as bacterial biomass. Ultrafiltration, as described earlier, concentrates viruses and most studies of HMWDOM characterization will include viruses.

2.1. Methods of isolation

2.1.1. Solid phase extraction

Several non-ionic chromatographic supports have been used for extracting DOM from natural waters on the basis of hydrophobic interactions. The most widely used supports include C_{18} (a silica-based matrix modified by an octadecyl bonded phase) and Amberlite® XAD resins (Aiken, 1985; Ertel et al., 1986; Leenheer, 1981; Mantoura and Riley, 1975; Thurman and Malcolm, 1981; McCarthy and Bronk, this volume). These resins fractionate organic matter based on their chemical composition. The XAD-8 support typically absorbs hydrophobic acids (fulvic acids) and XAD-4 absorbs hydrophilic acids (Aiken et al., 1985). The organic matter commonly absorbed from natural waters in this manner has been referred to as humic substances (i.e., humic or fulvic acids)—terminology adapted from the study of terrestrial organic matter. Commonly employed XAD resins include XAD 2, XAD 4, and XAD 8. The first two are cross-linked styrene-divinylbenzene resins with an average pore size of 50 and 90 Å, respectively and the third is an acrylic ester resin with an average pore size of 250 Å. These resins are often used in series to increase DOM extraction efficiencies (Druffel et al., 1992; Thurman and Malcolm, 1981). Based on $\delta^{13}C$ values, it is clear that most compounds within marine XAD extracts originate from autochthonous marine processes. For example, values of -20.8 ± 0.2‰ were reported for XAD-4 isolates, -22.0 ± 0.1‰ for XAD-2, and -23 ± 0.1‰ for XAD-8 isolates (Druffel et al., 1992).

Typically, seawater samples are acidified prior to extraction (pH 2–4) to protonate organic acids, and the compounds absorbed to resins are eluted with base for XAD resins and with organic solvents for C_{18} resins. Aiken et al. (1992) extracted 30–83% of the DOC in freshwater environments using XAD 8 and XAD 4 in series. For marine environments, Druffel et al. (1992) reported the isolation of 23–39% of the DOC when XAD 4 was used in series with either XAD 8 (30–39% of total DOC) or XAD 2 (23–28% of total DOC). More recently C_{18} resins have been used to isolate about 30% of total DOC from marine systems, and up to 50% from estuarine waters (Amador et al., 1990; Kaiser et al., 2003; Repeta et al., 2004; Simjouw et al., 2005). These resins are purportedly more successful than XAD-2, for example, at removing the colored DOM component from seawater.

The major disadvantage of solid phase extraction is that it provides a chemically biased view of the DOM reservoir, and seems to preferentially extract compounds with relatively low amounts of N. Other problems include sample contamination by various resins and incomplete elution of adsorbed DOM. Solid phase extraction remains, however, the only technique capable of reproducibly isolating DOM compounds within the entire range of MWs. Furthermore, the radiocarbon content (Δ^{14}C) of DOC isolated by XAD resins is depleted relative to the total dissolved inorganic carbon (DIC) reservoir (Δ^{14}C values for XAD isolates are between -300 and -500‰ (>3000 years); Druffel *et al.*, 1992) and so these isolates provide insight into the chemical structure of long-lived C- and N-containing organic compounds.

2.1.2. Tangential flow ultrafiltration

Over the last decade, studies of DOM structure, function, and cycling have relied primarily on the use of ultrafiltration to concentrate dissolved organic compounds and isolate them from the much more abundant inorganic compounds in seawater (reviewed in McCarthy and Bronk, this volume). The value of ultrafiltration was first demonstrated by Ogura (1974) but was not widely used by the oceanographic community until the 1990s. Ultrafiltration separates compounds based on size rather than chemical composition, thus providing a more comprehensive view of the structural diversity present in seawater DOM. In addition, since seawater is not pre-acidified, isolated compounds should retain their "natural" chemical character and conformation. However, following ultrafiltration, samples are often de-salted by diafiltration against Milli-Q water and this process likely affects molecular conformation. Theoretically, the intrinsic contamination from the ultrafiltration membrane is low, but poor cleaning of filters, for example, can result in cross contamination between samples and contamination from bacterial growth.

The most widely used nominal pore size for ultrafiltration is 1 nm, which is estimated to retain compounds with MWs >1000 Da. The 1 nm pore-sized membrane isolates $\sim20\%$ of the total DOC in surface and deep ocean waters and up to 55% of the DOC in coastal and estuarine environments (Benner *et al.*, 1997; Carlson *et al.*, 1985; Guo and Santschi, 1996). Ultrafiltration membranes with a smaller pore size are rare and do not show reproducible retention characteristics; filters with a larger pore size retain only a small fraction of total DOC and they are not widely used. In general, the actual MW retained and the isolation of reproducible quantities of DOC by ultrafiltration depends strongly on the membrane (e.g. construction material, manufacturer), sample type (e.g. river, coastal, open ocean), total DOC concentration, concentration factor, extent of desalting and operating conditions (Buesseler *et al.*, 1996; Guo and Santschi, 1996; Guo *et al.*, 2000). Losses to the ultrafiltration membrane can also be significant (Guo *et al.*, 2000) and depend primarily on the physiochemical characteristics of the particular molecule.

On average, approximately 20–25% of open ocean DOC is found in the >1000 Da or high MW (HMWDOC), fraction (Benner, 2002). In the Middle Atlantic Bight, Guo and Santschi (1997) found that approximately 54% of the C resided in the <1000 Da fraction while 25% of the DOC was found to be between 1000–3000 Da, 14% between 3000–10 000 Da and approximately 7% in the $>10\,000$ Da fraction. Carlson *et al.* (1985) reported that between 0 and

16% of the total DOC (isolated from the North Atlantic Ocean) was retained by a 10 000 Da filter and only 6% was retained by 30 000 Da filters. Together, these data show that most of the organic compounds dissolved in seawater have MWs below 1000 Da. Examples of molecules with weights close to 1000 Da that may be retained and concentrated by ultrafiltration include chlorophyll *a* and *b* (893.49 and 906.51, respectively), $\geq$ pentasaccharide, $\geq$ decapeptide, and large lipids such as the polar tetraether membrane lipids of the *Archaea* (few long chain, free lipids are expected to have MWs above 1000 Da).

The next few sections focus primarily on bulk and molecular level chemical characteristics of DOM isolated by ultrafiltration using membranes with a nominal pore size of 1 nm. This fraction is referred to as HMWDOM, HMWDOC, or HMWDON depending on the context.

3. BULK CHEMICAL COMPOSITION OF HIGH MOLECULAR WEIGHT DISSOLVED ORGANIC NITROGEN

3.1. Elemental composition

Only occasionally has the N content of solid phase extracts been reported. At a site in the Atlantic Ocean the carbon to nitrogen ratio (C:N) of XAD 8 and XAD 2 extracts fell in the range of 40–57 (57 ± 0.9 and 41.1 ± 3.3, respectively; Druffel *et al.*, 1992). In contrast, at the same site XAD 4, when used as the second resin in series with XAD 8 or XAD 2, extracted compounds with lower C:N ratios - 19–24 (21.0 ± 2.4). These values are only slightly higher than ratios reported for total DOM (see below). McKnight and Aiken (1998) reported a C:N value of 37 for DOM extracted by XAD 8 at one site in the Pacific Ocean; at other sites in the N. Pacific Ocean XAD 2 was found to extract DOM with a C:N ratio between 32 and 46.5 (Druffel *et al.*, 1992; Meyers–Schulte and Hedges, 1986). Bronk (2002, Table III) compiled various literature values and arrived at an average C:N ratio of 32.8 ± 19.5 for total ''humic substances'' isolated from a variety of aqueous environments (see McCarthy and Bronk, this volume).

The total DON extracted from the Sargasso Sea when using XAD 4 in series with either XAD 8 or XAD 2 is reported in Fig. 3.1A (Druffel *et al.*, 1992). If we assume a total DON concentration of 5.0 μM N in surface waters and 3.0 μM N in deep waters (estimated from Hansell and Carlson, 2001), the organic N extracted by XAD resins represents approximately 10% of the total DON present throughout the water column. The C/N ratio for DOM isolated by C_{18} resins has not been reported but is probably similar to that for other solid phase extracts (i.e., ≥ 30).

Figure 3.1B shows a vertical profile of the concentration of DON and HMWDON in the eastern North Pacific and Central Pacific, respectively. As is clear from this figure ultrafiltration only isolates a fraction ($<30\%$) of total DON. In the case of HMWDOM isolated from estuarine, coastal and open ocean areas the C:N ratio ranges from 11 to 25. These ratios are lower than those of DOM extracted onto chromatographic supports as discussed above. HMWDOM from marine sites are on the low end of this range with average ratios ($\pm 1\sigma$) of 16.0 (± 1.8; $n = 41$) for surface waters, 16.6 (± 3; $n = 9$) for mesopelagic waters (750–1200 m) and
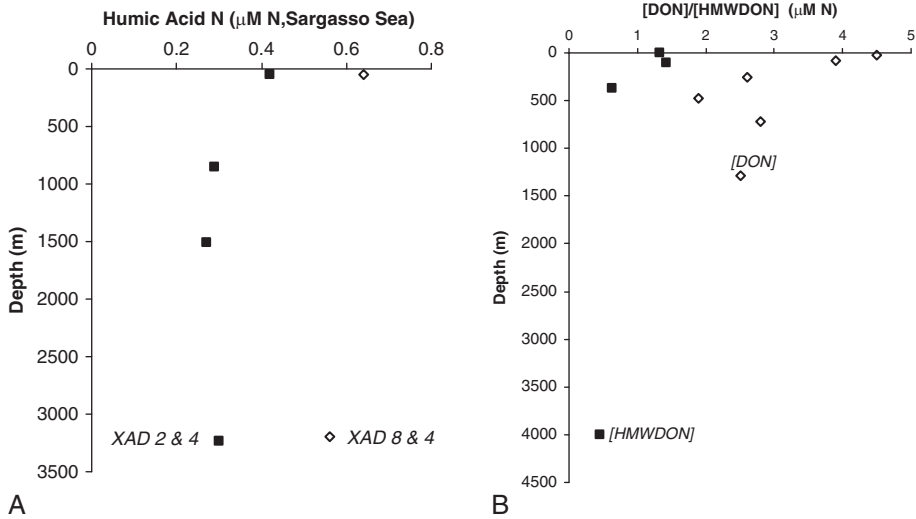
Figure 3.1 (A) Depth profile of humic DON isolated from the Sargasso Sea using XAD 8 + XAD 4 (diamonds) and XAD 2 + XAD 4 (squares). Adapted from Druffel *et al.* (1992). (B) Profile of total DON (North Pacific Ocean at 34°50′N, 123°0′W; Loh and Bauer, 2000) and HMWDON (Central Pacific Ocean at 12°S, 135°W; McCarthy *et al.*, 1998).

18.6(±0.7; $n = 4$) for bathypelagic waters (>2500 m) (Aluwihare *et al.*, 2002; Benner *et al.*, 1997; Guo *et al.*, 2003 (salinity >30); Meador *et al.*, 2007; Fig. 3.2). As shown in Fig. 3.2 total DOM has an average C:N ratio of approximately 14.6 (±3.3) in the surface ocean, 14.3 (±1.9) in the mesopelagic and 13.7 (±1.8) in the bathypelagic (Bronk, 2002; Hansell and Carlson, 2001; Loh and Bauer, 2000; De Jesus and Aluwihare, unpublished). Based on these data HMWDOM and DOM appear to share similar bulk C:N ratios in surface waters. Hopkinson and Vallino (2005) found that the C:N ratio of deep ocean DOM (primarily at sites in the Atlantic Ocean) was much higher than the ratio measured for surface waters—22 versus 14 (Fig. 3.2). These latter C:N ratios of total DOM are in better agreement with the C:N ratio of HMWDOM in surface and deep waters. Overall, data for both DOM and HMWDOM imply that N is preferentially lost from the dissolved organic reservoir with depth.

Benner (2002) estimated that the C:N:P of HMWDOM was 298:18:1 and 496:28:1 in surface and deep waters, respectively. In contrast, Hopkinson and Vallino (2005) calculated values of 374:27:1 for total DOM in surface waters and 3511:202:1 in deep waters. Both the C:N and C:N:P ratio of DOM and HMWDOM deviate significantly from the Redfield ratio (i.e., 106–132:16:1). However, vertical gradients in the C:N:P of total DOM suggests that the average DOM that is produced in surface waters and degraded at depth must have a ratio close to 199:20:1—similar to the Redfield ratio (Hopkinson and Vallino, 2005). In contrast, Loh and Bauer (2000) found little variation in the C:N:P of DOM with depth. Average values were shown to be 229-682:12-47:1; N:P ratios reported here are significantly lower at depth relative to values reported by Hopkinson and Vallino
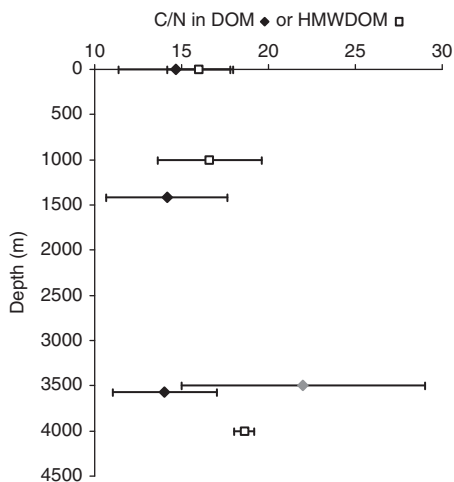
Figure 3.2 Vertical profile of C/N ratios in total DOM (closed diamonds) and HMWDOM (isolated via ultrafiltration through a 1000 Da membrane; open squares). Data for total surface (<50 m) DOM were derived from Bronk (2002) and De Jesus and Aluwihare (unpublished). Meso-pelagic (1000–2000 m) and bathypelagic (3000–4000 m) C/N values in total DOM (black, closed diamonds) were derived from Loh and Bauer, (2000) and Hansell and Carlson (2001). Also shown on the figure are more recent estimates of the C/N ratio in total DOM isolated from the deep ocean (from Hopkinson and Vallino, 2005; grey diamond). C/N ratios of HMWDOM were taken from Benner *et al.* (1997), Guo *et al.* (2003), Aluwihare *et al.* (2002), Meador *et al.* (2007) and Meador and Aluwihare (unpublished). Error bars depict the 1σ standard deviation for each data set. The depths at which the C/N values are located on the figure are for the general depiction of surface waters (near 2 m), mesopelagic (750–2000 m), and bathypelagic regions (depths >2500 m) only and do not correspond to the exact depth at which each sample was collected.

(2005). These observed discrepancies may reflect the challenge of accurately deter-mining DON and dissolved organic phosphorus (DOP) concentrations in the deep ocean where the concentration of the corresponding inorganic species is an order of magnitude higher. According to Kolowith *et al.* (2001) the C:N:P ratio of HMWDOM that is lost with depth is approximately 227:14:1 – significantly enriched in C compared to the Redfield ratio, but a similar N:P ratio as the Redfield ratio. In the case of DOM N and P are preferentially remineralized relative to the *accumulating* DOM reservoir. However, in the case of HMWDOM the accumulat-ing component and the component that is removed with depth have a similar C:N ratio, but P is enriched in the remineralized component. In order to balance what is observed for the total DOM reservoir, low molecular weight DOM compounds that are removed with depth must be enriched in N relative to HMWDOM.

Values discussed in the previous section represent an oceanic average and indi-vidual regions and parts of the water column may behave differently. For example, Abell *et al.* (2000, 2005) found that the DOM remineralized along isopycnals outcropping in the subtropical North Pacific Ocean is C-rich (C:N ∼ 30); based on this C:N ratio these authors concluded that DOM contributes between 70 and 90% of the total organic matter remineralized in the upper thermocline. As more data are collected, other sites in the ocean may show similar variability.

3.2. Stable isotope composition

Available stable C and N isotope values (δ^{13}C and δ^{15}N) for HMWDOM are listed in Table 3.1. Average δ^{13}C values for HMWDOM isolated from surface, mid–depth (between 100–1000 m) and deep waters (>2000 m) are −21.2‰ (±1.9), −21.7‰ (±0.3) and −21.7‰ (±0.3), respectively (Guo *et al.*, 2003 ($n = 4$; open ocean); Benner *et al.*, 1997 ($n = 21$); Meador and Aluwihare, unpublished ($n = 49$)). Some surface samples were influenced by local riverine inputs and this explains the greater variance in the surface ocean data set reported in Table 3.1. Reported values for the δ^{15}N signature of HMWDOM vary among studies; average values are 5.3‰ (±1.6) in the surface ocean, 6.4‰ (±2) at intermediate depths and 5.9‰ (±2.2) in the deep ocean. The observed spread in surface water δ^{15}N values (∼3.2–9.5‰) may reflect spatial or temporal (seasonal or longer time scale) changes in the isotopic composition of HMWDON. However, seasonal variations (i.e., temporal variations in primary production) are unlikely to influence the DON reservoir of the deep ocean. In addition, similar locations were sampled by multiple studies, so spatial variations alone cannot explain these differences. In fact, recent data from diverse marine sites show similar δ^{15}N values for HMWDON in the surface ocean despite spatial variations in inorganic N concentrations and N species as well as primary producer assemblages (Meador *et al.*, 2007). There is almost a 10 year lag between samples collected for the Benner *et al.* (1997) study—which reports high δ^{15}N values—and the Meador *et al.* (2007) study (for example). Therefore, decadal-scale variability could drive the observed difference. If the Benner *et al.* (1997) data are excluded, then the remaining surface ocean δ^{15}N values in Table 3.1 compare well with the δ^{15}N signature of the deep ocean nitrate reservoir (∼5‰; Sigman *et al.*, 2000).

There is less known about the δ^{15}N signature of total DON. At Station ALOHA, total DON in very near-surface waters was found to have a relatively low δ^{15}N signature, between 2 and 4‰ (Karl *et al.*, 2002). No such depletion in ^{15}N was recorded for HMWDOM isolated at or near this site (Meador *et al.*, 2007). However, δ^{15}N values for total DON below the euphotic zone at Station ALOHA were between 5 and 7‰ (Karl *et al.*, 2002), similar to values in Table 3.1. A one-year time series study at the Bermuda Atlantic Time Series (BATS) site found that the δ^{15}N signature of DON showed no seasonal variability and had an annually averaged value of 3.9‰ (Knapp *et al.*, 2005). Meador *et al.* (2007) reported a similar average δ^{15}N value for HMWDON isolated from the surface North Atlantic Ocean—4.1±0.64‰ (Table 3.1; $n = 25$). Unlike the relatively stable values reported for DOM and HMWDOM, the δ^{15}N signature of suspended organic particles is more variable and influenced by inorganic N concentrations and N species supporting primary production at specific sites (e.g. Altabet and Francois, 1994; Mino *et al.*, 2002; Rau *et al.*, 1998). These comparisons between PON and DON, and the minimal spatial and temporal (on < annual timescales) variability exhibited by δ^{15}N values of DON and HMWDON led Knapp *et al.* (2005) and Meador *et al.* (2007) to conclude that most of the DON/HMWDON reservoir accumulating in surface waters is refractory on annual timescales.

Table 3.1 Average carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope signature of HMWDOM isolated from several different sites in the Atlantic Ocean, Pacific Ocean, and the Gulf of Mexico

	$\delta^{13}C$ (HMWDOM)				$\delta^{15}N$ (HMWDOM)				Reference
	Atlantic	*Pacific*	*Gulf of Mexico*	*Arctic*	*Atlantic*	*Pacific*	*Gulf of Mexico*	*Arctic*	
Surface	−21.2 ± 1.2	−21.6 ± 0.2			4.1 ± 0.64	5.39 ± 0.75			Meador *et al.*, 2007
	−22.2	−21.6 ± 0.2			6.6	7.8 ± 0.5	9.5		Benner *et al.*, 1997
			−21.36		6.6		4.2		Guo *et al.*, 2003
				−21.1 ± 0.1				4.4 ± 0.3	Benner *et al.*, 2005
Mid-depth		−21.7 ± 0.3							
		−21.5 ± 1.3			5.56 ± 0.91	6.4 ± 0.98			Meador *et al.*, 2007
	−21.7	−21.6 ± 0.2	−21.3		8.9	7.7 ± 0.8	10.2		Benner *et al.*, 1997
			−22.2 ± 0.7		4.8		3.9 ± 1		Guo *et al.*, 2003
				−21.5 ± 0.3				4.6 ± 0.02	Benner *et al.*, 2005
Deep	−22.1	−21.6 ± 0.2			8.5	7.8 ± 0.5			Benner *et al.*, 1997
			−20.9		5.2 ± 0.1		3.46		Guo *et al.*, 2003
				−21.5 ± 0.3				4.4 ± 0.7	Benner *et al.*, 2005

Some values include ± 1 σ standard deviation; all values are expressed as ‰. Values reported by Meador *et al.* (2007) are from 2 m and 1000 m depth for surface and mid–depth values, respectively (analytical error on these measurements are ± 0.5‰). The data for Arctic Ocean samples were taken from Table 3.1 of Benner *et al.* (2005) and exclusively represent Nordic sea surface (<250 m), Arctic Ocean mid depth (200–600 m), and Arctic Ocean deep (>1000 m) samples because these were the only samples that were considered to be truly marine.

3.3. Functional groups in HMWDOM

The application of ultrafiltration to concentrate and isolate HMWDOM has allowed the scientific community to apply a variety of analytical tools to elucidate DOM chemical structure. Of particular note is the employment of ^{1}H, ^{13}C, ^{15}N and ^{31}P nuclear magnetic resonance (NMR) spectroscopy to study the chemical structure of DOM (e.g. Aluwihare *et al.*, 1997; Benner *et al.*, 1992; Hedges *et al.*, 1992; Kolowith *et al.*, 2001; McCarthy *et al.*, 1997; McCarthy and Bronk, this volume). Within the context of studying bulk HMWDOM, NMR spectroscopy provides a reproducible, quantitative method for identifying particular chemical functional groups. The *x*-axis of the NMR spectrum (as shown in Fig. 3.3; Eglinton and Repeta, 2004) identifies the chemical environment that surrounds each observed nucleus (e.g., in the case of ^{13}C NMR, various chemical substituents around the rare ^{13}C nucleus are observed) and the integrated area under each peak provides information on the relative abundance of various functional groups in HMWDOM. Based on functional group identification, we can infer (and quantify) the types of biochemicals present in HMWDOM.

Figure 3.3 shows ^{1}H, ^{13}C and ^{15}N NMR spectra of HMWDOM isolated from surface waters near Hawaii (modified from Eglinton and Repeta, 2004). The major functional group identified by both ^{13}C and ^{1}H NMR spectroscopy is HC-OH (between 3 and 4 ppm, ^{1}H-NMR; 50–80 ppm, ^{13}C-NMR), which is interpreted as arising from carbohydrates. Other carbohydrate specific resonances such as O-CH-O at the anomeric position (C-1 of the aldose ring) are also identifiable (between 5–6 ppm, ^{1}H-NMR; centered near 100 ppm, ^{13}C-NMR). Other major NMR resonances include alkyl/lipid functional groups (CH$_x$; 0.5–1.5 ppm, ^{1}H-NMR; 20–40 ppm; ^{13}C-NMR); acetate functional groups identified by ^{1}H-NMR spectroscopy as CH$_3$C $=$ O(O/N) (at 2.0 ppm) and manifested in the ^{13}C-NMR spectrum as carboxylic/amide (O $=$ C(O/N)) and carbonyl (C $=$ O) (between 170 and 180 ppm); and in some cases, aromatic functional groups (C $=$ C; 7–8 ppm, ^{1}H-NMR; 130–150 ppm, ^{13}C-NMR). In most open ocean samples, ^{1}H-NMR spectroscopy detects very little aromatic hydrogen suggesting that any aromatic compounds present in surface HMWDOM must be fully substituted (Aluwihare *et al.*, 1997, 2002). In general, 60–70% of C (O-CH-O+HC-OH) in surface HMWDOM can be attributed to carbohydrates, 10–12% to acetate (CH$_3$C $=$ O), 2–4% as non-acetate carbonyl/carboxylic/amide, and the remaining C to alkyl/lipid compounds which may also be found in compounds such as deoxy and methylated sugars (Fig. 3.4).

Proton and ^{13}C-NMR data compare well with each other and suggest that surface ocean HMWDOM has a H:C ratio of approximately 1.8–1.9 (Aluwihare, 1999; Benner *et al.*, 1992) and an O:C ratio between 1 and 1.1. These H:C and O:C ratios are very close to those of a pure carbohydrate with a general hexose structure (e.g., C$_6$H$_{12}$O$_6$). In comparison, humic substances isolated from seawater have an H:C ratio between 1.2 (direct elemental analyses) and 1.4 (based on NMR estimates) and are therefore, relatively C-rich (Hedges *et al.*, 1992). The H:C and O:C composition of phytoplankton as estimated by NMR spectroscopy is approximately 1.7 and 0.3, respectively (Hedges *et al.*, 2002). In comparison to phytoplankton
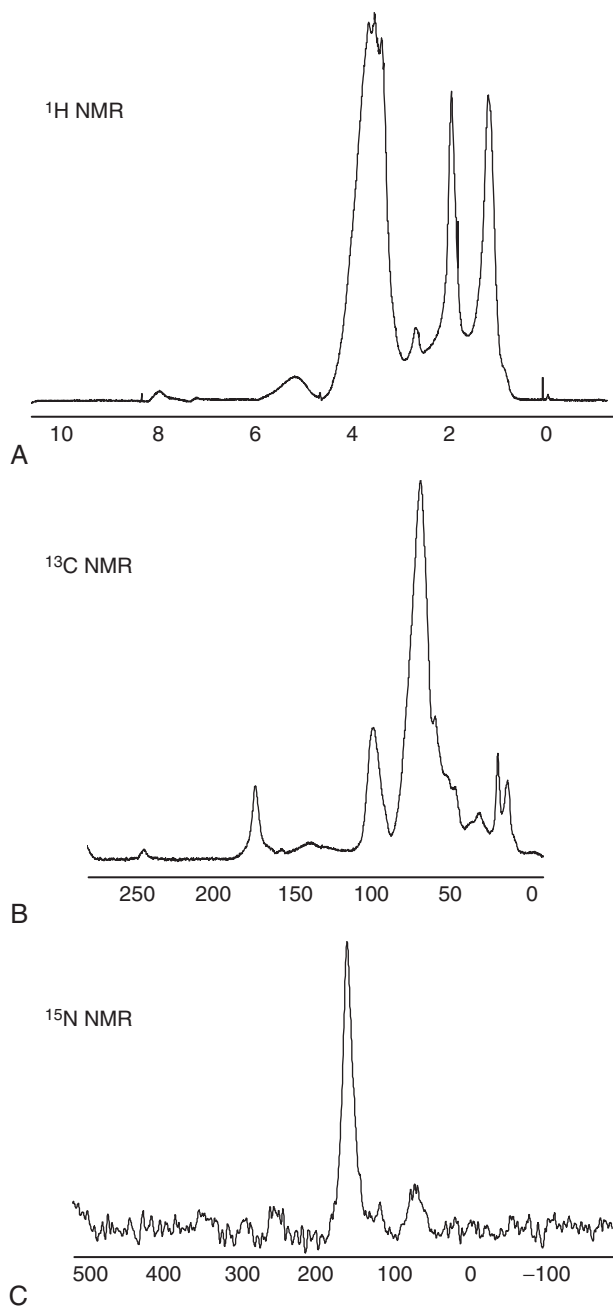
Figure 3.3 (A) Proton (^{1}H; 400 MHz, D_2O, δ), (B) carbon (^{13}C; 400 MHz, D_2O, δ), and (C) nitrogen (^{15}N; 400 MHz, solid-state, δ) nuclear magnetic resonances (NMR) spectra for HMWDOM isolated from surface waters of the North Pacific Ocean (after Eglinton and Repeta, 2004).

(where proteins dominate intracellular biochemicals) HMWDOM is rich in oxygen and deficient in hydrogen, consistent with a predominantly carbohydrate-like composition. While this discussion focuses primarily on the assignment of C, H and O in HMWDOM, it is necessary to understand this stoichiometry for the ensuing discussion on N-containing compounds in the marine environment.

At approximately 1000 m depth, sugars comprise 26–38% of HMWDOC while acetate comprises 4–6% of the total C (Fig. 3.4). At these depths, HMWDOM is also significantly enriched in lipid and olefinic C (C = C). Together, the surface and deep data support the existence of a large surface-derived carbohydrate reservoir that is removed with depth. Based on ^{13}C-NMR spectroscopy, the HMWDOM that is lost with depth has H:C and O:C ratios of 1.8 and 1.2, respectively. This component is slightly H poor and O rich relative to glucose (H:C = 2 and O:C = 1). As ^{1}H- and ^{13}C-NMR data cannot distinguish O from N in some functional groups (e.g., C-O-O vs C-O-N), part of the "O" that is lost may be N.

Shown in Fig. 3.3 (C) is the ^{15}N-NMR spectrum of HMWDOM isolated from a depth of 15 m near Hawaii. The one major resonance in the spectrum corresponds to amide N (124 ppm; >90% of the total N). Surface water samples sometimes show a minor resonance near 24 ppm (<10% of the total N), which corresponds to amine N. Amides are commonly present in biological systems within peptide bonds. Other amide containing biochemicals that may be common in oceanic environments include N-acetyl amino sugars such as *N*-acetyl glucosamine, *N*-acetyl muramic
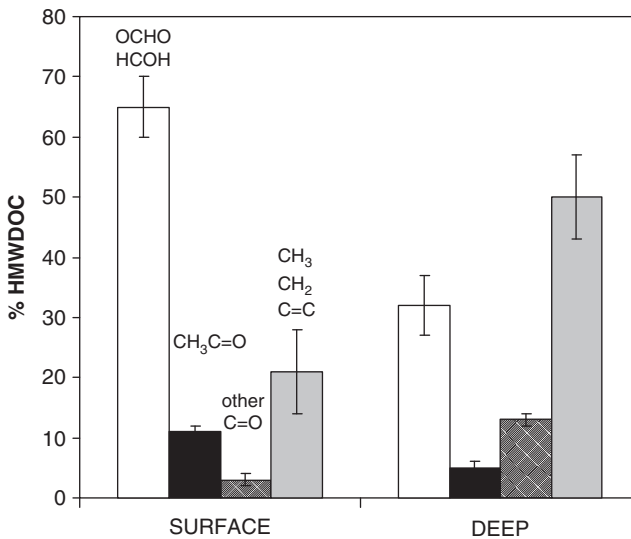
Figure 3.4 The amount of HMWDOM carbon present within various functional groups in surface and deep (1000 m) ocean waters. Functional groups are identified based on ^{1}H and ^{13}C-NMR spectroscopy (see text for details) and the quantification uses areas under each NMR resonance. The range of values (shown as "error" bars) represents direct measurements using the ^{13}C-NMR spectra and indirect calculations based on ^{1}H-NMR spectra and converting to carbon based on average C:H of identified functional groups. (Data are based on spectra in Figure 3.3 and Benner *et al.*, 1992)

acid, chitin (β (1→4)-(*poly*) *N*-acetyl D-glucosamine) and peptidoglycan (which contains a sugar backbone comprised of *N*-acetyl glucosamine and *N*-acetyl muramic acid). Based on the NMR resonance alone, we cannot assign the amide to any particular class of biochemicals. Amines are present in a variety of compounds including amino acids and amino sugars.

At 1000 m depth, the only discernible ^{15}N-NMR resonance corresponds to amide N. However, this amide peak centered near 124 ppm is much broader than the one observed for surface-ocean HMWDON and overlaps with regions in the NMR that correspond to resonances from functional groups such as pyrrole- and indole-N (130–215 ppm; Aluwihare *et al.*, 2005; McCarthy *et al.*, 1997). Results from ^{13}C- and ^{1}H-NMR spectroscopy can be used to indirectly estimate the N contributed by these compounds as they contain characteristic aromatic C and H resonances. According to this estimate, heterocyclic-N compounds represent <15% of the total-N in the deep ocean (Aluwihare and Repeta, unpublished). From the width of the amide peak in the ^{15}N-NMR spectrum, McCarthy *et al.* (1997) estimated that up to 26% of the N in subsurface waters is present in functional groups other than amide-N. The ^{1}H- and ^{13}C-NMR–based compositional analysis suggests that HMWDOM contains a variety of functional groups. However, despite the relatively "high" N content of HMWDOM, only a single type of N—amides (although potentially present in a variety of different biochemicals)—accumulates in this fraction of DOM. Therefore, certain amides (and primarily amides) are either resistant to biological degradation in the marine environment or produced throughout the water column.

As discussed above, NMR spectroscopy has alluded to the presence of many major biochemicals (carbohydrates, proteins and lipids) in HMWDOM. To determine which of these biochemicals are important and how each contributes to the observed NMR spectrum several investigators have performed molecular level analyses of HMWDOM. The discussion below will focus primarily on proteins and related compounds.

4. MOLECULAR LEVEL ANALYSES

4.1. Proteins

In living cells, proteins account for a major fraction of the cellular C and most of the N. We therefore expect that DON released from living organisms will contain proteins. In addition, the prominent amide resonance in the ^{15}N-NMR spectrum of HMWDON could be derived from peptides and therefore, it is reasonable to hypothesize that proteins dominate the DON reservoir. Direct quantification of proteins in DOM has proven to be difficult (as seen later), so to test the above hypothesis many studies have focused on identifying and quantifying total hydrolysable amino acids (THAA) in DOM. Amino acids are released from DOM following hydrolysis of peptides and proteins, and most marine DOM studies have used similar methods of hydrolysis and subsequent quantification (i.e., 6 M HCl hydrolysis with variable temperature and hydrolysis times, followed by HPLC

separation and fluorescence detection of *o*-phthaldialdehyde (OPA) derivatives). Using these techniques only 7–11% of total DON and 1–7% of total DOC can be recovered as THAA (Dittmar *et al.*, 2001; Hubberten *et al.*, 1995; Yamashita and Tanoue, 2003). In the case of HMWDOM, the amino acids quantified represent approximately 16–30% of the total N and 3–6% of the C in surface waters (Aluwihare, 1999; Aluwihare *et al.*, 2005; McCarthy *et al.*, 1996). Thus, either protein-N is not the dominant form of N accumulating in DOM, or current analytical methods underestimate the protein content of DOM. Possible analytical problems that could explain low N recoveries include interference by high salt concentrations, cross-reactions introduced by hydrolysis procedures, or, in the case of HMWDOM, adsorption of proteins to the ultrafiltration membrane. The relative distribution, D/L ratio and isotopic composition of amino acids and the presence of certain membrane proteins in DOM have provided insights into the composition of DON and its potential sources despite the low overall protein-based N yield. Below is a discussion of these studies and their various findings.

4.1.1. Amino acid composition

Early studies looked at both dissolved free and ''combined'' amino acids (DFAA and DCAA, respectively) in total DOM, where HMWDOM proteins are a subset of the DCAA fraction. Lee and Bada (1975) first reported DFAA concentrations in the range of 40–50 nM in surface Pacific waters and total hydrolysable amino acid (THAA = DFAA + DCAA) concentrations that were often 10 times higher. To date, the observed range of THAA is approximately 250–650 nM in surface waters (Dittmar *et al.*, 2001, 2004; Hubberten *et al.*, 1995; Yamashita and Tanoue, 2003). Lee and Bada (1975) observed an approximate three-fold decrease in THAA below the euphotic zone (at these depths DFAA became negligible). This trend was also seen in more recent studies where amino acid concentrations decreased to between 160 and 360 nM in mid-depth waters (Hubberten *et al.*, 1995; Yamashita and Tanoue, 2003). McCarthy *et al.* (1996) found that HMWDOM-DCAA concentrations were 178 and 278 nM in surface waters of the Sargasso Sea and North Pacific Ocean, respectively.

As discussed above DOM contains between 200 and 500 nM of DCAA (Lee and Bada, 1975, 1977) and HMWDOM contains between 178 and 278 nM DCAA (McCarthy *et al.*, 1998). Based on these values, between 30% and 100% of DCAA are in the >1000 Da fraction of DOM. Coffin (1989) found that ∼60% of all amino acids isolated from a Delaware estuary were in the <1 kDa fraction; and Sommerville and Preston (2001) showed that >90% of total DCAA (as analyzed by GC/MS) are released from peptides with MWs <3 kDa. Taken together, these studies suggest that peptides and small proteins (∼500 and 3000 Da) contain most of the amino acids detected in DOM so far. Unfortunately, precise MW information is not currently available for proteins in HMWDOM or DOM.

Amino acids identified in both total DOM (as THAA) and HMWDOM (as DCAA) include aspartic acid, glutamic acid, serine, histidine, glycine, threonine, alanine, arginine, tyrosine, valine, isoleucine, phenylalanine, leucine, and tryptophan (e.g., McCarthy *et al.*, 1996; Yamashita and Tanoue, 2003). Figure 3.5A shows the mole percent distribution of these amino acids in DOM and HMWDOM. All
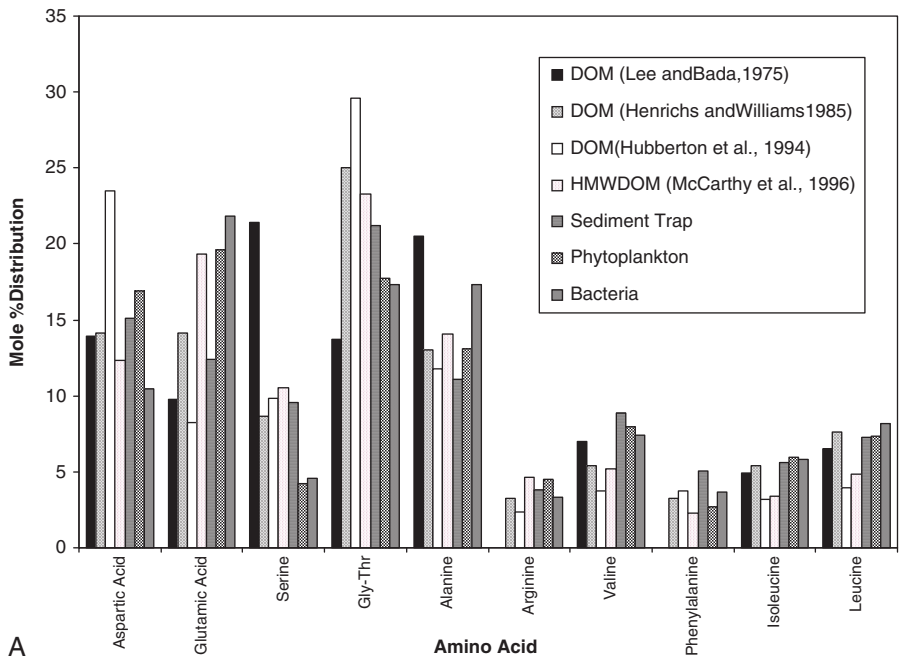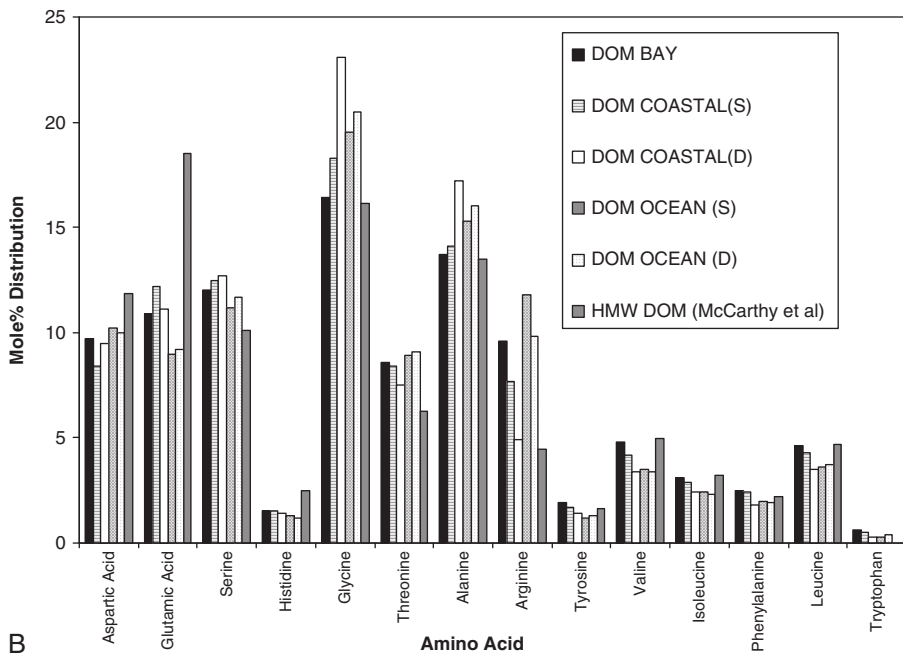
Figure 3.5 (A) The relative distribution of amino acids in total DOM (Lee and Bada, 1975; Henrichs and Williams, 1985; Hubberten *et al.*, 1994), HMWDOM (McCarthy *et al.*, 1996), phytoplankton, and bacteria (both from Cowie and Hedges, 1992). The data of Lee and Bada are a special case because not all the amino acids shown in this figure were quantified (see text for explanation). (B) The relative distribution on amino acids in total DOM, isolated from a variety of sites in the western North Pacific Ocean (data from Yamashita and Tanoue (2003). S and D represent surface and deep DOM samples, respectively. Distributions in HMWDOM are shown for comparison (McCarthy *et al.*, 1996).

DOM samples were obtained from surface waters and sampling locations include the Greenland Sea (Hubberten *et al.*, 1994), near Baja California (Henrichs and Williams, 1985), and the Eastern Equatorial Pacific (Lee and Bada, 1975). The HMWDOM sample was isolated from Station ALOHA (McCarthy *et al.*, 1996). Figure 3.5A also shows the distribution of amino acids in phytoplankton as represented by a net tow in Dabob Bay, WA (June 1982), a culture of the bacterium *Bacillus subtilis* and POM collected in a sediment trap at 60 m in Dabob Bay (July–August 1981; all data are from Cowie and Hedges, 1992). Similar acid hydrolysis conditions and liquid chromatography methods were used to determine the amino acid content of all samples shown in Fig. 3.5A. However, some differences do exist between methods, for example, Lee and Bada (1975) did not report individual concentrations for glycine, arginine, and phenylalanine, and Hubberten *et al.* (1994) omitted the analysis of threonine. Therefore, relative mole percent values shown in Fig. 3.5A for these studies are an overestimate relative to studies where the entire suite of amino acids was detected. Despite the various locations and times of sample collection and differences between methods identified above, the relative distribution of amino acids in both total DOM and HMWDOM as shown in Fig. 3.5A are similar.

Lee and Bada (1975) reported that DFAA were enriched in serine and alanine, and several studies have noted that THAA are enriched in alanine and glycine (e.g. Hubberten *et al.*, 1995; McCarthy *et al.*, 1996; Yamashita and Tanoue, 2003). In fact, glycine comprises between 20–25% of all amino acids detected in surface THAA by recent studies (Dittmar, 2004; Hubberten *et al.*, 1995; Yamashita and Tanoue, 2003). Glycine was also shown to be the most abundant amino acid in HMWDOM (McCarthy *et al.*, 1996). Therefore, most THAA–glycine is present in HMW dissolved proteins and/or ultrafiltration is isolating a representative fraction of total dissolved proteins. The major difference between HWMDOM and total DOM is the relatively high abundance of glutamic acid in HMWDOM. However, the relative abundance of this amino acid in living organisms (phytoplankton and bacteria; Fig. 3.5A) is similar to its abundance in HMWDOM.

The simple comparison of amino acid distributions in bacteria and phytoplankton reveals no obvious differences between these two possible autochthonous sources of dissolved proteins. Accordingly, the amino acid distribution in HMWDOM is similar to the distribution in phytoplankton, bacteria and sediment traps. The relationship between the amino acid distribution in total DOM and various particulate fractions is more variable. In general, it appears that amino acid distribution alone cannot identify the dominant source of dissolved proteins/peptides. However, assuming that the primary source of dissolved proteins in the ocean is bacteria and phytoplankton, these data support the interpretation that hydrolysable proteins in DOM and HMWDOM maintain a source-like amino acid distribution.

Several studies have suggested that the extent of organic matter degradation may be imprinted on the relative distribution of amino acids. For example, the depth-dependant relative enrichment of certain amino acids in POM has been interpreted as enhanced preservation of particular proteins. Hecky *et al.* (1973) showed that diatom cell wall proteins were relatively enriched in serine and glycine; Cowie and Hedges (1992) found that these amino acids were relatively unreactive in sinking POM and concluded that the observed pattern represented selective preservation of diatom cell wall material within POM. Glycine is enhanced in HMWDOM relative
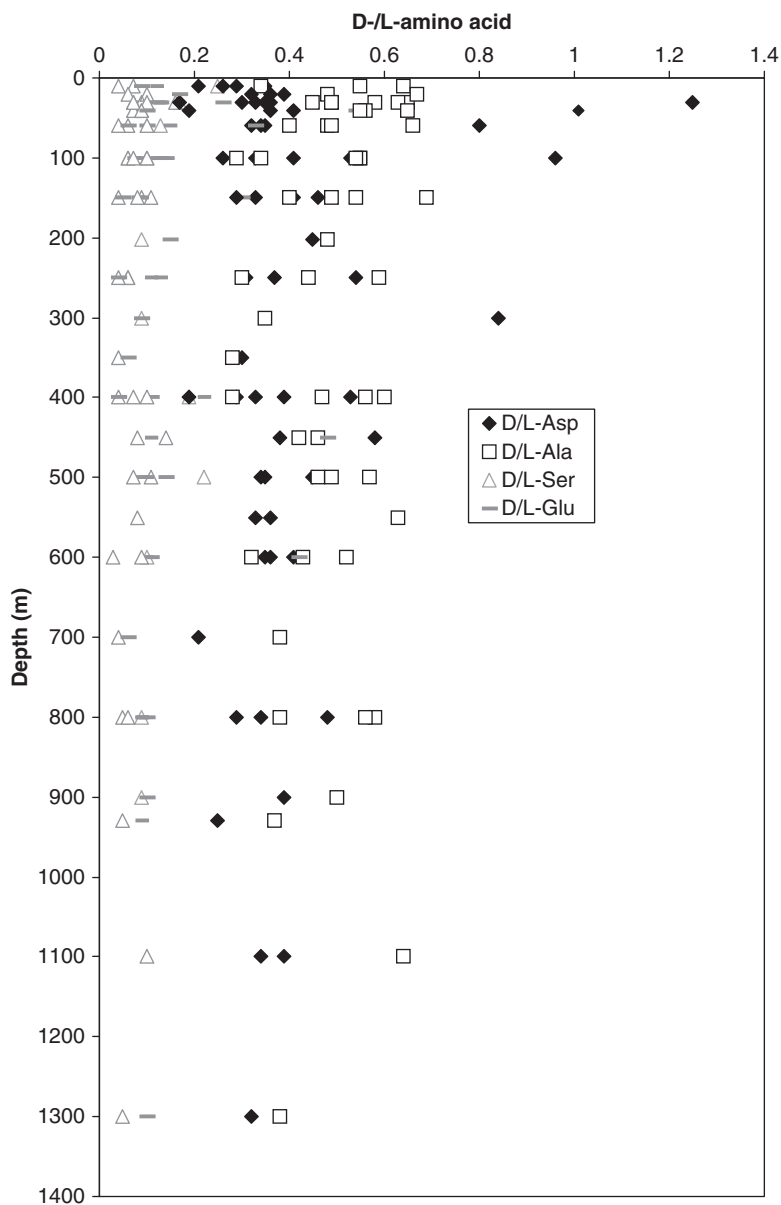
Figure 3.6 The spatial distribution of the D/L–aspartic acid (Asp), –glutamic acid (Glu), –alanine (Ala), and –serine (Ser). Adapted from Perez *et al.* (2003).

to POM, and both DOM and HWMDOM contain relatively more serine than phytoplankton and bacteria (Fig. 3.6A).

Yamashita and Tanoue (2003) examined vertical and horizontal variations in the amino acid composition of DOM (Fig. 3.5B) along a coastal to oceanic transect and found that the concentration of valine, isoleucine, phenyalanine and leucine varied together. Based on the spatial distribution of these amino acids (along the transect)

the authors concluded that these amino acids were present in bioavailable DOM. Alanine and glycine also varied together but their spatial distribution was better explained by their presence in diagenetically altered DOM. However Yamashita and Tanoue (2003) were unable to discern strong patterns of variability in just their open ocean samples (for example, surface (S) and deep (D) ocean amino acid distributions were similar; Fig 3.5B), which they attributed to the already diagenetically altered nature of open ocean DOM.

In the Arctic and Antarctic Ocean amino acids were also found in humic substances isolated from DOM by XAD-2 resins (Hubberten et al., 1995). The concentration of THAA in humic substances was between 233–246 nM, with all hydrolysable amino acids in the deep ocean and ∼60% of amino acids in the surface ocean residing in this fraction. Glycine was by far the most abundant amino acid detected in the humic fraction. These authors concluded that amino acids in the XAD-2 extracts represent a "refractory protein" background that is present throughout the ocean. The dominance of this "refractory protein" background in the surface and deep ocean could explain the relatively stable amino acid distribution observed by Yamashita and Tanoue (2003) at their open ocean sites.

Studies examining the amino acid distribution in DOM and HMWDOM have revealed some consistent trends. The general similarity of amino acid distributions across molecular size classes suggests that proteins isolated as HMWDOM are representative of total dissolved proteins; this similarity has also been used to suggest continuity in peptide subunits (Dittmar et al., 2001). The observed trends may also be consistently imparted by the nearly identical hydrolysis procedures used to analyze the composition of various DOM fractions. However, small differences, such as the relative enrichment of glutamic acid in HMWDOM, are noticeable and may be indicative of the relative "freshness" of this fraction. The latter conclusion is consistent with the strong correlation between amino acid distributions in HMWDOM and organisms (Fig 3.5A). In addition, biodegradation (Amon and Benner, 1996) and radiocarbon (Aluwihare et al., 1999) studies (see Section V) do suggest that total HMWDOM is enriched in a more labile, recently produced component. Several studies have also concluded that relative increases in the abundance of glycine and alanine reflect diagenetically altered DOM (Amon et al., 2001; Dauwe et al., 1999; Dittmar et al., 2001; Yamashita and Tanoue, 2003). It is important to note however, that amino acid composition and concentration studies target the protein fraction alone and cannot provide conclusive information about processes affecting total DOM; particularly since amino acids represent only a small fraction of the DOM reservoir.

4.1.2. The enantiomeric signature of amino acids

One potentially powerful indicator of source and diagenetic state is the D/L-amino acid ratio within DOM. Although proteins in nature contain primarily the L-enantiomer of amino acids, certain compounds, many of bacterial origin, do contain D-amino acids. Proteins do not contain D-amino acids but peptidyl compounds such as peptidoglycan (a structural component of bacterial cell walls; Osborn, 1969), peptide antibiotics (Bodanszky and Perlman, 1969) and peptide

siderophores (Martinez *et al.*, 2000) have all been shown to contain both the D and L enantiomer.

In 1977, Lee and Bada first reported the presence of D-amino acids in total DOM. They detected the D enantiomer of aspartic acid, glutamic acid, alanine, valine, isoleucine and leucine. This early study also observed both depth and site-related variations in the D/L-amino acid ratio within DOM. For example, the D/L-aspartic acid ratio was 0.14 in surface waters of the central North Pacific and 0.36 in the surface Sargasso Sea; and the D/L ratio of aspartic acid and glutamic acid decreased from surface to 4000 m depth in the central North Pacific but no such vertical trend was apparent in the Sargasso Sea. Recent studies in the North Atlantic by Perez *et al.* (2003) and Dittmar *et al.* (2001) also observed spatial (vertical and horizontal) variations in dissolved D/L-amino acid ratios, but no clear vertical trend was reported. Data from Perez *et al.* (2003) are shown in Fig. 3.6 and average D/L ratios in these samples were 0.49, 0.42, 0.15, and 0.09 for alanine, aspartic acid, glutamic acid and serine, respectively. McCarthy *et al.* (1998) reported the presence of D-amino acids in HMWDOM from the central Pacific; at this site alanine, aspartic acid, glutamic acid and serine had the highest D/L ratios (average values of 0.50, 0.37, 0.20, and 0.2, respectively). Serine was the only amino acid in HMWDOM that exhibited any vertical variation in its D/L ratio. The hierarchy of amino acid-specific D/L ratios and the magnitude of each ratio in HMWDOM are similar to those values reported for DOM above and confirm that HMWDOM is representative of total DOM.

Based on D/L–amino acid ratios and the dominant amide signal in the ^{15}N–NMR spectrum of HMWDOM McCarthy *et al.* (1997, 1998) argued that N in HMWDOM is contained primarily in peptidoglycan. D/L-amino acid ratios provide the strongest evidence in support of this conclusion as peptidoglycan commonly contains D-alanine and other D-amino acids such as D-glutamic acid and D-aspartic acid. The ^{15}N–NMR spectrum supports a strong amide contribution to HMWDON, but *protein*-N recoveries from HMWDON are low. As a result, McCarthy *et al.*, (1998) argued that amides must be present in the form of acetylated amino sugars, an essential component of peptidoglycan (Fig. 3.7). These interpretations led McCarthy *et al.* (1998) to conclude that D-amino acids and amides in HMWDOM are directly added from a bacterial source.

Alternatively, over very long time periods, L-amino acids can racemize to produce D-amino acids. However, measured D/L ratios for certain amino acids cannot be achieved based on known racemization rates of amino acids in seawater. For example, Lee and Bada (1977) calculated D/L ratios of 0.01 and 0.004 for aspartic acid and alanine, respectively, assuming an oceanic residence time of 3,400 years for these amino acids. These calculated values are much lower than the measured values and led Lee and Bada (1977) to conclude that the enhanced D-amino acid concentrations in marine DOM must be derived from a bacterial source. In a later paper, Bada *et al.* (1982) suggested that the near–racemic mixture (50% each of the D and L enantiomer) of alanine at depth in the ocean was a result of the dehydration of serine or threonine to produce racemic alanine. These authors also detected near racemic α–amino-*n*-butyric acid (ABA), which can be produced from the dehydration of threonine. This mechanism of D-alanine formation
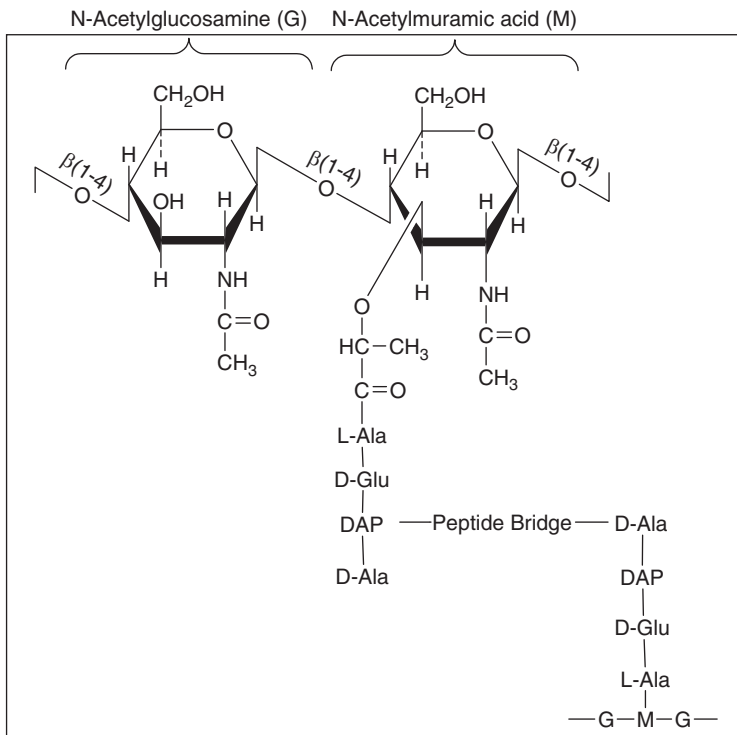
Figure 3.7 Model structure of peptidoglycan in gram–negative Bacteria. *N*-Acetylglucosamine (G) and *N*-Acetylmuramic acid (M) form the backbone, and the *N*-Acetylmuramic acid molecules are cross-linked with amino acids. There is some variation in amino acid composition of the peptides between organisms. For example, the DAP residue of the tetrapeptide is replaced by a lysine residue in gram–positive Bacteria. Any of the peptides of the tetrapeptide chain may occur in the peptide bridge along with glycine, threonine, serine, and aspartic acid. Figure is modeled after Madigan *et al.* (2000).

requires a vertical decrease in the concentration of serine and an increase in the D/L–alanine ratio, which was observed by Lee and Bada (1975). However, neither McCarthy *et al.* (1996) nor Perez *et al.* (2003) observed this trend in HMWDOM or DOM. In addition, McCarthy *et al.* (1998) found no ABA in their HMWDOM samples. In summary, recent studies examining D/L–amino acids in both HMWDOM and DOM conclude that D-alanine in HWMDOM is derived directly from bacterial production and not from post-production modification (through dehydration reactions for example) of peptides in the water column.

A study conducted in deep waters of the North Atlantic found no preferential microbial utilization of L– or D–amino acids during uptake experiments (Perez *et al.*, 2003); yet, the presence of D–amino acids may retard the *hydrolysis* rate of peptides. But D/L–amino acid ratios in DOM and HWMDOM rarely exceed 1 even in the deep ocean (some surface ocean samples analyzed by Perez *et al.* (2003) did show values >1 for D/L–aspartic acid). The ratio could approach or exceed 1 if peptides containing D–amino acids (e.g., peptidoglycan, which contains only a few amino acids) were preferentially preserved with depth. Instead, near constant D/L ratios

with depth and ratios below 1 for a variety of amino acids imply either no removal of the protein/peptide fraction of DOM with depth, or that a common source of the protein/peptide fraction exists throughout the water column. Either of these conclusions is also consistent with the near constant relative distribution of amino acids in DOM.

Relatively stable D/L ratios for diverse samples may also result from racemization during sample hydrolysis and processing. For example, in a comprehensive study Kaiser and Benner (2005) noted that D–amino acids in HMWDOM are overestimated following acid hydrolysis. However, based on the magnitude of the racemization blank and observed patterns within marine samples these authors concluded that the overestimation is negligible compared to naturally occurring concentrations of some D–amino acids (Dittmar et al., 2001; Kaiser and Benner, 2005; McCarthy et al., 1998). However, the distinct matrix of marine samples may introduce additional cross-reactions that have not been quantified. For example, one study noted a positive correlation between D/L ratios and salinity (Dittmar et al., 2001).

Rogers (1983) defined a relationship where the total peptidoglycan-N content of a sample could be estimated based on its D-Ala content (peptidoglycan-N = $5.7 \times$ D-Ala-N). Based on this relationship it was estimated that peptidoglycan contributed $7 \pm 2\%$ of THAA-N in Arctic rivers, $53 \pm 11\%$ of THAA-N in the deep waters of the Arctic Ocean (Dittmar et al., 2001), and 20–25% of the THAA-N (and 2.5% of the total DON) in North Atlantic surface waters (Perez et al., 2003). McCarthy et al. (1998) calculated a peptidoglycan-N content of 4.5–16% in HMWDON. However, intact peptidoglycans have not been isolated from any fraction of DOM, and D-amino acids identified in DOM could be present in other peptides.

4.1.3. Isotopic composition of amino acids

The stable C isotopic (δ^{13}C) signature of organic compounds is commonly employed for source apportionment, but few studies have applied this strategy for specific compounds within DOM. Recently, in an effort to identify specific HMWDOM sources McCarthy et al. (2004) compared the δ^{13}C signature of individual amino acids in HMWDOM to those amino acids isolated from a variety of autotrophic and heterotrophic organisms. Based on patterns of amino acid–specific δ^{13}C values these authors concluded that amino acids in HMWDOM were derived from an autotrophic source. However, this study focused on a limited sample set and considerable variations in δ^{13}C signature of amino acids was apparent.

Amino acid-specific δ^{13}C signatures reported by McCarthy et al. (2004) may also serve as a degradation index for certain amino acids in HMWDOM. To examine this possibility the δ^{13}C signature of individual amino acids in both HMWDOM and sinking POM collected at 105 m were compared (Fig. 3.8A, data from McCarthy et al., 2004). It is assumed here that POM represents relatively "fresh" organic matter. Relative to sinking POM the most ^{13}C-depleted amino acid in HMWDOM was glycine. This complements findings from other studies which have suggested that the relative abundance of glycine reflects the extent to which DOM has been degraded. Depth profiles of the distribution of ^{13}C in amino acids revealed additional trends (Fig. 3.8B). For example glycine, lysine, leucine and
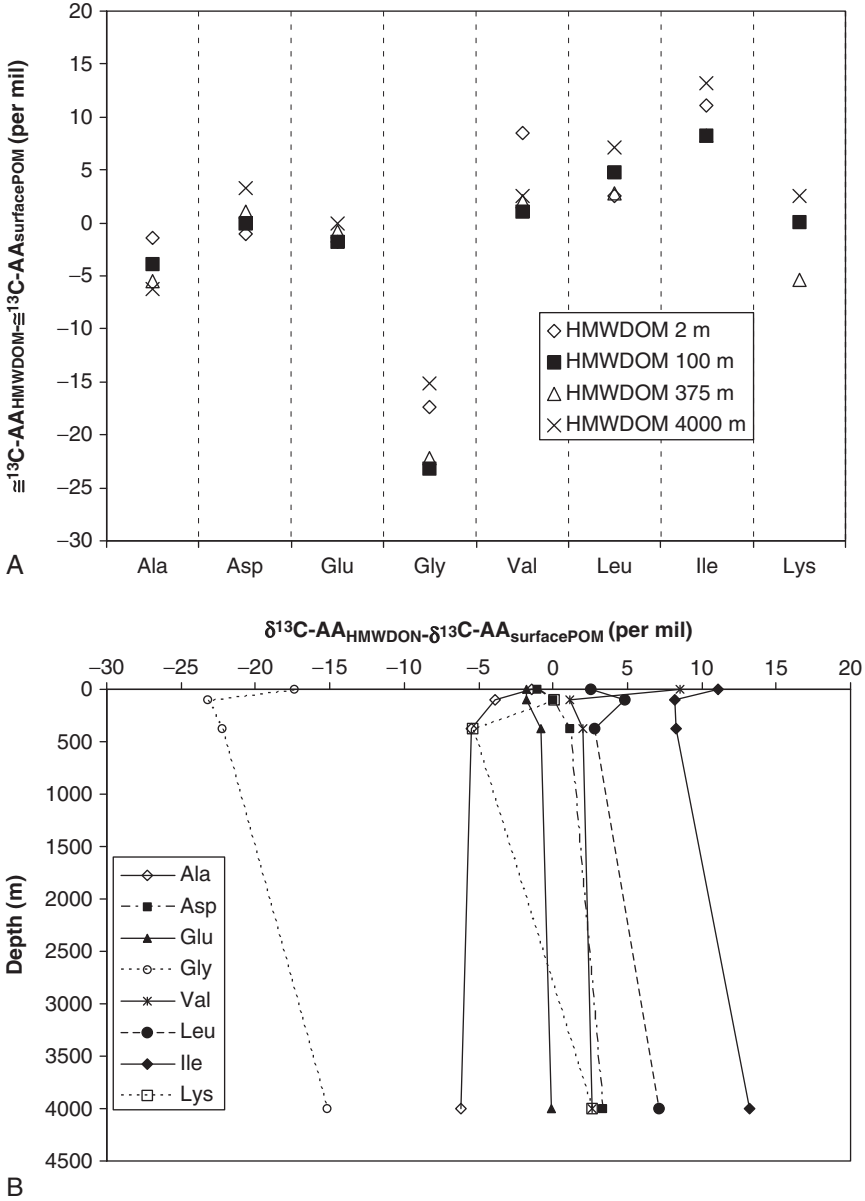
Figure 3.8 (A) Deviations in $\delta^{13}C$ values (‰) for amino acids in HMWDOM from amino acids in sinking POM ($\delta^{13}C\text{-AA}_{HMWDOM}-\delta^{13}C\text{-AA}_{POM}$) isolated from several depths in the central North Pacific Ocean (adapted from McCarthy *et al.*, 2004; only surface POM data from 2°N are used here). Note that we assumed a value of -23.9 for Ile *not* 23.9 (as shown in Table 3.1 of that paper). (B) Depth profiles of $\delta^{13}C_{HMWDOM}-\delta13C_{POM}$ for various amino acids.

isoleucine isolated from HMWDOM showed ^{13}C-enrichments below 500 m, but alanine did not. Also δ^{13}C signatures of all amino acids changed between the surface and 100 m—most values decreased over this depth range. The observed vertical variation in amino acid–specific δ^{13}C signatures may be explained by (1) unique amino acid–sources at different depths, (2) a similar biological source using a C source whose δ^{13}C composition is changing with depth or (3) amino acid–specific degradation/transformation processes. In general, the main conclusion provided by Fig. 3.8B is that all dissolved proteins/peptides do not share the same production and transformation history.

McCarthy *et al*. (2004) also measured the δ^{13}C signature of D–Ala isolated from HMWDOM. Within the error of their measurement, D and L enantiomers were found to have identical δ^{13}C signatures in surface waters and at 375 m depth in the central Pacific; based on these data the authors argued that both D- and L–Ala are derived from the same source. Using their early conclusion that amino acid–specific δ^{13}C patterns within HMWDOM resembled an autotrophic signature (see previous section), McCarthy *et al*. (2004) further inferred that alanine (both D and L) in HMWDOM is derived from an autotrophic source. Furthermore, as D–amino acids (including D–Ala) are commonly derived from bacteria the authors concluded that all of the amino acids preserved in HWMDOM are derived from autotrophic bacteria. However, it is not known whether D–Ala and L–Ala in bacteria have the same δ^{13}C signature (i.e., no fractionation takes place during racemization). Second, only a few of the amino acids found in HMWDOM are likely to be present in peptidoglycan (<50% of the THAA-N), and so, the total amino acid–specific δ^{13}C pattern does not argue for an autotrophic source of peptidoglycan (if present in HMWDOM). Finally, identical δ^{13}C signatures of D- and L–alanine do not require that all amino acids in HMWDOM are derived from bacteria. Ascribing source information based on δ^{13}C signatures alone is difficult in the current context as these values reflect only an average for each amino acid and each amino acid is likely to be found in many different dissolved proteins/peptides. Therefore, more work in this area is needed before the source of HMWDOM can be deduced from δ^{13}C signatures alone. Recently, McCarthy *et al*. (2007) have provided δ^{15}N isotopic signatures for amino acids in HMWDOM. Because the ^{15}N isotope composition of specific amino acids has been shown to either remain unaltered, or exhibit identifiable fractionation effects during N trophic transfer (Montoya *et al.,* 2002), this compound specific analysis has the potential to identify both amino acid source and degree of heterotrophic processing. McCarthy *et al*. (2007) found no statistically significant variation in δ^{15}N patterns of HMWDOM with depth. They also could not identify distinct source δ^{15}N patterns in HMWDOM. Thus, the transformation of marine DON evident in DON concentration profiles does not appear to be captured by the nitrogen isotope signature of HMW amino acids. This finding is consistent with the small contribution of hydrolyzable proteins to total DON. Again, this isotopic study was limited to a few HMWDOM samples (n = 4) and should be developed further by 1) expanding sample sets of HMWDOM, autotrophic and heterotrophic plankton cultures, and environmental, size fractionated POM, and 2) examining dual stable C and N isotopic composition of amino acids important in identifying trophic N transfer.

4.1.4. Isolation of proteins from HMWDOM

The analyses described above have provided valuable insights and raised interesting questions about the cycling of DOM. However, they only focus on amino acids that are released from dissolved proteins by acid or base hydrolysis. It is imperative that the field now focus its efforts toward separating and isolating intact dissolved molecules for chemical characterization. Recently, efforts have been made by Eiichiro Tanoue's group for example, to isolate, sequence, and identify intact dissolved, marine proteins isolated from HWMDOM.

Tanoue's group (e.g., Tanoue, 1995) reports results for HMWDOM isolated from seawater following pre-filtration through GF/F filters (Whatman; 0.7 μm pore size) and concentration by tangential flow ultrafiltration using a MW cutoff of 10 000 Da. A direct contribution to ''HMWDOM'' (10 kDa–0.7 μm) from microbial cells cannot be ruled out on the basis of the pre-filtration step used in the isolation of HMWDOM. In all cases, 0.01% (w/v) SDS and in some cases NaN_3 (5 mM) was added to the seawater before ultrafiltration. Both of these compounds may have enhanced the dissolution of cell-surface and intracellular proteins of organisms passing through the GF/F filter; in fact SDS is often used to lyse cells because it can effectively dissolve the phospholipid and protein components of cell membranes.

Tanoue et al. (1995) and Tanoue (1995) first reported the isolation and characterization of a 48 kDa protein isolated from HMWDOM as above. Since these initial studies, several papers from the same group have identified the presence of this and other proteins (e.g., 34, 37, and 39 kDa proteins) at many sites and depths in the Pacific, Indian and Antarctic Oceans (Suzuki et al., 1997; Tanoue, 1995; Tanoue et al., 1996; Yamada and Tanoue, 2003). The N–terminal amino acid sequence of the 48 kDa protein isolated from a depth of 45 m in the western North Pacific Ocean was GTVTTDGADIVIKT, a sequence that resembles bacterial porin P of the gram-negative bacterium Pseudomonas aeruginosa (Tanoue et al., 1995). Based on the homology between the 48 kDa protein and porin P, Suzuki et al. (1997) developed a fluorescence based immunochemical assay to detect these proteins in seawater. Natural populations of bacteria throughout the water column stained positive for this assay indicating that the 48 kDa protein is derived from bacteria and has a ''fresh'' source at all depths. The abundance of positively stained bacteria was approximately $10^{5.5}$ cells/L in surface waters; for comparison the total abundance of microorganisms in surface waters is approximately 10^9 cells/L. Yamada and Tanoue (2003) showed that a 40 kDa dissolved protein was also derived from a bacterial source based on homology to an outer membrane protein (OmpA) of Acinetobacter spp. Intact HMW proteins identified to date appear to be derived primarily from a bacterial source; in some cases these proteins have an in situ source throughout the water column.

Are the proteins identified in the previous section selectively preserved in DOM? In general, porins are expected to be resistant to bacterial degradation (Powell et al. (2005) and reference therein), and modification of these proteins, by saccharide moieties for example, may further retard their bacterial degradation. For example, in a laboratory experiment Keil and Kirchman (1993) noted longer turnover times for glycosylated ribulose 1,5-bisphosphate carboxylase (RuBPCase) when compared with unmodified RuBPCase. In accordance with this report Suzuki et al. (1997) suggested that the glycosylation of porins could help to explain their enhanced preservation/accumulation in seawater. Subsequently Yamada and Tanoue (2003)

showed, using a periodate oxidation method to oxidize these dissolved proteins, that the 48 kDa protein and a 34 and 39 kDa protein were indeed glycosylated. Based on the experimental conditions (i.e., strong or weak oxidation) these authors were able to distinguish sialic acids (a saccharide) and other saccharides in the 48, 34, and 39 kDa proteins. Sialic acids are primarily present in animals of the deuterostome lineage and are rare in bacteria and even plants, where their presence is still controversial (Varki, 2004). In fact, the few sialic oligosaccharides detected in bacteria to date have been found in pathogenic bacteria where it is hypothesized that genes for synthesis may have been acquired by gene transfer from a host eukaryote (Varki, 2004). The suggestion that sialic acids may be present in DOM is intriguing. In pathogenic bacteria, when sialic acids are present, they occur primarily on the outer membrane of the cell, consistent with the detection of porin membrane proteins in HMWDOM.

Based on the results discussed in this section it appears that bacterial membrane components, particularly glycosylated proteins, may be selectively preserved in DOM. However, based on the filtration method discussed above we cannot rule out the possibility that these membrane components represent contamination by living organisms where extracellular proteins were most easily released during the analysis. Also, most of the proteins that have been identified by Tanoue and co-workers are closely related to proteins of pathogenic bacteria. The link to pathogenic bacteria is interesting but could have several different explanations. For example, pathogens are the best studied bacteria and are well-represented in the protein data base; therefore, when any new protein sequence is entered into the data base and searched against known similar proteins, there is a high probability that proteins from pathogens will be recovered (Yamada and Tanoue, 2003).

Recently, other investigators have also successfully used SDS–polyacrylamide gel electrophoresis (PAGE) to isolate dissolved proteins. For example, Jones *et al.* (2004), working in the everglades, reported the presence of a 48 kDa protein (along with several other proteins) in one of their marine DOM samples. A recent study by Powell *et al.* (2005) identified ≤25 dissolved proteins (as gel electrophoresis bands) with MWs >25 kDa. To identify these proteins Powell *et al.* (2005) applied a liquid chromatography–mass spectrometry method to characterize peptide fragments produced from the enzymatic degradation of intact dissolved proteins separated from DOM by SDS–PAGE. Mass spectra of the peptide fragments were compared to theoretical mass spectra of known proteins in NCBI's databases to identify which protein families were present in DOM. Using this technique, Powell *et al.* (2005) detected some membrane/envelope proteins that appeared to be primarily of bacterial origin. Although porin P as identified by Tanoue and coworkers was not detected, the predominantly bacterial origin of identified proteins is consistent with the finding of Tanoue *et al.* (1996). Proteins in the database with similar amino acid sequences included long chain fatty acid acyl CoA synthetase, a component of anthranilate synthase, RuBisCo and a luminal binding protein (the latter is a bacterial outer membrane protein). Powell *et al.* (2005) added 0.01% SDS to seawater before pre-filtration and ultrafiltration (as did Tanoue and coworkers) in at attempt to keep dissolved proteins in solution during ultrafiltration The effect of SDS on dissolving proteins that were originally attached to living or non-living particles is not discussed and could explain the presence of previously undetected proteins.

The analytical approach applied in the Powell *et al.* (2005) study represents a major advancement in our current study of the chemical structure of DOM. SDS-PAGE remains one of the few methods that will allow separation and purification of intact dissolved proteins; proteomics, as applied by Powell *et al.* (2005), is now routinely applied in the biochemical and biomedical fields but is rarely applied in the environmental sciences. A major advantage of these mass spectrometry based techniques (i.e., proteomics) is the relatively small quantity of material required for the analysis; this opens up the possibility for analyzing peptides and proteins in total DOM with little or no pre-concentration. However, the presence of salts still needs to be minimized before effective mass spectra can be generated. A recent review by Mopper *et al.* (2007) highlights the application of high-resolution analytical techniques to study marine DOM composition, and we refer the reader to this review for a more comprehensive discussion of recent analytical advances.

Proteins identified in DOM contribute only a small fraction of N to total DON. Therefore, future studies should also seek to improve our ability to recover and quantify marine, dissolved proteins. For example, Section IIIc discussed the abundance of amide-N resonances in the ^{15}N-NMR spectrum of HMWDON; proteins are a likely source of these resonances but current inventories based on amino acid analyses and protein mass spectrometry are too low. Alternatively, protein-N may indeed be rare in HMWDOM and other molecule could be responsible for the amide-N identified in HMWDON. The next section focuses on polysaccharides as some of these biochemicals can contain amide functional groups.

4.2. Polysaccharides

4.2.1. Monosaccharide composition

Carbohydrates comprise a large percentage (20–40%) of the dry weight of phytoplankton and bacterial cells where they often play a role in structural integrity or energy storage. Accordingly, dissolved carbohydrates have been detected in the media of several laboratory phytoplankton cultures and in the field. In general, a major fraction of total identifiable C in both sinking POM and total DOM is comprised of carbohydrates suggesting that this biochemical in abundant in a variety of organic phases (Aluwihare *et al.*, 1997, 2002; Biddanda and Benner, 1997; Cowie and Hedges, 1984; Hamanaka *et al.*, 2002; Mopper, 1977; Tanoue and Handa, 1987; Wakeham *et al.*, 1997). Although ^{1}H- and ^{13}C-NMR spectra suggest that carbohydrates dominate HMWDOM, only 10–20% of C is recoverable as monosaccharides following the acid hydrolysis of HMWDOM. Despite the application of a variety of acid hydrolysis conditions and detection techniques, the same novel distribution of seven neutral monosaccharides has been described in HMWDOM – four hexoses galactose, glucose, mannose and xylose; two deoxysugars rhamnose and fucose; and the pentose arabinose (e.g. Aluwihare *et al.*, 1997; Borch and Kirchman, 1997; McCarthy *et al.*, 1996; Skoog and Benner, 1997). The yield of neutral monosaccharides decreases with depth in the ocean, but the relative abundance of individual monosaccharides is nearly the same irrespective of sample location, depth, or time of collection.

Of particular relevance to this discussion is the detection of amino sugars in DOM. The presence of amino sugars in HMWDOM was first quantified by

GC-MS and shown to be a minor component of hydrolyzed HMWDOM (Aluwihare et al., 1997, 2002). Boon et al. (1998), using direct temperature resolved mass spectrometry (DT-MS) following chemical (NH$_3$) ionization, confirmed the presence of N-acetyl amino sugars in HMWDOM but were unable to quantify their contribution to HMWDOC and HMWDON. In 2000, Kaiser and Benner developed an HPLC method to quantify amino sugars (N-acetyl glucosamine, N-acetyl galactosamine and muramic acid) following HCl hydrolysis of total DOM. Using this method Benner and Kaiser (2003) reported that 2–3% of total C and 2–7% of total N can be recovered from HWMDOM as amino sugars. Based on monosaccharide recoveries they were able to estimate that amino sugars contributed 22.9 ± 4.6% of all carbohydrate-C in HMWDOM. The two most abundant amino sugars detected in HMWDOM are N-acetyl glucosamine and N-acetyl galactosamine (Aluwihare et al., 2002; Benner and Kaiser, 2003; Kaiser and Benner, 2000) and their surface water concentrations are approximately 17–56 nmol/L and 10–36 nmol/L, respectively. Deep water concentrations are 2.3–5.8 nmol/L. Benner and Kaiser (2003) also detected muramic acid, the acetylated amino sugar unique to bacterial cell wall polymers, in HMWDOM; the concentration of this N-species in the surface ocean is reported to be in the range 0.12–0.78 nmol/L. The ratio of N-acetyl glucosamine to N-acetyl galactosamine in HMWDOM is between 1 and 2, and is fairly stable throughout the water column. This relatively constant composition is reminiscent of the near-constant ratio of various neutral monosaccharides in HMWDOM as discussed above. Additionally, both amino sugars and neutral monosaccharides exhibit a marked depth-dependent decrease in their contribution to total HMWDOC (and HWMDON). These lines of evidence could be indicative of a surface contribution to HMWDOM from a family of reactive polymers that contains all of these monosaccharides.

The presence of muramic acid in HMWDOM suggests that intact or degraded fragments of the polysaccharide backbone of peptidoglycan (alternating residues of N-acetyl glucosamine and muramic acid) are present in HMWDOM, consistent with the conclusion of McCarthy et al. (1997, 1998). However, the accumulation of intact peptidoglycan in HMWDOM has yet to be demonstrated and the data of Benner and Kaiser (2003) and Aluwihare et al. (2005) confirm that most N-acetyl amino sugars present in HMWDOM are not associated with peptidoglycan.

4.3. Molecular reconstruction of nitrogen in HMWDOM

The amino acid and amino sugar recoveries discussed above account for only a small fraction of the total C and N in HMWDOM. Either these biochemicals are scarce in HMWDOM, or strong acid hydrolysis conditions that are typically used in the analysis of dissolved proteins and polysaccharides result in a low yield of individual monosaccharides or amino acids. Aluwihare et al. (2005) developed a method based on mild hydrolysis of HMWDOM coupled with ^{15}N-NMR spectroscopy to identify the chemical environment surrounding amide bonds. Given the prominence of these bonds in DON (Fig. 3.3), these authors were particularly interested in quantifying any contribution to amide N from N-acetyl amino sugars. Mild acid hydrolysis of acetylated amino sugars yields acetic acid without depolymerizing the

molecule. That is, for each molecule of acetic acid that is generated, a mole of amide–N is converted to an amine, but the glycosidic bonds remain intact. In the case of proteins, mild acid hydrolysis is expected to leave the polymer untouched, in which case the N of the peptide bond will remain in the amide form. If hydrolysis of the peptide bonds does occur, then amino acids will be released and so this depolymerization of the protein will convert amide-N to amine-N. In either case, the products of the hydrolysis (acetic acid and amino acid) can be directly quantified, while the conversion of amide to amine can be monitored and quantified by ^{15}N–NMR spectroscopy. A simple schematic of this hydrolysis is shown in Fig. 3.9 for a protein/peptide and N-acetyl glucosamine.

Mild acid hydrolysis affected the chemical environment surrounding amide-N in both surface and deep HMWDOM. These changes were recorded by ^{15}N-NMR spectroscopy as shown in Fig. 3.10 (after Aluwihare *et al.*, 2005). Based on NMR quantification and molecular level analyses of acetic acid and amino acids released during the hydrolysis, Aluwihare *et al.* (2005) reported that half ($\sim$45%) of the N in surface HMWDOM was present in acetylated amide groups such as those found in N-acetyl amino sugars. Of the remaining N, approximately 20% was recovered as amino acids (following additional acid hydrolysis at 6 M HCl) but the remaining
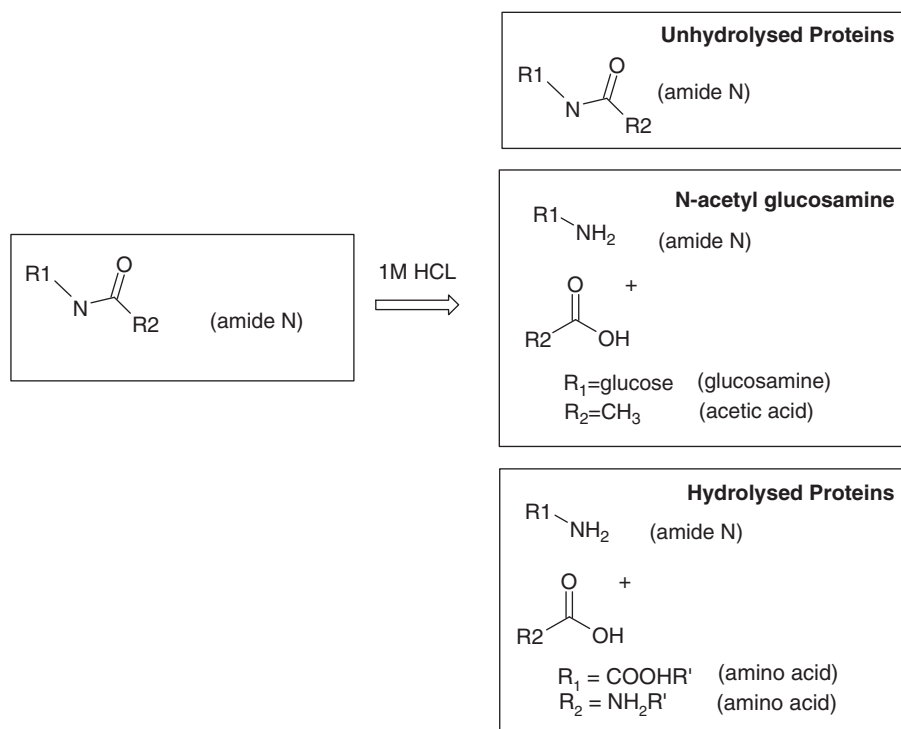
Figure 3.9 A simplified scheme for the mild acid (1 M HCl) hydrolysis of an amide containing molecule. Products of the hydrolysis for proteins and *N*-acetyl glucosamine are shown. After Aluwihare *et al.* (2005).
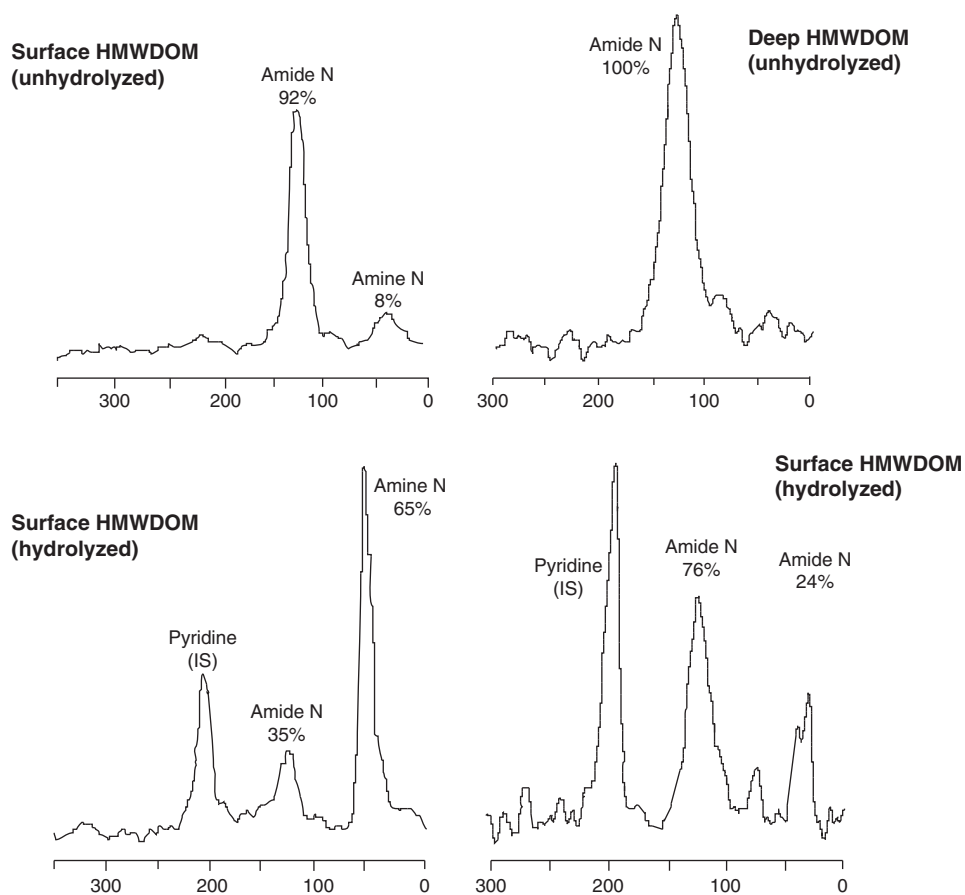
Figure 3.10 ^{15}N-NMR spectra of HMWDOM from surface and deep waters before and after mild acid (1 M HCl) hydrolysis (after Aluwihare *et al.*, 2005). Pyridine is used as an internal standard (IS) to quantify N recoveries.

amide N was not transformed by acid hydrolysis; this is consistent with previous findings for the amino acid content of HMWDOM (e.g. McCarthy *et al.*, 1996, 1998). Aluwihare *et al.* (2005) also reported that the unhydrolyzable amide-N dominated the HWMDON inventory in the deep ocean (Fig. 3.10); only ~30% of the amide-N in the deep ocean was hydrolyzable (detected as either amino acids (~15% of N) or inferred to be present in *N*-acetyl amino polysaccharides). As mentioned previously in Section 3.3, aromatic N compounds may also be present in the deep ocean but cannot be quantified by ^{15}N-NMR spectroscopy. The abundance of acetylated amides (amino sugars) in the surface ocean was surprising; however, more intriguing is the fact that ~30% of the amide N is surface waters and ~70% of the N is deep waters cannot be transformed by (strong) acid or base hydrolysis (Fig. 3.11). Low recoveries of *N*-acetyl amino sugars following hydrolysis of HMWDOM imply that these polysaccharides are resistant to hydrolysis. Amino
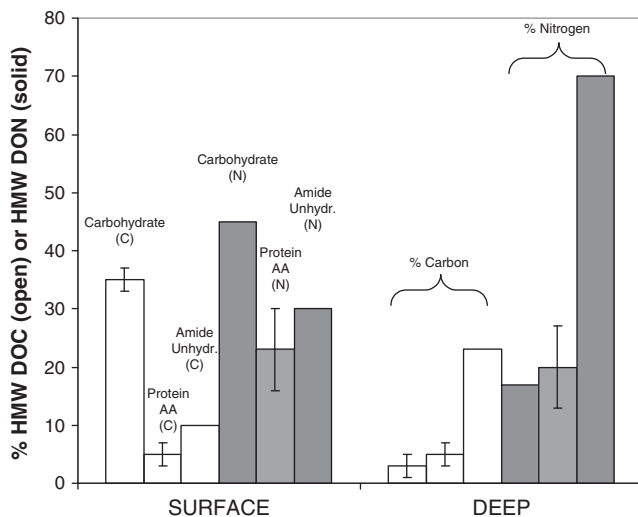
Figure 3.11 Amounts of carbon or nitrogen present as various biochemicals in HMWDOM isolated from the surface and deep (1000 m; open symbols) ocean. Carbon percentages in each sample are shown as open bars (and (C) notation); nitrogen percentages are shown as solid bars (and (N) notation). The range of calculated values is noted by the "error" bars. Biochemicals are assigned based on the functional groups identified by ^{1}H, ^{13}C, and ^{15}N-NMR spectroscopy and molecular level analyses. Carbohydrate C is calculated based on monosaccharide recoveries and amino sugar C estimated by ^{15}N-NMR (see text). Carbohydrate N and unydrolyzable amide N and C (assuming a C:N ratio of 4) is calculated from ^{15}N-NMR spectroscopy (see text). Amino acid recoveries (noted Protein AA in the figure) are based on molecular level analyses. Data are taken from Aluwihare *et al.* (2002, 2005) and McCarthy *et al.* (1998).

sugars may be present in a homopolymer, or in a homogeneous region of a hetero–polymer, that is not completely depolymerized; alternatively, Maillard type cross–reactions as shown in Fig. 3.12 may render them unhydrolyzable. Other explanations for low recoveries include the possibility that amino sugars are destroyed during hydrolysis and that amide-N is present within other unidentified molecules.

Given the general structure of *N*-acetyl glucosamine and the quantity of acetic acid recovered following acid hydrolysis, an estimated 20% of C in surface waters is
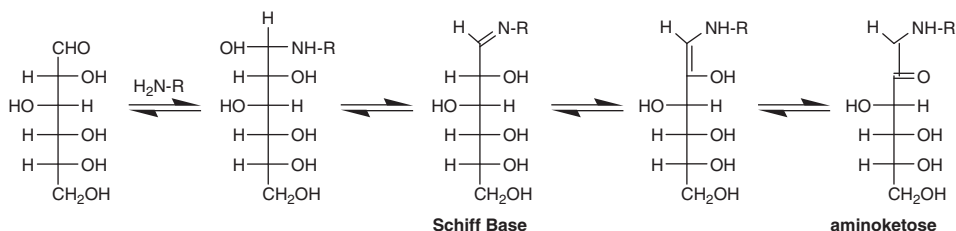
Figure 3.12 The Maillard Reaction. Shown are the preliminary rearrangements of functional groups (common to the proteins and sugars in DOM) that could serve as the foundation for addi–tional cross–reactions that may occur during *in situ* transformations of DOM or during analytical hydrolysis protocols. This could provide a mechanism for the formation of hydrolysis resistant amides that prevent bulk and monomer level characterization of DON.

present within acetylated amino sugars (assuming that all of the acetic acid is present in acetylated amino sugars). Together with directly determined neutral monosaccharide recoveries (between 10–20% C) carbohydrates account for approximately 30–40% of the total C in surface HMWDOM (Fig. 3.11); closer to NMR estimates of the carbohydrate content of HMWDOM (60–70%).

5. SOURCES AND SINKS BASED ON CHEMICAL INFORMATION

So far, this review has focused on establishing what is known about DON composition, but has also alluded to the important role that chemical composition plays in establishing DON sources and sinks. In fact, a major impetus for identifying the chemical composition of DOM is the hope that this information will allow us to better constrain the sources, inventory, residence times and sinks of various DOM components in the marine environment. In the following discussion we highlight the progress that has been made in this pursuit. Readers are also referred to Carlson (2002) for a thorough review of processes controlling DOM production and removal.

5.1. Sources

Source identification has relied primarily on chemical characteristics of *bulk* DOM or HMWDOM. For example, stable C isotope signatures of total DOM have proved very useful in establishing the predominantly marine origin of DOM (e.g., Williams and Druffel, 1987). More recent studies of the $\Delta^{14}C$ content of HMWDOM components, particularly polysaccharides (Santschi *et al.*, 1998; Aluwihare *et al.*, 2002; Repeta and Aluwihare, 2006) are also consistent with a primarily marine origin. In coastal and estuarine environments elevated C/N ratios of HMWDOM may be indicative of a terrestrial contribution from humic substances which have been shown to have relatively high C/N ratios. But in most cases the multiple components that are averaged in the bulk C/N ratio make it a poor source indicator. Bulk spectroscopic (e.g., NMR) or spectrometric (e.g. fluorescence, mass spectrometry) techniques do allow us to compare DOM samples and gain insight into chemical structure, but they do not suffice for unequivocal source assignment.

The $\delta^{15}N$ signature of DOM (Knapp *et al.*, 2005) and HMWDOM (Benner *et al.*, 1997; Guo *et al.*, 2003; Meador *et al.*, 2007) is comparable to the $\delta^{15}N$ value of deep ocean NO_3^- (Sigman *et al.*, 2000) and appears to vary independently of the $\delta^{15}N$ signature of suspended POM (Knapp *et al.*, 2005; Meador *et al.*, 2007). However, interpreting these $\delta^{15}N$ data in the context of N sources has proven challenging because $\delta^{15}N$ signatures of various inorganic N and particulate N reservoirs show marked variability in marine and terrestrial systems (e.g. Sigman and Casciotti, 2001). However, our ability to apportion sources based on $\delta^{15}N$ signatures will improve as studies aim to assign $\delta^{15}N$ values to all (or most) N reservoirs (e.g., suspended and sinking PON, DON, DIN etc) simultaneously at a

given location. In addition, compound-specific $\delta^{15}N$ measurements may allow DON source apportionment to particular marine biogeochemical processes such as N_2-fixation (Meador *et al.*, 2007).

Monomer composition studies of HWMDOM have revealed surprising similarities in the composition of marine HWMDOM isolated from various sites and depths; and studies seeking to assign sources based on these analyses have provided some clues but still lack complete and convincing data. For example, Cowie and Hedges (1992) concluded that amino acid composition served little use as a source indicator in marine environments. This was confirmed by the work of Yamashita and Tanoue (2003) who found no discernible spatial pattern in the relative distribution of amino acids in DOM isolated from various open ocean sites. Alternatively, Cowie and Hedges (1984) found that the ribose and fucose content of organic matter could be used to differentiate terrestrial and marine DOM; other studies have observed that the relative abundance of rhamnose is higher and fucose is lower in riverine DOM (e.g., Amon and Benner, 2003). Although these monomers are all present in HMWDOM, it is still difficult to conclusively identify sources based on monosaccharide composition alone because most monosaccharides are widely distributed in a variety of polysaccharides. In addition, Cowie and Hedges (1984) note that differentiating between various marine sources based on monomer composition is particularly difficult because small, metabolically active organisms have variable and diverse biochemical compositions.

Several authors have also compared bulk chemical properties between marine HMWDOM and HMWDOM produced in culture (Aluwihare and Repeta, 1999; Biersmith and Benner, 1998; Ogawa *et al.*, 2001). By comparing HMWDOM produced in phytoplankton cultures with oceanic HMWDOM, Aluwihare and Repeta (1999) noted that various phytoplankton produced dissolved compounds that were compositionally similar to HMWDOM accumulating in seawater. Proton NMR spectroscopy of some phytoplankton exudates even exhibited the dominant acetate peak observed in marine HMWDOM suggesting that *N*-acetyl amino sugars were produced by these organisms. These authors also showed that most exudates contained the same variety of neutral monosaccharides and that upon degradation, the general monomer distribution tended towards that observed for marine HMWDOM. In general, this monosaccharide distribution developed through a loss in neutral hexoses and a relative increase in the amount of deoxy sugars (rhamnose and fucose) over the course of the degradation experiment. Amon *et al.* (2001) and Amon and Benner (2003) also observed similar changes in the sugar composition of DOM during degradation, and additionally noted that the relative abundance of glucose increased as a result of diagenesis. Other investigators have also noted variations in the relative abundance of glucose in organic matter fractions (see references in Amon and Benner, 2003). In summary, these analyses have yielded mixed results—some have suggested that polysaccharides in HMWDOM are eventually sourced from phytoplankton products (Aluwihare and Repeta, 1999; Biersmith and Benner, 1998) while others studies have suggested that refractory HMWDOM is derived from bacteria (e.g. Ogawa *et al.*, 2001).

Cell membrane and cell wall components may also represent part of the accumulating DOM reservoir; these compounds may be selectively preserved as a result

of complex structural features that are not easily accessible to common bacterial enzymes (McCarthy et al., 1998; Nagata et al., 1998). Tanoue (1995) identified the presence of bacterial Porin P in HMWDOM, and subsequent studies from this laboratory have suggested that other structural polymers derived from bacteria are also present in seawater (e.g. Yamada and Tanoue, 2003). Based on their functional significance, a variety of microbial cell wall components may be designed to escape enzymatic hydrolysis and therefore, accumulate in DOM.

Short-term laboratory experiments have shown that the bacterial degradation of known biochemicals can result in the formation of refractory DOM (Brophy and Carlson, 1989; Ogawa et al., 2001; Stoderegger and Herndl, 1998). Brophy and Carlson (1989) added glucose and glutamate (at marine concentrations) to a natural population of marine microbes and showed for the first time, that heterotrophic organisms formed biologically refractory HMW organic compounds. Bacteria utilized only 17% of the C within the newly formed HMWDOM over a 6-month period. Ogawa et al. (2001) similarly showed that a natural bacterial assemblage grown on simple compounds such as glucose and glutamate produced DOM that was only slowly degraded on annual timescales.

Boon et al. (1998) used the presence of methylated and amino sugars, both also reported by Aluwihare et al. (1997) as minor constituents of HMWDOM, to argue that bacteria produce HMWDOM. However, methylated sugars and amino sugars have also been detected in eukaryotes (see below). Other authors have also arrived at a similar conclusion based on the work by McCarthy et al. (1997, 1998) which suggested that D-amino acids in HMWDOM are derived from peptidoglycan, a compound rich in acetylated amino sugars and found only in bacteria. Muramic acid, one of the two acetylated amino sugars present in peptidoglycan, has been detected in small quantities in HMWDOM (Benner and Kaiser, 2003; Kaiser and Benner, 2000). The N-acetyl glucosamine:muramic acid ratio detected in HMWDOM is in the range ∼20–120 (Benner and Kaiser, 2003), whereas this ratio is 1:1 in peptidoglycan. Bacteria are the only known source of muramic acid and the presence of this sugar in HMWDOM explicitly indicates a bacterial contribution to HMWDOM, albeit small. The low abundance of muramic acid in HMWDOM could result from low recoveries following acid hydrolysis of peptidoglycan. In fact, Benner and Kaiser (2003) report that muramic acid recoveries from peptidoglycan were on the order of 63%—lower than recoveries for N-acetyl glucosamine. However, lactic acid, which is present in muramic acid, is easily released upon treatment with base. When this method is applied to HMWDOM, only a small amount of lactic acid is detected. In fact, lactic acid recoveries from HMWDOM are too small for the sugar backbone of peptidoglycan to be a dominant component of DOM (Aluwihare et al., 2005). Although separate from the origin of N-acetyl amino sugars, it is still possible and likely that D-amino acids are present in peptides (not proteins) that are derived from bacteria. For example, several compounds derived from bacteria including antibiotics and siderophores do contain these amino acids (e.g. Bodanszky and Perlman, 1969; Martinez et al., 2000). Some reports also suggest that Archaea and eukaryotes may contain peptidyl D-amino acids (Nagata et al., 1998). In most cases, D-amino acids are present in non–protein peptides; however, there are several other amino acids in HMWDOM

and bulk DOM that are probably present in proteins which are not necessarily derived from bacteria.

The abundance of peptidoglycan in HMWDOM was inferred primarily from the strong amide resonance observed by ^{15}N-NMR spectroscopy (Fig. 3.7). However, other compounds could give rise to the observed amide resonance. For example, chitin, a homopolymer of β (1→4)–(*poly*) *N*-acetyl D-glucosamine is likely the most abundant polysaccharide in the ocean (similar to the ubiquity of cellulose in terrestrial environments). Since chitin is abundant in marine eukaryotes (e.g., diatoms, zooplankton, and fungi; this compound does not occur widely in prokaryotes) it is also abundant in detritus (Hashimoto *et al.*, 1997; Minor and Eglinton, 1999). The presence of chitin in the cell walls of diatoms and other algae has been well documented (Smucker, 1991; Shahgholi *et al.*, 1996 for example). In diatoms, investigators have shown that chitan is present in the fully acetylated, highly crystalline form with little or no cross-linking with amino acids or glycans. Chitan can contribute as much as 34% of the total cell mass (including hard-parts) of diatoms (McLachlan *et al.*, 1965). A study of cell wall carbohydrates in *Chlorella* sp. (from freshwater; Kapaun and Reisser, 1995) found a molecular ratio of amino sugars to neutral sugars in the range of 1:2–1:3. This is similar to the distribution of amino and neutral sugars in marine HMWDOM (Aluwihare *et al.*, 2002, 2005). Acid hydrolysis of the *Chlorella* polysaccharide released neutral monosaccharides (arabinose, xylose, rhamnose, fucose, galactose, mannose and glucose) but amino sugars were not released until strong acid hydrolysis conditions were applied (4 or 6 M HCl). Benner and Kaiser (2003) examined several different microorganisms for the presence of amino sugars and consistent with reports above, *N*-acetyl glucosamine was commonly present. *N*-acetyl galactosamine was found to be equally ubiquitous, and smaller quantities of mannosamine were also detected in several of the organisms. Although chitin and related, soluble polysaccharides are common in the marine environment, current molecular-level analytical methods have not been able to recover significant quantities of these polysaccharides from HMWDOM. Chitinase genes are ubiquitous in both free and particle-attached marine bacteria and have been shown to be predominant in the marine α-proteobacteria and the *Cytophaga-Flavobacter* clades (Cottrell *et al.*, 2000); these genetic data could imply that chitin is an important substrate for bacterial growth in the marine environment.

The compositional studies discussed in this paper have provided some insights into the DOM cycle. For example, monomer level studies have shown that the chemical composition of HMWDOM is homogeneous in the surface ocean (Aluwihare *et al.*, 1997; McCarthy *et al.*, 1996). Compositional differences between the surface and deep ocean have demonstrated that polysaccharides are reactive and removed with depth (Aluwihare *et al.*, 2002; Benner *et al.*, 1992); furthermore, the relative abundance of amides resembling those found in *N*-acetyl glucosamine also decreases with depth suggesting that these compounds are reactive and likely derived from surface ocean productivity (Aluwihare *et al.*, 2005; Benner and Kaiser, 2003). Amino acid compositions and quantities exhibit no vertical trend and certain amino acids could have a common source throughout the ocean (McCarthy *et al.*, 1998). D enantiomers of several amino acids are also ubiquitous in HMWDOM and indicate the presence of dissolved peptides derived from bacteria.

What is clear from these discussions is that intact molecules must be isolated from HMWDOM before a more accurate picture of the DOM cycle can be generated. So far studies have focused on the primary structure (or monomer composition) of complex biochemicals and this level of structural information cannot provide conclusive source information. Recently, gel electrophoresis and proteomics have been applied to characterize individual proteins in DOM. These techniques are very promising and cell membrane proteins and proteins involved in particular biosynthetic pathways have already been identified. In addition to providing source information, these techniques will enable us to identify chemical characteristics that favor the preservation of certain molecules in seawater. In the case of polysaccharides, attempts have been made to determine secondary structure through linkage analysis for example (Aluwihare *et al.*, 1997, 1999). However, beyond showing the predominance of highly branched heteropolysaccharides (interpreted to be present primarily in structural polysaccharides) over straight chain, homopolysaccharides (storage polysaccharides) these analyses have failed to identify specific sources.

5.2. Sinks and transformations of DON

Existing protocols for sampling DOM provide, at best, only a snapshot of a matrix of compounds that are cycling on dramatically different timescales and are subject to a variety of degradation processes. Carlson and Ducklow (1995) identified three types of DOM based on reactivity: (1) a labile fraction with rapid turnover rates and little to no accumulation in surface waters; (2) a semi labile fraction that is present at depths <1000 m and is only slowly degraded over seasonal timescales, and (3) a refractory fraction which is well mixed throughout the water column and is degraded on millennial timescales. Based on this reactivity scheme and an assumed biological source for the entire DOM reservoir, organisms in the surface ocean must synthesize biopolymers with widely differing rates of biological reactivity. Also, post-production modification of labile polymers may render them less susceptible to degradation. Bacterial uptake is responsible for the rapid turnover of the so-called labile fraction of DOM (Carlson and Ducklow, 1995) and tight coupling between production and consumption of labile DOM implies that these compounds do not accumulate in seawater and therefore, escape chemical characterization. Using the dissolved remnants from biological and abiotic transformation processes studies of DOM thus focus on reconstructing the cycles of semi-labile and refractory DOM.

Enzymatic assays can be applied in the marine environment to provide indirect information on dissolved compounds that are available to fuel bacterial production. Approaches that have been commonly applied include measuring hydrolytic enzyme activities in seawater and monitoring degradation rates of model compounds. Protein hydrolysis in seawater is rapid as expressed by model protein studies (e.g., Nunn *et al.*, 2003; Pantoja and Lee, 1999). This rapid and selective removal of dissolved proteins explains the relatively minor contribution from proteins to the accumulating DOM reservoir even though proteins are by far the most abundant intracellular biochemical. In an elegant study, Nunn and coworkers (2003) used matrix assisted laser desorption/ionization (MALDI) time of flight (TOF) mass

spectrometry (MS) to follow structural changes associated with the degradation of bovine serum albumin (BSA) in seawater. BSA was degraded rapidly in seawater and enzymatic attack occurred at a variety of sites on this protein. Based on these results the authors concluded that either non-specific proteases or a diverse collection of specific proteases were abundant in seawater. Consistent with these findings, the $\delta^{15}N$ signature of HMWDOM and proteins isolated from HMWDOM indicate that dissolved proteins are turning over more rapidly than bulk HMWDOM (Meador et al., 2007).

In the case of carbohydrates, bacteria produce a variety of ectoenzymes that are capable of hydrolyzing many different glycosidic bonds (Arnosti, 2000). Studies in the field have focused primarily on glucosidases, chitinases and cellulases because their assays are well developed (Pinhassi et al., 1999; Riemann and Azam, 2002) but degradation experiments with model polysaccharides have also indirectly monitored the activity of other glycosidases (Arnosti, 2000). These and other studies have shown that enzymes capable of degrading a variety of different polysaccharides are ubiquitous in seawater. Furthermore, genetic studies confirm the cosmopolitan existence of free bacteria capable of expressing many of these enzymes (e.g., Cottrell et al., 2000; Venter et al., 2004).

Despite the ubiquity of enzymes involved in polysaccharide and protein hydrolysis and the observed rapid degradation of model polymers, many biopolymers still accumulate in seawater. Can the chemical composition of semi-labile and refractory dissolved compounds provide insight into the extent to which these fractions of DOM have been transformed relative to labile biochemicals? Can chemical composition help identify processes responsible for these transformations? It has been suggested that the hydrolysis-yield of amino acids, normalized to the C content of the sample, serves as an index of organic matter diagenesis. Specifically, relative enrichments of glycine and alanine relative to distributions in phytoplankton, POM etc are expected to be indicative of highly degraded DOM (Amon et al., 2001; Dauwe et al., 1999; Yamashita and Tanoue, 2003). Various DOM fractions do appear to be enriched in glycine relative to phytoplankton and bacteria (see Fig. 3.5A) but no obvious vertical trend in amino acid yield or composition has been recorded. The absence of a vertical trend is consistent with the hypothesis that DOM and HMWDOM present at all depths in the water column has already undergone substantial degradation (Dauwe et al., 1999). However, since recoverable amino acids represent such a small fraction of total HMWDON, factors controlling amino acid production and preservation do not necessarily reflect those factors controlling the cycling of major HMWDON components.

Some laboratory degradation studies have shown the selective preservation of particular polysaccharides suggesting that certain structural features may render recently biosynthesized compounds unavailable for rapid bacterial utilization (Aluwihare and Repeta, 1999; Nanninga et al., 1996). Mechanistic studies imply that monomer composition, carbohydrate stereochemistry, linkage pattern, degree of cross polymerization and/or chemical substituents other than monosaccharides contribute significantly towards retarding the degradation rate of polysaccharides (e.g. Arnosti, 2000). A classic example is the degradation rate of starch versus cellulose (articles in Aubert et al., 1988). The monomer composition of these two

polysaccharides is identical (repeating units of glucose) but the β (vs α) stereochemistry about the glycosidic bond renders cellulose resistant to rapid bacterial degradation. This simple case expresses the importance of determining the absolute structure of intact molecules before a particular chemical characteristic of DOM can be applied as a degradation index.

In the case of refractory DOM, additional abiotic transformation processes resulting from chemical interactions are often considered (Degens, 1970; Harvey et al., 1983; Hedges, 1978; Ishiwatari, 1992). Like the Maillard reaction (see Fig. 3.12) these proposed transformations are expected to create bonds that are resistant to both biological and chemical hydrolysis; these processes have often been collectively referred to as humification. Hedges (1988) proposed that labile monomers (amino acids, sugars) could react through abiotic processes and form refractory organic matter. Ishiwatari (1992) proposed a similar mechanism by which the remnants of abundant biopolymers such as carbohydrates and proteins react to form refractory molecules. Alternatively, labile proteins may be physically protected from enzymatic attack by other cell wall materials (Nagata et al., 1998). Other physical processes may also be important for removing organic compounds from the dissolved phase. For example, the aggregation of dissolved compounds is thought to contribute to the formation of transparent exopolymer particles (TEP) and marine snow (Logan et al., 1995; Passow, 2002 and reference therein). Once formed, these particles can be exported from the surface ocean and so aggregation processes also represent a DOM sink. The carbohydrate composition of marine snow is very similar to the carbohydrate composition of HMWDOM (Mopper et al., 1995); and recently Engel et al. (2004) showed that up to 30% of the C in POM of an Emiliania huxleyi laboratory culture was derived from DOM. Staining with dyes such as Alcian Blue have shown the importance of charged functional groups in promoting the aggregation; and Chin et al. (1998) showed that carboxylic acid groups play a critical role in the spontaneous aggregation of natural DOM to form micrometer sized particles. Although aggregation processes could play an important role as a DOM sink few studies have examined whether/how DON is explicitly transferred to the particulate phase.

A number of studies have also examined the photochemical conversion of DOM into its inorganic components and into labile fragments that may then be rapidly utilized by bacteria (e.g. Benner and Biddanda, 1998; Kieber et al., 1989; Mopper et al., 1991; Moran et al., 2000). Mopper et al. (1991) suggested that 12–48% of the photochemically reactive DOC is degraded by sunlight during each ocean-mixing cycle. Based on this turnover rate the authors calculated a residence time of 1000–4000 years for the photoreactive fraction of DOM. A similar residence time for refractory DOM ($\sim$6000 years) has been estimated based on ^{14}C measurements (e.g., Druffel et al., 1992). Therefore, photodegradation could be the dominant removal process for refractory DOM.

Based on the above discussion, it is expected that chemical structure plays some role in controlling rates of DOM removal by biological and non-biological processes. For example, some studies have suggested that the glycosylation of proteins retard their bacterial degradation in seawater; other studies suggest that heteropolysaccharide structure and linkage patterns control dissolved polysaccharide

degradation rates in seawater; carboxylic acid functional groups appear to assist in transforming DOM to POM through their involvement in aggregation processes. However, few of these studies have quantified the relative incorporation of N into POM or refractory DOM. A firmer understanding of DOM structure will undoubtedly provide greater insights into processes controlling DOM preservation and accumulation in seawater.

5.3. Residence time of DOM components

Determining the chemical structure of refractory compounds is the definitive approach to understanding how and why compounds escape degradation and accumulate in the ocean on long timescales. However, refractory compounds must first be identified and we suggest that compound-specific Δ^{14}C measurement techniques must be developed and applied in DOM studies to identify semi-labile and refractory DOM components. For example, the refractory nature of the marine DOM reservoir was first conclusively demonstrated using Δ^{14}C measurements (Williams and Druffel, 1987).

Recent Δ^{14}C measurements have shown that HMWDOM is enriched in labile compounds relative to total DOM (Aluwihare et al., 2002; Loh et al., 2004; Repeta and Aluwihare, 2006). In fact, some of these studies have shown that polysaccharides (Aluwihare et al., 2002; Repeta and Aluwihare, 2006) and protein-like fractions (Loh et al., 2004) isolated from surface ocean HMWDOM have modern ^{14}C signatures—i.e., a Δ^{14}C signature that is within the range of values observed for the post-1950s DIC reservoir in the surface ocean (e.g., Williams and Druffel, 1987). In the case of polysaccharides, studies confirm that these compounds are probably synthesized and recycled on sub-decadal timescales in the surface ocean (Repeta and Aluwihare, 2006). Meador et al. (2007) found that dissolved proteins isolated from HWDOM at a site in the N. Atlantic Ocean had a Δ^{14}C value consistent with a sub-decadal residence time in the upper ocean. The modern radiocarbon content of carbohydrates and proteins suggests that these biochemicals are turning over on short timescales relative to an average residence time of 6000 years for total DOM in the deep ocean. However, more compound specific radiocarbon measurements are needed to conclusively establish the residence time of N-containing molecules.

Several studies have labeled various inorganic N pools and shown that labeled DON is released by phytoplankton during growth (Antia et al., 1991; Bronk, 2002 and references therein). However, the extent to which these processes contribute DON to the oceanic semi-labile reservoir is not well constrained. Stable N isotopes may be helpful in constraining the residence time of DON on short and perhaps even seasonal time scales. For example, δ^{15}N values of HMWDOM do not show large temporal or spatial variations although nitrate concentrations and δ^{15}N values of suspended PON at the same location are more variable, suggesting that the majority of DON is cycling on > annual timescales (Meador et al., 2007). Therefore, DON production measured during incubation studies probably represents a fraction that is rapidly recycled by mixotrophic and heterotrophic organisms and does not accumulate in seawater.

6. Summary and Future Direction

The preceding discussion on the chemical composition of DON in seawater demonstrates that bulk analytical methods (e.g., bulk stable isotope measurements, elemental analysis, ^{1}H-, ^{13}C-, and ^{15}N–NMR spectroscopy) have made significant progress in identifying and quantifying various components of this N reservoir. However, molecular-level investigations are required before definitive sources and sinks for these molecules can be identified in the marine environment. On the basis of amino acid analysis and monosaccharide analysis, proteins and amino sugars, which could contribute to the dominant amide functional groups as observed in HWMDOM by ^{15}N–NMR spectroscopy, have been shown to constitute only ∼15% of total DON. In the deep ocean as much as 70% of the N accumulate as amides that are resistant to common analytical methods (such as acid hydrolysis) and has so far escaped identification at the compound level. Our conclusion is that the methods employed in the study of HMWDOM structure—acid hydrolysis induced depolymerization followed by identification and quantification of monomers—is inadequate for making progress toward identifying the chemical composition of oceanic DON. Current hydrolysis methods (including acids such as TFA, HCl and H_2SO_4) likely induce cross-reactions and/or cannot effectively hydrolyze particular bonds present in DON. Therefore, we suggest that analytical approaches should focus on isolating individual components and intact molecules from DOM for further analysis. To date, ultrafiltration, which isolates only ∼25% of total DOM, seems to be the best method for concentrating N-rich compounds for this analysis. In addition, the application of tools routinely used in biomedical and immunological research (e.g., synthesis of antibodies, lectin affinity chromatography, proteomics) to study HMWDOM structure may provide the most useful insights into the DON cycle. Recent studies coupling gel electrophoresis, enzyme degradation, and mass spectrometry have identified intact proteins in HMWDOM, providing a window into what the future holds. The application of techniques used in molecular biology, such as enzymatic hydrolyses, may also help to deconvolute complex HMWDOM structures by producing identifiable and representative monomers and oligomers that are easier to quantify by common analytical methods such as HPLC. As always, structural investigations must strive to include as much of the DOM reservoir as possible and recent advances in DOM isolation methods combining reverse osmosis and electrodialysis (Mopper *et al.*, 2007) holds promise for isolating a major fraction of DOM. This will be most effectively achieved if we continue to develop sensitive analytical techniques such as mass spectrometry and methods involving fluorescent detection (such as antibody labeling).

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Nitrogen Fixation in the Marine Environment

Edward J. Carpenter *and* Douglas G. Capone

Contents

1. Introduction

Tremendous progress has been made in the past several decades in the realm of marine nitrogen (N_2) fixation. The spectrum of organisms recognized to contribute to N_2 fixation in the sea has been greatly expanded, and there has been a virtual revolution

in our understanding of its quantitative significance, the interplay between N_2 fixation and the C cycle and the major controls on this process. The pace of discovery in this area has increased rapidly with the infusion of new geochemical and molecular approaches augmenting the traditional microbiological and physiological methods. Several recent reviews have summarized aspects of progress on marine N_2 fixation (Capone, 2001; Karl et al., 2002; Mahaffey et al., 2005). See Mahaffey et al. (2005) for a comprehensive synthesis. The purpose of this review will be to provide a current synopsis and update to recent developments.

Diazotrophic organisms are found across the tree of life (Fig. 4.1A and B), and marine representatives of clades I and III have been either isolated or identified as environmental clones (Table 4.1). Phylogenetic trees of diazotrophic sequences have been generated from nifH sequences and related genes (hetR, nifD, nifK) are very congruent with analyses based on 16s rRNA (Zehr et al., 2003; Raymond et al., 2004). See Zehr et al. (2003) for a comprehensive treatment of nitrogen fixer phylogeny including marine specific examples.

The earliest organisms identified as diazotrophic in the sea were heterotrophic bacteria grown on nitrogen (N)-free media or isolated into pure culture (see e.g., Capone, 1988; Guerinot and Colwell, 1985; Maruyama et al., 1970; Pshenen, 1963; Waksman et al., 1933). However, with the recognition that some heterocystous cyanobacteria were diazotrophic (Fogg, 1942), attention was largely focused for a time on marine representatives in various nearshore ecosystems (Fogg, 1978; Stewart, 1965; Wiebe et al., 1975). Generally, heterocystous cyanobacteria are largely distributed/ confined to fresh and brackish waters, and benthic environments. One exception are the heterocystous cyanobaterial symbionts (cyanobionts) of various open ocean diatoms.

In 1961, Dugdale et al. (1961) first reported that the planktonic non-heterocystous cyanobacterium Trichodesmium was also diazotrophic, an observation which was greeted with considerable skepticism at the time (e.g., Fogg, 1974). Trichodesmium had been studied by planktologists for decades (Wille, 1904) and was known to be cosmopolitan throughout the world's oligotrophic ocean (Carpenter, 1983a,b) and to form extensive blooms (Carpenter and Capone, 1992), but since it did not possess heterocysts and lived in an aerobic environment, it was initially thought by many that it was unlikely to be a diazotroph.

The development of a convenient field assay for N_2 fixation in the late 1960s (Stewart et al., 1967; Hardy et al., 1968) which was rapidly adapted for marine studies (see Capone 1983, 1993), led to a flurry of field efforts in a range of open ocean and shallow water and benthic marine environments. We will first summarize some of the recent advances and insights relative to N_2 fixation in benthic marine environments, move on to the water column, summarize the controls on N_2 fixation and comment on the broader, biogeochemical impacts of N_2 fixation.

2. BENTHIC NITROGEN FIXATION

Several reviews have previously summarized benthic N_2 fixation (Capone, 1983, 1988; Herbert, 1999; Howarth et al., 1988a,b). More recent research in marine benthic environments has focused on seagrass (see also Chapter 23 by
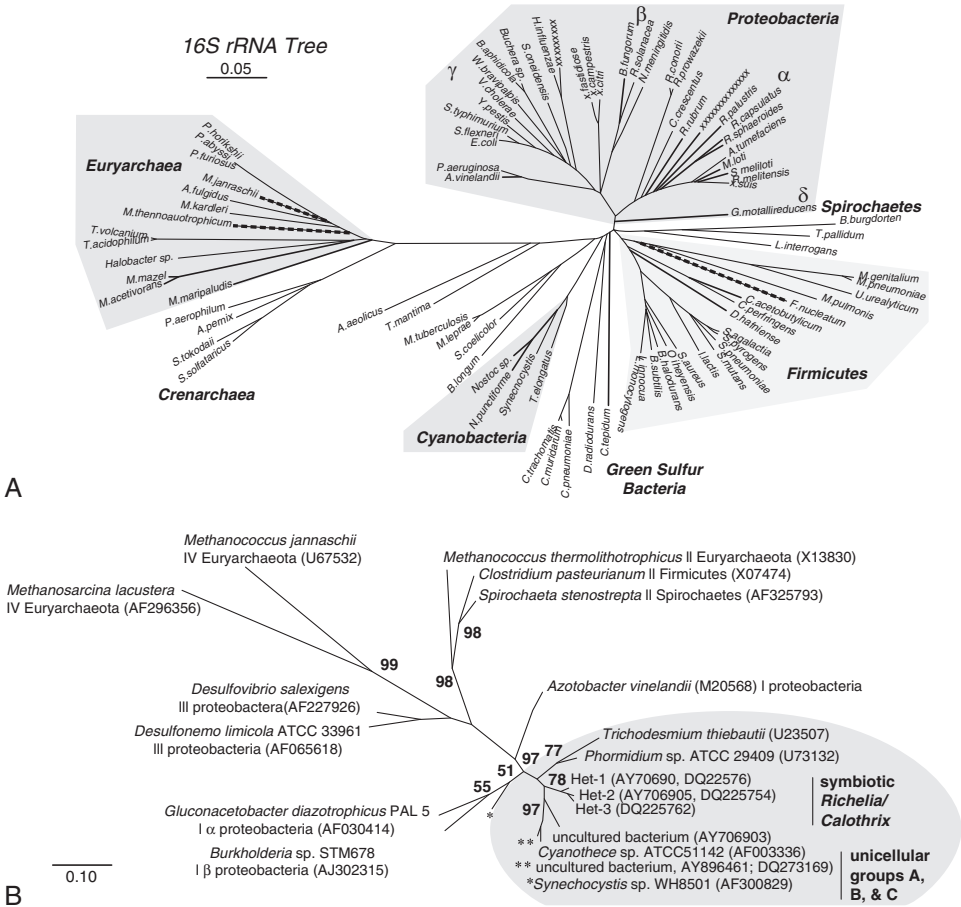
Figure 4.1 Phylogenetic trees showing the diversity of diazotrophs. A. 16S rRNA tree indicating position of diazotrophs within the major clades (from Raymond *et al.*, 2004) with permission. Bold lines indicate lineages with the nif operon. Dashed lines have nif homologues. Shaded major phyla indicated those containing diazotrophs. B. An unrooted tree of inferred amino acids of selected *nifH* sequences recreated with a *nifH* database courtesy of J.P. Zehr and based on Fig. 4.2 from Short *et al.*, 2004. Sequences and GenBank accession numbers, *nifH* group, and phylogenetic affiliation are provided for reference with results from 1000 bootstrap analyses using a Kimura correction. With the exception of *Phormidium* sp., the sequences highlighted in gray designate the most common open ocean diazotrophic phylotypes to date. Figure was courtesy of R.A. Foster.

McGlathery, this volume) and coral reef habitats (see Chapter 21 by O'Neil and Capone, this volume).

2.1. Deep sea sediments

Deep sea sediments are vast microbial habitats covering almost two thirds of the planet's surface and often with 100s of meters of accumulated sediment (D'Hondt *et al.*, 2004). Relative to near shore environments which are much more accessible,

Table 4.1 Some Representative Groups of Marine Diazotrophs and their Habitats Observed using Molecular Approaches

Habitat	Group	Sub-group	Life Style		Taxon notes	References
OPEN OCEAN						
Photic zone	I	Cyanobacteria	E, I	Free living	*Trichodesmium* & *Katagnymene* spp.	Janson *et al.*, 1999a,b; Orcutt *et al.*, 2002; Lundgren *et al.*, 2005
Photic zone	I	Cyanobacteria	E	Free living★	Coccoid type A	Zehr *et al.*, 2001; Falcon *et al.*, 2004; Langlois *et al.*, 2005
Photic zone	I	Cyanobacteria	E, I, E2	Free living★★	Coccoid type B (*Crocosphaera* like)	Zehr *et al.*, 2001; Falcon *et al.*, 2004; Langlois *et al.*, 2005
Photic zone	I	Cyanobacteria	E	Free living★	Coccoid type C (*Cyanothece* like)	Langlois *et al.*, 2005; Foster *et al.*, 2007
Photic zone	I	Cyanobacteria	E2	Free living	coccoid cyanobacteria	Mazard *et al.*, 2004
Photic zone	I	Cyanobacteria	E, I	Endosymbiont/ diatom	*Richelia intracellularis*	Villareal, 1992; Foster and Zehr, 2006
Photic zone	I	Cyanobacteria	E	Endosymbiont/ diatom	*Calothrix rhizosoleniae*	Janson *et al.*, 1999a,b; Foster and Zehr, 2006
Photic zone	I	Cyanobacteria	NA, E2	Ectosymbiont/ dinoflagellate	coccoid cyanobacteria	Foster *et al.*, 2006
Photic zone	I	Cyanobacterium	E2	Endosymbiont	*Cyanothece* sp.	Carpenter and Janson, 2000
Near surface	I	α, β, & γ Proteobacteria	E, I	Free living	distantly related to *Vibrio*	Zehr *et al.*, 2001; Langlois *et al.*, 2005; Hewson *et al.*, 2007

Photic zone	I	γ Proteobacteria	I	*Trichodesmium* aggregate	*Rhizobia* or *Azotobacter* like	Kirshtein *et al.*, 1991
Upper water column	I	γ Proteobacteria		Free living	*Vibrio natriegens* and *Azotobacter vinelandii*	Bird *et al.*, 2005
Near surface	III	Green sulfur	E	Free living	*Chlorobium* (83%)	Langlois *et al.*, 2005
Near surface	I	β, & γ Proteobacteria	E, I	Zooplankton gut	*Chromatium*	Braun *et al.*, 1999
Near surface	III	δ Proteobacteria	E, I	Zooplankton gut	*Desulfotobacter, Desulfonema, Chlorobium*	Braun *et al.*, 1999
At depth	I	α & γ Proteobacteria	E	Free living		Mehta *et al.*, 2003; Hewson *et al.*, 2007
At depth, hot vent area	IV	Archaea	E,I	Free living	Euryarchaeota	Mehta *et al.*, 2003, 2005, 2006
BENTHIC						
Seagrass	I	γ Proteobacteria	I	Rhizosphere/ *Ruppia*	*Rhizobia* like	Kirshtein *et al.*, 1991
Seagrass	III	Firmicutes	E	Rhizosphere/ *Halodule*	*Clostridia* like	Kirshtein *et al.*, 1991
Seagrass	III	δ Proteobacteria	E	Rhizosphere/ *Thalassia*	Sulfate reducing bacteria	Smith *et al.*, 2004
Salt marsh	I	α, β, and γ Proteobacteria	E, I	Rhizosphere/ *Spartina*	*Vibrio, Enterobacter, Azotobacter, Spirilla, Psuedomonas, Rhizobia*	Bagwell *et al.*, 1998; Lovell *et al.*, 2000

(*Continued*)

Table 4.1 Some Representative Groups of Marine Diazotrophs and their Habitats Observed using Molecular Approaches (*continued*)

Habitat	Group	Sub-group		Life Style	Taxon notes	References
Coral reef	I	Cyanobacteria	NA	Endosymbiont/ coral/ *Montastrea*	Coccoid	Lesser *et al.*, 2004
Estuary	I	γ Proteobacteria	E	Sediment	*Azotobacter*	Burns *et al.*, 2001
Estuary	III	δ Proteobacteria	E	Sediment		Burns *et al.*, 2001
Deep sea	I	α Proteobacteria	I,	Sediment	*Rhizobium* (98% 16s)	d'Hondt *et al.*, 2004
Deep sea	I	γ Proteobacteria	I,	Sediment	*Vibrio diazotrophicus* (99% 16s)	d'Hondt *et al.*, 2004
Intertidal	I	Cyanobacteria	E	Mats	Lyngbya,	Zehr *et al.*, 1995; Steppe and Paerl, 2005
Intertidal	I	γ Proteobacteria	E	Mats	*Azotobacter, Rhizobia*	Zehr *et al.*, 1995; Steppe and Paerl, 2005
Intertidal	III	δ Proteobacteria	E	Mats	Desulfovibrio-like	Zehr *et al.*, 1995; Steppe and Paerl, 2005
Intertidal	III	Fimicutes	E	Mats	*Clostridia*	Zehr *et al.*, 1995; Steppe and Paerl, 2005

E – environmental *nifH* sequences; E2 – environmental 16S rRNA; I – Isolate, *nifH* sequence; NA – nitrogenase antibody.
★ Speculative based on *nifH* phylogeny.
★★ Evidence for an exception of cyanobionts of *Climacodium frauenfeldianum* (Carpenter and Janson, 2000).

deep sea sediments have only been preliminarily characterized microbiologically and with respect to their global biogeochemical importance. Recent culture-dependent approaches within the International Deep Ocean Drilling Program (IODP) have isolated a number of potential diazotrophic bacteria, including organisms closely related to *Rhizobia* and *Vibrio diazotrophicus* (based on 16S rRNA) (Biddle *et al.*, 2005) (Table 4.1). Diazotrophic bacteria have also been recently isolated from Mediterranean sapropels (Süß *et al.*, 2004).

2.2. Macrophyte communities

A variety of macrophyte communities, including seagrass, salt marsh and mangrove ecosystems, have been examined for the importance of N_2 fixation to plant nutrition and biogeochemistry, and much of the early findings have been summarized (Capone, 1983, 1988; Welsh, 2000) (See also Chapter 23 by McGlathery, this volume).

A comprehensive study of seagrass N_2 fixation was recently undertaken by Welch and his colleagues in meadows of *Zostera noltii* in the Bassion d'Arcachon. N_2 fixation represented an important input of nitrogen in this system contributing about 6–12% of the nitrogen requirement of the seagrass (Welsh *et al.*, 1996a,b,c). This is comparable to earlier reports for *Z. marina* (Capone, 1982).

Sulfate respiring bacteria (SRBs) were reported to be an important component of the diazotrophic flora of these systems based on both experimental studies. Using molybdate (MoO_4) inhibition, a specific inhibitor of sulfate reduction, (Oremland and Capone, 1988), severely reduced nitrogenase activity in rhizosphere sediment, and led to seasonal patterns of rhizosphere N_2 fixation and sulfate reduction. Similar observations with regard to the importance of SRBs to rhizosphere N_2 fixation have been made for sea grass communities of *Thalassia testudinum* (Capone *et al.*, 1977) and *Z. marina* (Capone, 1982; McGlathery *et al.*, 1998). Later studies determined that acetate was a major metabolite supporting nitrogenase activity, and an appreciable fraction (about 17%) of the energy derived from sulfate reduction supported N_2 fixation (Nielsen *et al.*, 2001; Welsh *et al.*, 1996a,b,c).

Controls on rhizosphere N_2 fixation appear to be a combination of organic substrate supply from the plant to support heterotrophs such as SRBs, balanced by the negative feedback of elevated ammonium concentrations in porewaters (Welsh *et al.*, 1997). For a salt marsh sediment with high ammonium concentrations, Capone and Carpenter (1982a,b) had earlier shown that removal of ammonium greatly stimulated nitrogenase activity. Interestingly, about 30% of the nitrogenase activity remained after addition of 1 mM NH_4Cl (Welsh *et al.*, 1997) which contrasted with the study of (McGlathery *et al.*, 1998) which found that N_2 fixation in the rhizosphere of *Z. marina* beds from a fjord in Denmark were not sensitive to ammonium additions.

Two recent studies have assessed the importance of nitrogenase activity associated with roots and rhizomes of seagrasses. Nielsen *et al.* (2001) reported that roots and rhizomes accounted for about 31% of rhizosphere N_2 fixation in *Z. nolti*, while they accounted for 91% of the activity in *Spartina maritima*. McGlathery *et al.* (1998) found that in *Z. marina*, about 39% of depth-integrated N_2 fixation was associated

with roots with only 4% associated with rhizomes, and the root–rhizome activity could account for about 65% of the total plant N demand.

The overall importance of N_2 fixation to seagrasses has been evaluated recently in a few studies employing a $\delta^{15}N$ stable isotope approach. Relatively low $\delta^{15}N$ values for seagrass tissue ranging from 1.1 to 2.2 per mil (with individual samples as low as –1.2 per mil) have been reported by Anderson and Fourqurean (2003) for Florida Bay. Similarly low $\delta^{15}N$ values were reported for several species of seagrass from various tropical sites in the western Pacific (Yamamuro *et al.*, 2003) and for *Posidonia oceanica* at several sites in the northwest Mediterranean. These observed low $\delta^{15}N$ values provide independent evidence that a substantial input by N_2 fixation does occur in seagrass communities.

2.2.1. Molecular ecology

Molecular approaches have been extremely effective in discerning the identity and dynamics of N_2 fixer activity in marine sediments and macrophyte communities (Table 4.1) (also see Chapter 30 by Jenkins and Zehr, this volume). Kirshtein *et al.* (1991) isolated and characterized the *nifH* sequence from 2 *Klebsiella* sp. isolates obtained from the roots of the seagrass *Ruppia marina*, and subsequently obtained *nifH* sequences from another seagrass, *Halodule wrightii*, which had greatest sequence similarity to *Clostridium pasteurianum*. Burns *et al.* (2002) obtained and compared *nifH* sequences from the sediments of the Chesapeake Bay and Neuse River, finding sharp differences between the sites. The Chesapeake Bay sequences clustered with each other and showed similarity to several anaerobic clades (e.g., related to *nifH* sequences from species of *Desulfobacter, Cholorobium* and *Acetobacterium*), while the sequences derived from Neuse River samples were highly divergent. As noted above, much of the diazotrophy in seagrass sediments is associated with SRBs. In this regard, (Smith *et al.*, 2004) using the dissimilatory bisulfite reductase (dsrA and B), found very high diversity and largely unknown identities (and therefore physiologies) of sulfate respiring bacteria in a *Thalassia testudinum* seagrass bed.

Lovell and colleagues have undertaken perhaps the most comprehensive study of the physiological and molecular ecology of N_2 fixation in a marine macrophyte community. Initial studies isolated hundreds of diazotrophic strains from the salt marsh rhizosphere and subjected them to physiological characterization. They found representative species of the *Enterobacter, Vibrio, Azotobacter, Spirilla, Pseudomonas* and *Rhizobia*, as well as numerous unknown clone sequences using *nifH* primers (Bagwell *et al.*, 1998) (Table 4.1). Piceno *et al.* (1999) compared the activity and diversity (using a *nifH* fingerprinting approach) of diazotrophs in tall and short *Spartina alterniflora* stands, finding that while nitrogenase activity differed between and within these communities, there was a relatively high stability in the composition of the diazotrophic flora both spatially and temporally. Perturbations with short term nutrient additions (Piceno and Lovell, 2000) and clipping or shading plots to reduce plant organic input to the sediments, also resulted in relatively insignificant shifts in nitrogenase activity and diazotroph composition, reinforcing the considerable short-term stability of these assemblages. While rhizoplane diazotrophs were

responsive to longer term fertilization studies with respect to their activity and relative occurrence of phylotypes, none were fully displaced by the experimental treatment, thereby indicating their resilience, perhaps through niche specialization (Bagwell and Lovell, 2000a,b). Sequencing partial fragments of the *nifH* gene from the rhizoplane, revealed three major clusters: a large clade composed of sequences with high identity to γ-proteobacteria, and many unknown sequences, a second group composed of sequences similar to α-, β- and γ- proteobacteria and a third cluster of sequences derived from anaerobic bacteria and more unknown phylotypes (Lovell *et al.*, 2000). Most recently, (Brown *et al.*, 2003) demonstrated that many of the organisms they have implicated as diazotrophs through culture-dependent and culture independent techniques do in fact express *nifH* mRNA in the *S. alterniflora* rhizosphere.

Several studies have compared diazotrophic assemblages from *S. alterniflora* with other salt marsh plants including *Juncus roemerianus* (Bagwell and Lovell, 2000a,b) and *Spartina patens* (Bergholz *et al.*, 2001). Bagwell and Lovell (2000a,b) used whole genome hybridization and substrate utilization patterns to differentiate among 50 isolates determined to be diazotrophic by the presence of *nifH*. Interestingly, They found that strains that were phylogenetically related could be physiologically diverse in their metabolism, while phylogenetically diverse strains often displayed similar substrate utilization patterns, providing support for functional redundancy in these systems. Sequences derived from isolates of *S. patens* (Bergholz *et al.*, 2001) form two major clades, the isolates were largely microaerophilic, and except for 1 strain, were physiologically distinct from the isolates from the other two grasses. Surveys of rhizosphere diazotrophs from two tropical seagrass beds yielded sequences most similar to anaerobes, with some sequence identity to β- and γ-proteobacteria and showed some similarity to the communities in *S. alterniflora* communities (Bagwell *et al.*, 2002).

2.3. Microbial mats

Microbial mats have been a longstanding focus of N_2 fixation studies (Paerl *et al.*, 1996, 2000) (Table 4.1). Most recently, applied molecular approaches have revealed much more information about these systems. Zehr *et al.* (1995) examined a microbial mat from an intertidal mat from coastal North Carolina and found a wide variety of diazotrophic bacteria were present, with the majority of sequences related to *nifH* sequences of anaerobes (sulfate reducers and *Clostridia*), particularly deeper in the mat.

Using MoO_4 inhibition (see above), Steppe and Paerl (2002) showed that SRBs could account for the majority of the dark activity, but that MoO_4 had little effect during the day, suggesting a predominance of phototrophic-based activity. Reverse transcription (RT)-PCR with *nifH* revealed transcripts from diverse anerobes including SRBs. In this same system, Steppe *et al.* (2001) have also recently reported on the diel patterns of both nitrogenase activity and *nifH* gene expression, and responses to the specific photosynthetic inhibitor dichorophenyl-dimethyl urea (DCMU) and the protein synthesis inhibitor chloramphenicol.

Their studies revealed a diverse assemblage of diazotrophs contribute distinct diel patterns that vary over the seasonal cycle depending on the exact composition of the diazotrophic assemblage.

Moisander *et al.* (2005) developed a *nifH* microarray with 96 oligonucleotide probes with broad representation of the major clades of diazotrophs. They examined mat community in Guerrero Negro (GN), Baja California, portions of which had been fertilized. Fertilized plots, although showing no *nifH* expression, had higher diversity than the unfertilized plots.

Steppe *et al.* (2001) have also examined modern stromatolites, laminated sedimentary structures with associated microbial populations similar to microbial mats, finding a temporal separation of photosynthesis and nitrogenase activity. *NifH* sequences of diverse cyanobacteria, α & δ proteobacteria were recovered.

2.4. Coral reefs and associated habitats

Coral reefs have been long recognized as active sites of N_2 fixation (Wiebe *et al.*, 1975) (see also Chapter 21 by O'Neil and Capone, this volume). Earlier results that indicated the importance of N_2 fixation in these environments were previously summarized by Capone (1996, 1988). More recent findings have confirmed these studies in a broader range of locations and with newer techniques.

Coral reef sediments are sites of N_2 fixation (O'Neil and Capone, 1989), and can account for a substantial fraction of N flow in the organic poor carbonate sediments surrounding reefs (Capone *et al.*, 1992). Results from the Red Sea found high rates of nitrogenase activity in substrates such as coral rubble and carbonate sands (Shashar *et al.*, 1994a,b), consistent with earlier reports from the Great Barrier Reef (Capone *et al.*, 1992; Crossland and Barnes, 1976; O'Neil and Capone, 1989). Carbonate sands accounted for more than 70% of the overall activity on the reef. Lower rates were associated with macroalgae and living corals. Activity that was associated with living corals appeared to be associated with bacteria as it was stimulated by glucose (Shashar *et al.*, 1994a,b). Charpy-Roubaud *et al.* (2001) examined nitrogenase activity associated with lagoon sediments, cyanobacterial mats and limestone surfaces in Tikehau Lagoon, French Polynesia. Activity was greater in the light than dark, apparently due to the presence of cyanobacteria in each of these substrata. Rates associated with the sediments ranged between 30 and 280 µmol N m^{-2} day^{-1} and were about 140 µmol N m^{-2} day^{-1}. N_2 fixation in the lagoon benthos could account for about 25% of the N demand of benthic primary production. A subsequent study examined the reef rim and found similarly high areal rates largely associated with cyanobacterial mat communities and accounting for an average of 332 µmol N m^{-2} day^{-1} of new N input, or roughly 28% of total N_2 fixation in the entire lagoon (Charpy-Roubaud and Larkum, 2005).

Yamamuro *et al.* (1995) were the first to use a ^{15}N–natural abundance approach in a coral reef environment, surveying various components of the ecosystem and concluded that much of the nitrogen demand on the reef is met by N_2 fixation.

France *et al.* (1998) surveyed benthic macroalgae in the reef environments of Puerto Rico, comparing them to macroalgal samples from the northeast coast of the U.S. and freshwater environments, finding further support for the notion that N_2 fixation is associated with macroalgal–cyanobacterial consortia on the reef.

Rohwer *et al.* (2002) initiated studies of the bacterial flora associated with corals, and has identified 16s rRNA sequences from potentially diazotrophic taxa (e.g., *Rhodobacter, Vibrio, Clostridium*). Specific studies examining the molecular ecology of N_2 fixation in reef communities remain to be undertaken. Hewson and Fuhrman (2006) have directly examined the diversity of diazotrophs, first using a rapid DNA fingerprinting approach, and more recently by culture independent approaches including development of clonal libraries, and direct amplification of *nifH* DNA and mRNA. Phylotypes of both oceanic cyanobacteria as well as cyanobacteria and proteobacteria of both groups I & III (Fig. 4.1), presumptively indigenous to the reef environment, were identified.

With regard to other diazotrophic symbioses on reefs, Paerl (1984) reported evidence for nitrogenase activity associated with an intact *Procholoron*-Ascidian association (*Lissoclinum* sp.). However, activity could not be directly associated with the isolated symbiont. Later, Odintsov (1991) provided evidence that the nitrogenase activity associated with encrusting ascidians was in fact not associated with the symbiont. More recently, Kline and Lewin (1999) concluded that, based on the low isotopic ratio of $^{15}N/^{14}N$ in *Lissoclinum* sp. associations, at least a portion of the cellular N may be derived (directly or indirectly) from N_2 fixation.

An important recent observation was the presence of putatively diazotrophic coccoid cyanobacteria in the tissues of certain *Montastrea* corals (Lesser *et al.*, 2004). In this initial study, immunological evidence indicated the presence of nitrogenase enzyme. Subsequent research has directly determined nitrogenase activity and reported low isotopic ratios of $^{15}N/^{14}N$ in zooxanthellae from orange pigmented corals containing the cyanobacterial symbiont (Lesser *et al.*, 2007). Further research is needed to quantify the importance of these organisms in introducing new N to coral reef ecosystems.

2.5. Directions in benthic nitrogen fixation

While progress has clearly been made in understanding the process of N_2 fixation in a range of benthic environments, research in this area has not been nearly as intensive as in water column systems (see below). Nonetheless, we now know that SRBs appear to be very important components of the diazotrophic flora of seagrass, salt marsh and mat communities. However, their physiology remains unclear and more remains to be done in defining the composition and dynamics of diazotrophs in such environments.

Given the preliminary geochemical and microbiological evidence for a potential role for N_2 fixation in both hot vent and cool seep environments, these are clearly systems requiring closer scrutiny with regard to the ecological importance of this process. Similarly, for coral reefs where the quantitative importance of N_2 fixation is not debated, we have only begun to scratch the surface with regard to the identity and diversity of diazotrophic populations.

3. PELAGIC NITROGEN FIXATION

3.1. Pelagic diazotrophs

In the open ocean, N_2 fixation research has focused most intensively on the cyanobacterium, *Trichodesmium* (Carpenter, 1973; Saino, 1977). Much of the earlier work has been summarized by Carpenter (1983a,b), Howarth *et al.* (1988a,b) and more recently by Karl *et al.* (2002). In its colonial form, or as surface slicks, *Trichodesmium* is obvious and relatively easy to collect by bucket or plankton tow. There are at least five species of *Trichodesmium* extant (Janson *et al.*, 1995), and samples of the five species from the Atlantic and Pacific Oceans and Caribbean Sea can be separated into three clades on the basis of 16S rDNA and *het*R sequences (Janson *et al.*, 1999a,b) (Table 4.1). One clade contains *T. hildebrandtii* and *T. thiebautii*, the second consists of *T. contortum* and *T. tenue*, and the third has *T. erythraeum*. A second recent study on *Trichodesmium* diversity was carried out by Orcutt *et al.* (2002), and three different genetic techniques were used on samples from the Sargasso Sea and Indian Ocean. These investigators reported that *T. thiebautii, T. hildebrandtii, T. tenue* and the non–heterocystous *Katagnymene spiralis* displayed remarkable similarity based on three independent analyses (i.e., DNA fingerprinting of a highly iterated palindrome, intergenic spacer analysis and DGGE of a hetR fragment). The largest genetic variation was found between *T. erythraeum* and all other species of *Trichodesmium. Katagnymene* has long been recognized in the tropical ocean plankton (Wille, 1904); however, it was only recently that its diazotrophic nature was confirmed (Lundgren *et al.*, 2001). More detailed analysis by Lundgren *et al.* (2005) has further shown that despite the morphological differences of *K. spiralis and K. pelagica,* based on *hetR* and partial 16sRNA sequences, they are a common species, and fall directly within the *Trichodesmium* cluster.

Early reports also described N_2 fixation in the pelagic zone associated with the cyanobacterial epiphytes *(Dichothrix fucicola)* of the brown macroalga, *Sargassum* (Carpenter, 1972) and cyanobacterial endosymbionts of certain oceanic diatoms (Mague *et al.*, 1977; Villareal, 1994). The diazotrophic cyanobacterial endosymbiont *Richelia intracellularis* is found in several species of diatoms within the genera *Rhizosolenia* and *Hemiaulus* (Villareal, 1992). Unlike *Trichodesmium*, this cyanobacterium has a single, terminal heterocyst. Genetic analysis (*het*R) of *R. intracellularis* samples from these diatom genera plus a similar appearing cyanobacterium, *Calothrix rhizosoleniae,* which is an epiphyte on several species in the diatom genera *Chaetoceros* and *Bacteriastrum*, revealed that they all belong to the same clade but diverge into separate lineages (Janson *et al.*, 1999a,b). Similarly, Foster and Zehr (2006) recently concluded the cyanobionts associated with *H. hauckii, R. clevei*, and *C. compressum* were distinct strains based on analysis of their *hetR* and *nifH* sequences. The *nifH* lineages derived from the symbionts of *Rhizosolenia, Hemiaulus*, and *Chaetoceros,* have been designated as het–1, het–2, het–3, respectively; and there is some evidence for a further delineation of the het–2 group into het–2A and het–2B (see Foster and Zehr, 2006). The same three cyanobiont lineages were however, highly similar (>98%) based on their 16 S rRNA sequences (Foster and Zehr, 2006).

Some unicellular coccoid cyanobacteria in the plankton of the subtropical North Pacific have now been shown to express *nifH* and are capable of N$_2$ fixation Ocean (Zehr *et al.*, 1998, 2001; Montoya *et al.*, 2004), (see Chapter 30 by Jenkins and Zehr, this volume). Closely related cyanobacteria have also been observed in the tropical Atlantic Ocean (Falcon *et al.*, 2002). These cyanobacteria, approximately 3–10 mm in diameter, are larger than the abundant picoplankter *Synechococcus*, and two clades are currently recognized (Zehr *et al.*, 1998). Group A have distant phylogenetic affinities with *Cyanothece*, and group B are most similar to *Crocosphaera watsonii* WH8501 while a recently observed Group C has close phylogentic affinities to *Cyanothece* (Langlois *et al.*, 2005; Foster *et al.*, 2007) (Table 4.1).

Diatom/*Richelia intracellularis* associations can be spatially extensive and contribute intensive input of N through N$_2$ fixation in the upper water column. For instance, a *Hemiaulus hauckii*/*Richelia* bloom encountered by Carpenter *et al.* (1999) off the northeast coast of South America in Oct 1996 was observed over a linear cruise track of 2500 km and accounted for very substantial levels of N$_2$ fixation (see below). The symbionts for individual diatoms such as *Hemiaulus* and *Rhizosolenia* while morphologically similar are genetically distinct and discrete probes for each association have been developed and applied in the Amazon River plume to enumerate the relative densites of these two associations (Foster *et al.*, 2007).

Novel groups are continuing to be uncovered. Foster *et al.* (2006) also reported that some coccoid cyanobacteria living symbiotically with dinoflagellates might possess nitrogenase and that some symbionts posess 16S rRNA sequences most similar to other known diazotrophs (i.e., *Cyanothece* sp.).

Coccoid cyanobacterial endosymbionts within the planktonic diatom *Climacodium frauenfeldianum* are potential planktonic N$_2$ fixers (Carpenter and Janson, 2000). (see also Chapter 27, Foster and O'Mullin, this volume). These symbionts have 16S rRNA sequences most closely related to the coccoid diazotroph *Cyanothece*, and likely fix N$_2$ at night within the host diatom. Using a coupled immunocytochemistry-TEM approach, a symbiotically associated *C. frauenfeldianum* was collected and preserved at night. The symbionts of the *C. frauenfeldianum* were coccoid in morphology, had cell diameters approximately 5–7 μm, possessed large glycogen stores, and localized both nitrogenase and phycoerythrin antiseras (Foster, unpubl.). One filamentous heterocystous cyanobacterium, *Anabaena gerdii*, has been collected within the plankton of the South Pacific Ocean and Arabian Sea (Carpenter and Janson, 2001), but concentrations of *A. gerdii* and *C. frauenfeldianum* are low, and it is doubtful that they are important in a global sense in N$_2$ fixation.

NifH gene diversity studies reported a significant fraction of *nifH* sequences clustered with proteobacteria rather than cyanobacteria, thus suggesting that components of this clade also are involved with N$_2$ fixation (Falcón *et al.*, 2004; Langlois *et al.*, 2005; Zehr *et al.*, 2001) (Table 4.1). Bird *et al.* (2004) examined the distribution of an uncultured diazotrophic γ-proteobacteria most closely related to *Vibrio natriegens* and *Azotobacter vinelandii* in the Arabian Sea. While they found it most

commonly amplified and expressing transcript in warm, near surface waters, they also observed active samples from below the nutricline expressing nitrogenase. (Church *et al.*, 2005) determined the abundance of Cluster III *nifH* copies in DNA in the oligotrophic North Pacific using qPCR. Langlois *et al.* (2005) also found Cluster III and proteobacterial *nifH* sequences to be abundant in the tropical Atlantic Ocean. Interestingly, Hewson *et al.* (2007), using both amplification and cloning of *nifH* sequences as well as a micro-array approach, have reported the presence of *nifH* sequences closely related to non-cyanobacterial diazotrophs, including α, β & γ proteobacteria, derived from samples obtained below the euphotic zone from the north Atlantic and northeast Pacific. The physiology and metabolism of these diazotrophic proteobacteria remains to be determined and could potentially include heterotrophs, methylotrophs, chemotrophs, and potentially some types of phototrophs (Kolber *et al.*, 2000; Beja *et al.*, 2001). Resolving the contribution of different physiological groups to N_2 fixation will be a challenge, but could be significant since nitrogenase activity could potentially take place through the entire diel period rather than just at night and, also possibly, deeper in the water column.

Diverse N_2 fixers have been detected in the water column of temperate estuaries such as the Chesapeake Bay (Affourtit *et al.*, 2001) (Table 4.1). Affourtit *et al.* (2001) found a variety of diazotrophs from Cluster I (which includes cyanobacteria and most proteobacteria) with rarer samples yielding Cluster II (some δ-proteobacteria, spirochaetes and some Archaea, some alternative nitrogenases) and III (firmicutes, spirochaetes and Archaea) in samples from the Neuse River. Short *et al.* (2004) reported the densities of two representative *nifH* sequences in the Chesapeake Bay using a quantitative PCR approach, and they detected specific seasonal and spatial patterns for each. Interestingly, expression of *nifH* was never detected.

Other unique sites of N_2 fixation remain to be discovered. Dense populations of Archaea are also found throughout the water column of the worlds' oceans (DeLong, 1992; Fuhrman *et al.*, 1992), and some methanogens have the capacity for diazotrophy (Murray and Zinder, 1984). Indeed, diazotrophic archaeal *nifH* sequences have been obtained from a marine vent community and in deep sea waters away from vents (Mehta *et al.*, 2003, 2005). They also obtained an isolate capable of growth and N_2 fixation up to 92°C (Mehta and Baross, 2006). N_2 fixing bacteria, including purple sulfur bacteria, are also associated with the intestinal flora of zooplankton (Braun *et al.*, 1999; Proctor, 1997).

Attention has also been lately focused on Oxygen Minimum Zones (OMZs) as sites for a recently discovered pathway in the nitrogen cycle, the Anammox reaction (Kuypers *et al.*, 2005) (see Chapter 6 by Devol, this volume). OMZs feature both low oxygen and combined nitrogen concentrations through denitrification and anammox and relatively low N:P ratios, characteristics which would make them suitable habitats for some groups of diazotrophs. Furthermore, recent geochemical evidence suggests that OMZs may also promote conditions that foster N_2 fixation in surface waters affected by the OMZs (Sigman *et al.*, 2005; White *et al.*, 2007, see below). The recent global analysis by Deutsch *et al.* (2007) examining the decrease in excess P (expressed as P★) in waters upwelled from OMZ areas and advected offshore provides strong supporting evidence for this (Capone and Knapp, 2007).

3.2. What happens to all that fixed nitrogen?

We still lack a good understanding of pathways of fixed N from marine diazotrophs to other members of the marine plankton. However, there is plenty of evidence that newly fixed N is utilized by other members of the plankton community. From the distribution of N⋆ anomalies in the range of 10°N–40°N in the N. Atlantic Ocean, it is strongly suggested that much of the nitrate in near-surface water originated through diazotrophy (Gruber and Sarmiento, 1997; c.f. Hansell et al., 2004). Furthermore, the low δ^{15}N of zooplankton in broad areas of the tropical Atlantic Ocean clearly originates from either the direct or indirect consumption of nitrogen originating from diazotrophs (Montoya et al., 2002). The magnitude of this signal indicates that N$_2$ fixation is a major source of "new" N in this area.

During an extended station in a Trichodesmium bloom in the Indian Ocean, sediment trap material from that station clearly reflected diazotrophic input, compared to stations away from the bloom (Capone et al., 1998). Another example was observed during the massive bloom of R. intracellularis within H. hauckii off the NE coast of South America (Carpenter et al., 1999). Suspended particles and zooplankton collected within the bloom were depleted in ^{15}N, reflecting the dominant contribution of N$_2$ fixation to the planktonic N budget.

There are multiple pathways for newly fixed N to be taken up by non-diazotrophs. Importantly, the fate of recently fixed N may be quite distinct among the various potential agents of N$_2$ fixation. Whereas Trichodesmium is not readily grazed by planktonic copepods (O'Neil and Roman, 1992) and much of its recently fixed N may enter food webs as DON after exudation (Capone et al., 1994; Glibert and Bronk, 1994) or lysis. Coccoid cyanobacteria and heterotrophic bacteria are directly grazed by protists (Caron et al., 1991). Recent research has shown the existence of specific viruses which lyse Trichodesmium (Hewson et al., 2004), and conservative calculations suggest that in samples collected near Hawaii, 0.3–6.5% day^{-1} of trichomes could potentially be lysed, and this represents 3–64% fixed N day^{-1}. Yet a third fate may be autocatalytic programmed cell death (PCD) which can be induced by a number of stressors such as iron or phosphorus starvation, high irradiance and oxidative stress (Berman-Frank et al., 2004).

Whereas Trichodesmium is buoyant and resides largely in the upper layers of the water column, endosymbiotic Richelia may be more prone to gravitational settlement to deeper layers in their diatom hosts (Scharek et al., 1999a,b; Subramaniam et al., 2008) (Fig. 4.2). Tropical river plumes may be particularly important sites of diazotroph-diatom associations (Voss et al., 2006; Subramaniam et al., 2008).

The microbial and metazoan community associated with Trichodesmium is rich, and in a study conducted near Bermuda, 85% of the Trichodesmium colonies examined harbored associated organisms (Sherman et al., 2002). These included bacteria, fungi, diatoms, dinoflagellates, chrysophytes, ciliates, amoebae, hydroids, copepods and juvenile decapods. One organism thought to be involved in N transfer is the harpactacoid copepod Macrosetella gracilis. This copepod uses the Trichodesmium colonies as a physical substrate for juveniles and adults, but also as a food source (Roman, 1978; O'Neil, 1998). Both O'Neil and Roman carried out studies on feeding of M. gracilis on Trichodesmium, and the results indicated that it was a
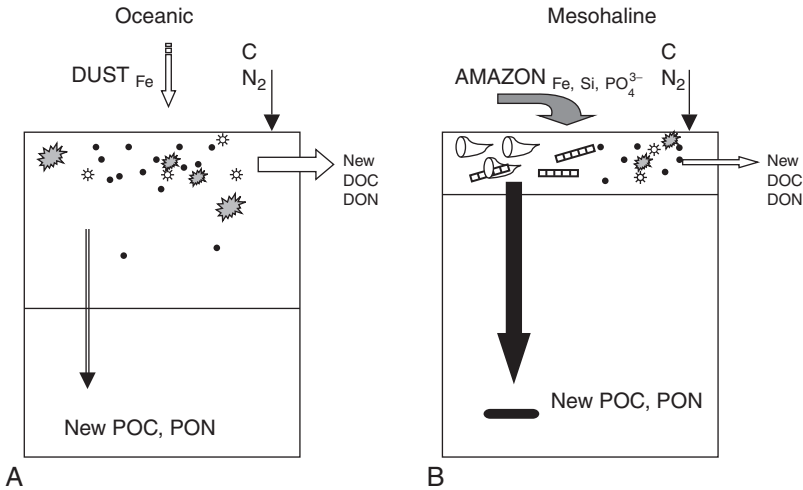
Figure 4.2 Conceptual model for differing fates of filamentous cyanobacterial diazotrophs (A) compared to diatom–diazotroph associations (B), from Subramaniam *et al.*, 2008.

voracious consumer. Roman (1978) found that adults could ingest from 90–126% of its body carbon per day when feeding on *Trichodesmium*. However, Eberl and Carpenter (2007), working in the Central North Pacific, found no significant correlation between *M. gracilis* and *Trichodesmium* population distributions. Further-more, natural abundance of stable isotopes of N and C suggested that the diet of *M. gracilis* was not predominately composed of *Trichodesmium*.

Trichodesmium is fed upon directly by larger nekton. Bryceson (1980) introduced juvenile clupeid fish into aquaria containing *T. erythraeum* from coastal waters of Tanzania, and the cyanobacterium was eaten rapidly. In the Indian Ocean, it has been observed to be eaten by two young fish species *Sardinella gibbosa* and *Mugil waigiensis* (Devanesan, 1942; Devanesan and Chidambaram, 1948), and there are data indicating that 60–90% of the diet of two other fish species, *Hilsa kanagurta* and *Rastrelliger kanagurta* consists of *T. erythraeum* (Ramamurthy, 1970).

We suggest that salps and perhaps other jellies are major consumers of *Trichodes-mium*. This very weakly supported supposition is primarily based on plankton net contents on numerous tropical cruises in which we observed that when *Trichodes-mium* is abundant, there are few salps, and when salps are abundant, the concentra-tions of *Trichodesmium* are almost nonexistent. In the western Pacific Ocean, Nemoto (1975) noted the possible feeding of *Salpa fusiformis* and *Weelia cylindrical* on *Trichodesmium*. In the Atlantic, Montoya *et al.* (1992) analyzed 37 *Salpa aspera* samples and obtained a mean δ^{15}N of *ca.* 4 per mil, values that were lower than other particulate material collected in the same area. The low values suggest that these salps had a diet that may have partially consisted of diazotrophs.

3.3. Directions in pelagic nitrogen fixation

The infusion into the field of innovative approaches is revolutionizing our under-standing of this process in the world's oceans. Sensitive new direct tracer methods of quantifying N_2 fixation and improvements in mass spectrometry in parallel

with modern molecular genetic approaches has catalyzed the current avalanche of new information. Molecular methods continue to improve and the advent of means to reliably and rapidly identify diazotrophs, quantifying their abundance (using qPCR) as well their physiological state with respect to nitrogenase gene expression using RT-PCR will continue to promote advances in the field. Similarly, new analytical approaches, the addition of moored systems with molecular biological capabilities provide the prospect for high density data streams which will allow detailed monitoring of the population dynamics of diazotrophs.

4. WHAT LIMITS NITROGEN FIXATION

Numerous factors, physical, chemical and biotic, can affect the extent of N_2 fixation in an ecosystem (Capone, 1988; Howarth *et al.*, 1988a,b; Karl *et al.*, 2002). Many factors which bear on nitrogenase activity are interdependent such as light, temperature, oxygen and turbulence. Indeed, a variety of different factors may limit the growth and activity of diazotrophs in various areas of the world's oceans at different times of the year.

4.1. Temperature

One key factor for tropical diazotrophs may be water temperature. For example, the distribution of *Trichodesmium* spp. is roughly limited by the 20°C isotherm, and other planktonic cyanobacteria are likewise primarily tropical or subtropical in distribution. Metabolically active populations of *Trichodesmium* have been observed at 18.3°C in the North Atlantic (McCarthy and Carpenter, 1979), but activity was low, and substantial growth is typically not seen until water temperature exceeds 20°C (Carpenter, 1983a,b). Moreover, water temperature co-varies inversely with surface nutrient concentrations (Kamykowski and Zentara, 1986). Indeed, in previous studies we have used sea surface temperature as a proxy for oligotrophic waters in order to estimate the areal range of *Trichodesmium* (Capone *et al.*, 2005).

Low, but measurable N_2 fixation rates were recently reported by Needoba *et al.* (2007), from the inter-convergence zone, 200 km off the coast of Northern California, where sea surface temperatures were >19°C. In addition, they reported the temporal dynamics of mRNA abundance for *nifH* down to 80 m, where temperatures ranged 14–19°C, and expression was dominated by a unicellular Group A phylotype, and to a lesser extent by Group B. Similarly, Holl *et al.* (2007) reported N_2 fixation in the upper water column of warm core and cold core eddies at temperatures at or below 19C in the Leeuwin Current off the west coast of Australia.

As for most enzyme mediated reactions, increases in temperature often increase activity in a characteristic fashion. The response of an enzyme to temperature increases, often evaluated as the Q_{10} parameter (i.e., the increase in activity over a 10°C range), varies among and within enzyme systems and can show compensation. Staal *et al.* (2003) have evaluated Q_{10} responses for N_2 fixation on short time scales in a range of cyanobacteria including *Trichodesmium*, which exhibited a Q_{10} of 1.12 for dark N_2 fixation over the temperature range of 20–35°C, and a much

higher value of 2.06 over the range 15–20°C. Light N_2 fixation showed less variation over the 2 temperature ranges at 1.64 for 15–20°C and 1.84 for 20–35°C.

In contrast to the tropics, high latitude (>50° latitude) marine waters are generally characterized by low temperatures and higher nutrient concentrations, and there is little evidence of marine diazotrophy in the water column of these systems. However, it should be recognized that low temperature does not intrinsically restrict nitrogenase activity, which has been observed at temperatures near 0°C in terrestrial environments (Englund and Meyerson, 1974; Fogg and Stewart, 1968; Vincent, 2000). One other exception to this generalization is the Baltic Sea which experiences extensive blooms of diazotrophic cyanobacterial blooms (*Aphanazominon, Nostoc* and *Nodularia*) during the summer when surface temperatures are warmest and water column stability greatest (Finni *et al.*, 2001; Wasmund *et al.*, 1998).

4.2. Light

For photoautrophic diazotrophs, nitrogenase activity is intimately linked to photosynthesis (Gallon, 2001). Thus, light is an obvious and important factor potentially regulating or constraining this process. The general influence of light on photoautrophic processes in the sea is covered comprehensively elsewhere (Falkowski and Raven, 1997; Kirk, 1995). There are potential interactions of light with other physical and chemical factors in the ocean such as water column stability and turbulence (Howarth *et al.*, 1995a,b), depth, particle density and the concentration of dissolved organic matter, all of which can affect the quantity and quality of the light field and in turn the activity of photosynthetic diazotrophs. Amongst the photoautotrophic diazotrophs, cyanobacteria have received the most attention and in marine systems this has largely been focused on *Trichodesmium* spp. in the open ocean and in cyanobacteria in shallow mat communities.

Carpenter (1983a,b) has summarized much of the early marine work largely relating to *Trichodesmium* spp. with respect to their relationship to light. Whereas many non-heterocystous cyanobacteria fix nitrogen during the night, and thereby uncouple N_2 fixation from photosynthesis (although photosynthesis is the ultimate source for energy and reductant for even these obligate autotrophic organisms, Gallon and Stall, 1992), *Trichodesmium* fixes nitrogen exclusively during the light period and shows a strong diel pattern of activity with maxima during midday (Berman-Frank *et al.*, 2001a,b; Capone *et al.*, 1990; Saino and Hattori, 1978). Indeed, an endogenous rhythm, rare in prokaryotes, underlies this pattern (Chen *et al.*, 1998). Mechanisms by which this happens have been proposed and are debated (see next section on Oxygen).

Natural populations of *Trichodesmium* which are often found in the upper layers of the euphotic zone (see Chapter 16 by Karl *et al.*, this volume), appear to be adapted to high light with a relatively shallow compensation depth (typically 100–200 μmol quanta m^{-2} s^{-1}) for photosynthesis (Carpenter, 1983a,b; LaRoche and Breitbarth, 2005). Several early studies considered the light–photosynthesis relationships of *Trichodesmium* (e.g., Lewis *et al.*, 1988; Li *et al.*, 1980). Half saturation (I_k) constants for photosynthesis are reported to be about 300 μmol quanta m^{-2} s^{-1} (based on results from four studies, LaRoche and Breitbarth, 2005). See LaRoche and Breitbarth (2005) for a recent comprehensive summary of observed physiological and photosynthetic parameters for *Trichodesmium*.

While several studies have reported photosynthetic parameters for *Trichodesmium*, there have been far fewer studies which have considered the relationship of light and N_2 fixation. N_2 fixation has recently been incorporated as an explicit feature of many ecosystems models (e.g., Fennel *et al.*, 2002; Hood *et al.*, 2001, 2004; Moore *et al.*, 2002, see also Chapter 33 by Hood and Christian, this volume). This has required parameterization of N_2 fixation and that has been done most often using an approach similar to that for photosynthesis. This involves representation of the relationship between light and N_2 fixation (assuming this is largely a photoautotrophic associated process). Hood *et al.* (2002) modeled the photosynthetic parameters, P_{max} and alpha, for field data from a series of stations in the tropical N. Atlantic; the model empirically examined the effect of light intensity on N_2 fixation by *Trichodesmium* (original data reported in Capone *et al.*, 2005). Values for alpha ranged from about 4 to 26 μmol N (mg chl a)$^{-1}$ (μmol quanta m^{-2} s^{-1})$^{-1}$ with a mean of about 11 μmol N (mg chl a)$^{-1}$ (μmol quanta m^{-2} sec^{-1})$^{-1}$. The data suggested a potential for photoinhibition at about 1200 μmol quanta m^{-2} s^{-1}.

Two recent studies have directly examined the effect of light on N_2 fixation in *Trichodesmium*. Breitbarth et al. (submitted) found diazotrophic growth in axenic cultures of *Trichodesmium* IMS 101 to be light saturated above 180 μmol quanta m^{-2} s^{-1} and did not vary significantly at higher light. During mid-exponential phase, N_2 fixation saturated at about 300 μmol quanta m^{-2} s^{-1} and was stable above that. They also noted photoinhibition of N_2 fixation at photon fluxes greater than 1100 μmol quanta m^{-2} s^{-1}.

Finzi (2006, Finzi-Hart *et al.* in review a) compared alpha and P_{max} for natural populations of *Trichodesmium* in the tropical N. Atlantic and N. Pacific finding consistently lower values in the N. Atlantic and hypothesized that this might be due to differences in major limiting factors in the two regions. Finzi (2006, Finzi-Hart *et al.* in review b) went on to demonstrate the effect of iron and P stress on these parameters in cultures of *Trichodesmium* IMS 101. They found that in both cases, both alpha and P_{max} for both carbon and N_2 fixation were severely reduced.

Benthic cyanobacteria also show strong diel patterns which depend on the composition of the cyanobacterial population. Mats dominated by heterocyctous cyanobacteria generally show a daytime maximum while those dominated by non-heterocystous forms exhibit maximum nitrogenase activity at night (e.g., Paerl *et al.*, 1988; Stal and Krumbein, 1987; Steppe and Paerl, 2005).

The relationship to light of the newly discovered planktonic coccoid groups is still being resolved. Group B coccoids appear to temporally segregate N_2 fixation with activity largely associated with the dark phase while Group A enigmatically exhibit maximum activity during daylight hours (Montoya *et al.*, 2004; Zehr *et al.*, 2001, 2007).

Over the last decade or so, two other groups of light-dependent prokaryotes have been discovered in the upper ocean: the Aerobic Anoxygenic Photobacteria or AAPs are found largely among the *Erythrobacter* and *Roseobacter* clades of the proteobacteria (Stackebrandt *et al.*, 1996). There is active debate as to the relative ecological importance of these groups in oceanic photosynthesis (see Kolber *et al.*, 2000) *cf.* (Goericke, 2002; Allgaier *et al.*, 2003). Another recent discovery is that of the presence of light-sensing rhodopsin molecules, termed proteorhodopsins, in a range of proteobacteria and cyanobacteria (Beja *et al.*, 2001), and perhaps other organisms. Proteorhodopsins have been implicated in energetic metabolism and sensory responses, and defining the

physiological role of these molecules is a current area of research. Neither AAP nor proteorhodopsin containing diazotrophs have been yet reported. However, many conventional anoxygenic (anaerobic) phototrophs are diazotrophic (Zehr *et al.*, 2003) and it is conceivable that diazotrophs of both types will soon be discovered.

4.3. Oxygen

The upper water columns of most of the world's oligotrophic oceans are well oxygenated. However, the molecular strategies by which *Trichodesmium*, diazotrophic coccoids and heterotrophic proteobacteria reconcile nitrogenase activity, high O_2 concentrations and, in the case of oxygenic photoautotrophs, active O_2 production in *Trichodesmium*, have been a particular enigma. In contrast to many other non-heterocystous cyanobacteria, *Trichodesmium*, fixes N_2 during the day while actively photosynthesizing. Berman-Frank *et al.* (2001a,b) have presented evidence to show that this may be accomplished by (1) the segregation of active nitrogenase into specialized cells termed "diazocytes" (El-Shehawy *et al.*, 2003) and (2) the down regulation of oxygenic photosynthesis during mid-day allowing a temporal window of opportunity. Staal *et al.* (2003) have put forth a solution to the puzzle regarding the paucity of heterocystous cyanobacteria in tropical seas and illustrate well the interaction of physical factors. As water temperature increase, nitrogenase activity also increases, but the differential diffusion of N_2 into the thick-walled heterocyst does not keep up with the increased nitrogenase activity, thus limiting the enzyme for substrate.

With respect to the low O_2 requirement of nitrogenase, oxygen minimum zones (or OMZs) in the water column exist globally both in the open ocean (e.g., eastern tropical North and South Pacific, Arabian Sea) and in more restricted areas (e.g., Cariaco Basin, Black Sea, Gulfo Dulce). These zones are globally important sites of denitrification, but may have elevated concentrations of ammonium in their anoxic cores. Recently, the anammox reaction has been reported to be important in several OMZ systems (Dalsgaard *et al.*, 2003; Kuypers *et al.*, 2005; Thamdrup *et al.*, 2006; Hamersley *et al.*, 2007), and combined nitrogen concentrations were relatively low even in the core of these anoxic plumes. Such sites may be suitable sites for N_2 fixation, particularly near the oxic/anoxic interface where both O_2 concentration and combined nitrogen may be reduced. However, N_2 fixation has not generally been considered in these systems.

In addition to affecting N_2 fixation directly through interaction with the nitrogenase enzyme, oxygen may also an important role in the global control of this process. As mentioned, OMZs are sites of removal of combined nitrogen by denitrification and Anammox, thereby affecting the relative availability of nitrogen to phosphorus. As mentioned above, modeling evidence by Deutsch *et al.* (2007) indicates this may indeed be the case. Several other modeling efforts have considered the interaction of OMZs with marine N_2 fixation (Canfield, 2006; Moore and Doney, 2007). Moreover, the redox associated with OMZs may affect the relative availability of key trace metals necessary for N_2 fixation.

4.4. Turbulence

Since conventional nitrogenase is uniformly very O_2 sensitive across the spectrum of physiological types (Gallon, 1992), physical mixing in planktonic systems has been recognized as a potentially important factor in regulating N_2 fixation, particularly for

non-heterocystous forms such as *Trichodesmium*. Carpenter and Price (1976) found agitation rapidly inactivates nitrogenase activity in *Trichodesmium*, and a strong inverse correlation exists between sea state and the extent of fixation by natural populations of this cyanobacterium. However, relatively dense populations of *Trichodesmium* are routinely encountered throughout the Trade Wind belts (Carpenter *et al.*, 2004) with their steady 20–30 knot breezes. How turbulence might affect planktonic microbial diazotrophs remains to be determined (e.g., see Howarth *et al.*, 1993, 1995; Paerl *et al.*, 1995).

Factors controlling N_2 fixation in reef environments have been considered in several recent studies. Williams and Carpenter (1998) investigated the importance of natural water flow on nitrogenase activity associated with algal turfs of coral reef environments and found a positive correlation between flow and activity. Higher activity was associated with oscillatory flow compared to unidirectional flow.

4.5. Salinity

Fu and Bell (2003a,b,c) have recently reported that a *Trichodesmium* isolate from the Great Barrier Reef was relatively euryhaline, showing growth over the range of 22–43. However, maximum growth and nitrogenase activity occurred over a much narrower range of 33–37. Recent evidence that Diatom-*Richelia* symbioses are tolerant to lower salinities was reported by Foster *et al.* (2007). They found significant *nifH* gene copy abundances for 2 of the 3 heterocystous cyanobiont lineages within the fresh water lens of the Amazon River plume (salinity >26). In addition, *Trichodesmium nifH* gene copies were also detected within the lower salinity water, however, abundances sharply increased for *Trichodesmium* below the lens (Foster *et al.*, 2007).

4.6. Trace metals

Over the last three decades, we have moved from the monolithic view that nitrogen is the primary limiting nutrient for phytoplankton in the world's oceans (e.g., Thomas, 1966; Ryther and Dunstan, 1971), to the recognition that iron, silicon or phosphorus may at times be in shortest supply. Further, it is recognized that separate groups with distinct biogeochemical functions exist within the phytoplankton that may concurrently exhibit limitation by different nutrient factors (Dugdale and Wilkerson, 1998).

N_2 fixers represent an important functional group that includes a variety of physiological types with unique nutrient requirements. Furthermore, N_2 fixers have unique or elevated cell quotas for certain metals as a result of possessing the nitrogenase enzyme system which requires both molybdenum and iron.

Since diazotrophs can be successful in oligotrophic regions due to their ability to utilize the largest reservoir of nitrogen, N_2 gas, their growth there must ultimately be constrained by the availability of other nutrients such as iron, phosphorus or molybdenum. Early attention focused on molybdenum as a possible growth constraint for marine diazotrophs through the interference with its uptake by the stereochemically similar species, sulfate, which occurs in very high concentrations in seawater (Howarth and Cole, 1985). However, molybdenum concentrations are relatively high (Millero, 1996), and subsequent experimental work has indicated that it is generally not growth limiting (Paerl *et al.*, 1987; Paulsen *et al.*, 1991). Recently, however, Marino *et al.* (2003) have reported on further experiments with diazotrophic lake planktonic that suggest sulfate inhibition of Mo transport.

The *in situ* chemical controls on oceanic N_2 fixation, in particular iron and phosphorus, are the topic of much current research effort and debate. Fueling these efforts has been the observation of extensive areas of excess N (relative to Redfield regeneration stoichiometry, often expressed as positive $N\star$ anomalies) relative to P in subeuphotic zone waters in the North Atlantic that roughly correspond to regions which receive substantial aeolian dust deposition (Michaels *et al.*, 1996; Gao *et al.*, 2001; Gruber and Sarmiento, 1997). Wet and dry deposition of mineral aerosols fertilize the surface ocean with Fe (along with N and P) (Mahowald *et al.*, 1999).

Hence, considerable attention has been focused and substantial progress made in evaluating the role of Fe with respect to *Trichodesmium* growth. Rueter (1988) first presented evidence that Fe can regulate *Trichodesmium* growth and nitrogenase activity. He found increases in CO_2 and N_2 fixation rates and cellular chlorophyll *a* content in natural samples of *Trichodesmium* collected near Barbados amended with Fe. Similarly, Paerl and others (Paerl *et al.*, 1994) reported stimulation of growth and nitrogenase activity by Fe in cultures and natural samples of *Trichodesmium*.

Several recent studies (Berman–Frank *et al.*, 2001a,b; Kustka *et al.*, 2003; Sañudo-Wilhelmy *et al.*, 2001) estimate that Fe requirements of photosynthetic diazotrophs are at most 10-fold greater than for strictly photosynthetic organisms assimilating NH_4^+, much less than earlier theoretical estimates which were 100 times higher (Raven, 1988). However, even an amplified cell quota of 10-fold is likely to be critical in waters where N_2 fixation occurs, as dissolved Fe levels are generally <1 nM (Wu *et al.*, 2000). Fe limited cultures of *Trichodesmium* strain IMS101 were shown to have reduced N_2 fixation rates compared to Fe-replete cultures (Berman–Frank *et al.*, 2001a,b). N_2 fixation rates in cultures of *Trichodesmium* were more greatly stimulated by Fe additions than were cell yields (Fu and Bell, 2003a,b,c). Various natural organic compounds may facilitate, or impede, how accessible Fe is to diazotrophs (Achilles *et al.*, 2003). No information is currently available on the Fe requirement of microbial diazotrophs.

A number of studies have speculated on diazotroph response to mineral dust Fe fertilization in the Atlantic (Mahaffey *et al.*, 2003), and Pacific Oceans (Johnson *et al.*, 2003). Circumstantial evidence for dust stimulation of marine diazotrophic biomass has recently been reported. A 1999 Saharan dust event coincided with increases in dissolved Fe concentrations on the west Florida shelf and a 100-fold increase in *Trichodesmium* biomass. N_2 fixation rates were not measured, but DON concentrations doubled, presumably due to exudation by N_2 fixers (Lenes *et al.*, 2001). A recent Saharan dust addition experiment to surface water samples collected along a west African cruise transect ($35°W–17°W$), found a minimal increase in CO_2 fixation and a large stimulation in N_2 fixation, suggesting that diazotrophs were co-limited by both P and Fe (Mills *et al.*, 2004). Note however, analysis of aerosol dust shows that while providing Fe, it also supplies P and combined N (Baker *et al.*, 2003; Mills *et al.*, 2004; Ridame and Guieu, 2002).

Due to the presumed elevated Fe requirement of photosynthetic diazotrophs and low atmospheric dust deposition to many oligotrophic areas (low nutrient, low chlorophyll, or LNLC), some have suggested that Fe limits N_2 fixation globally (Falkowski, 1997; Rueter *et al.*, 1992;). Combining data on *Trichodesmium* Fe quota and data on aeolian dust fluxes, (Berman–Frank *et al.*, 2001a,b) estimate that N_2 fixation is Fe limited in 75% of the world's oceans, a conclusion echoed by Moore *et al.* (2002) (Figs. 4.3 and 4.4).
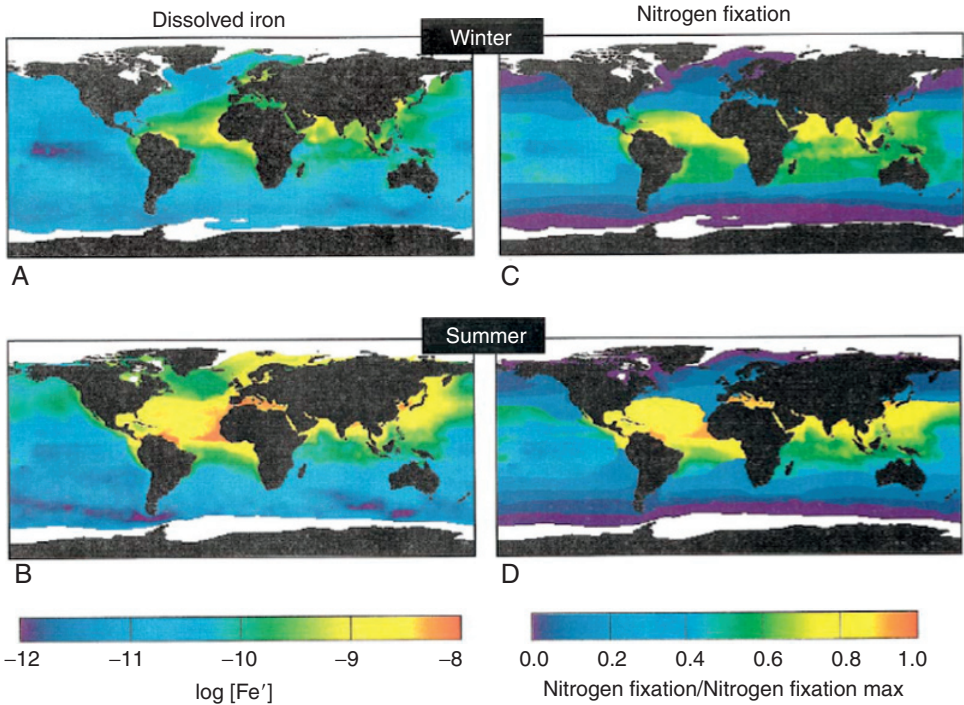
Figure 4.3 Seasonal maps showing the potential for nitrogen fixation in the world oceans. Maps were generated by converting global dust iron fluxes to total dissolved iron and using the relationship shown in between log[Fe'] and nitrogen fixation: maximum nitrogen fixation. From Berman–Frank *et al.*, 2001 (Fig. 4.6) with permission.
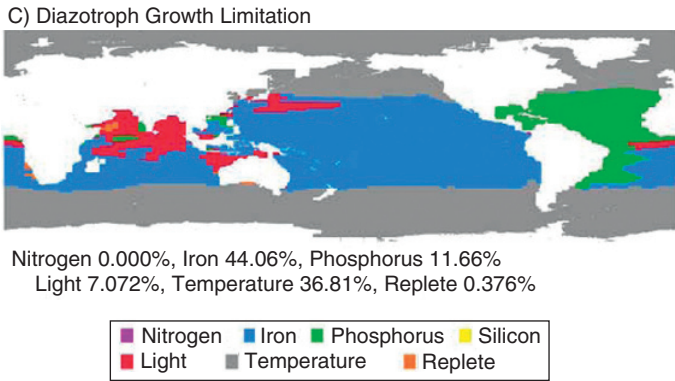

Figure 4.4 Modeled areas of diazotroph nutrient limitation. Nutrient-limitation patterns for the diazotrophs during summer months. Areas where all nutrient cell quotas are >97% of the maximum cell quota values are arbitrarily defined as nutrient-replete. Also shown is the percentage of total ocean area where each nutrient is limiting growth. From Moore *et al.* (2004) with permission.

4.7. Inorganic nutrients

4.7.1. Phosphorus limitation

In contrast to the general sense that Fe is the predominant limiting factor for oceanic N_2 fixation, Wu *et al.* (2000) reported that the subtropical North Atlantic is more depleted in P in comparison to the sub-tropical North Pacific. They suggested that this occurs because more Fe reaches the subtropical North Atlantic than the subtropical North Pacific via aeolian dust deposition (Prospero and Lamb, 2003). They posit that the available Fe in the North Atlantic increases N_2 fixation, leading to a draw-down of all available P. With the accumulating observations in the North Atlantic and North Pacific, there is indeed a suggestion of somewhat higher rates of N_2 fixation for at least *Trichodesmium* in the North Atlantic (Capone et al., in prep.), consistent with the hypothesis that P is more severely limiting for diazotrophs in the North Atlantic while Fe is more limiting in the North Pacific. Corroborating this suggestion, Sañudo-Wilhelmy *et al.*, (2001) found that internal nutrient stoichiometry of field populations of *Trichodesmium* exhibits a stronger relationship of N_2 fixation with cellular phosphate concentration compared to Fe content (Sañudo-Wilhelmy *et al.*, 2001) indicating that *Trichodesmium* from the North Atlantic are more likely limited by P. More recently; Sañudo-Wilhelmy *et al.* (2004) reported that a substantial fraction of the inorganic P associated with *Trichodesmium* (and other algae) is adsorbed to the surface. Dyhrman *et al.* (2002) also provided evidence of P stress in populations of *Trichodesmium* from the Atlantic.

In the Central North Pacific Ocean (CNPO) it has also been reported that phosphorus availability limits N_2 fixation and primary production (Karl *et al.*, 2001a,b) (but iron availability may also play a role as suggested by Wu *et al.* (2000). Karl *et al.* (2001a,b) contend that since the mid 1970s there has been an enhanced stratification in the CNPO and a decreased inorganic nutrient availability which selects for diazotrophs and shifts from a N-limited phytoplankton population to one that is either P or Fe limited (see Karl *et al.*, Chapter 16, this volume).

In contrast, Wu *et al.* (2003) have concluded that N rather than P is limiting to phytoplankton in the South China Sea, and that a relative paucity of Fe, despite considerable atmospheric deposition, limits the ability of diazotrophs to compensate for the N deficiency.

Diazotrophs such as *Trichodesmium* exhibit a relatively high and variable cellular N:P ratio (Krauk *et al.*, 2006; Letelier and Karl, 1996, 1998; Mague *et al.*, 1977; White *et al.*, 2006). Considering surface absorption, intracellular N:P ratios are substantially higher than bulk measurements. The ability of diazotrophs (like many organisms) to sequester P coupled with their relative plasticity in N:P stoichiometry of diazotrophs may allow them to sustain relatively high growth over a range of P concentrations. Such factors need to be considered in the interpretation of short-term measurements of P uptake and growth.

Also, inorganic P is not the only P source for *Trichodesmium* in the open ocean (Mulholland *et al.*, 2002). *Trichodesmium* produces alkaline phosphatase (APA), which cleaves PO_4^{-3} from dissolved organic P compounds to provide an additional P source to the organism. APA activity in natural populations of *Trichodesmium* can indicate P stress to some degree. APA was much higher in the North Atlantic, where inorganic P concentrations are extremely low, than off the northern coast of

Australia where P concentrations are perhaps 10-fold higher (Mulholland et al., 2002). APA was also found to be 10-fold higher in P-deplete cultures of Trichodesmium strain WH9601 compared to P-replete cultures (Stihl et al., 2001). Sohm and Capone (2006) report that APA can account for a major fraction of the P uptake by this organism. However, APA is not a direct index of general P limitation, but an indication that Trichodesmium are experiencing low levels of inorganic P (Scanlan and Wilson, 1999) and may be exploiting organic pools of P.

Several studies have examined the P uptake kinetics of Trichodesmium (Table 4.2). McCarthy and Carpenter (1979) first reported a relatively low affinity (Ks = 9 μM) and long P- based doubling times (>200 d) for natural populations of Trichodesmium. More recently, Fu et al. (2005) determined half saturation values of 0.4–0.6 μM and 0.1–0.2 μM for a strain they isolated from the Great Barrier Reef and for the widely used strain IMS 101 (Prufert-Bebout et al., 1993), respectively.

In contrast, Moutin et al. (2005) reported that natural populations of Trichodesmium, mostly as free trichomes, in the SW Pacific, had relatively high affinity for phosphate, with a Ks of 630 nM and a V_{max} of about 0.31 h^{-1} (Table 4.2). They stated that these are the first accurate determinations of these parameters at environmentally relevant concentrations. They further asserted that the high Ks values presented in the text of McCarthy and Carpenter (1979) were due to a calculation which used an incorrect value for the colony specific rate of 1.2 pmol colony^{-1} h^{-1}, and which Moutin et al. (2005) state should have been 1.2 nmol^{-1} colony^{-1}. This would result in values more in line with their observations. However, McCarthy (personal communication to DGC) stated that the original calculation and values given in the text of McCarthy and Carpenter (1979) are correct but that Fig. 4.4 in McCarthy and Carpenter (1979) had an incorrectly labeled y axis.

Most recently, Sohm and Capone (2006) compared kinetic constants of bulk plankton and Trichodesmium colonies on a transect in the subtropical and tropical N. Atlantic. They found a K_s of about 0.015 μM and V_{max} of about 12 nmol P (μg chl a h^{-1}) for the plankton. K_s for Trichodesmium colonies were substantially higher (0.38–1.1 μM) and V_{max} estimates much lower (5–15 nmol P μg chl a^{-1} h^{-1} or 0.19–0.5 nmol P colony^{-1} h^{-1}).

Sohm et al. (in press) have extended their studies of APA and P uptake kinetics by Trichodesmium colonies in the N. Atlantic to a comparative study of these two indices in the tropical N. Pacific and coastal northern Australian waters as well. They detected sharp contrasts among the sites with much higher V_{max} and APA values in their tropical N. Atlantic stations compared to the N. Pacific and northern Australia indicating more severe P limitation in the tropical N. Atlantic. Dyhrman et al. (2002) also reported evidence of severe P stress in Trichodesmium in the N. Atlantic based on a enzyme linked fluorescence (ELF) assay. A follow up study also saw less evidence of P stress in samples from the N. Pacific (Hynes et al., Pers. Comm.).

Results by Zehr et al. (2007) from a recent comprehensive experimental study examining the response of diazotrophic populations from station ALOHA in the N. Pacific gyre to phosphorus additions saw little effect on either the composition of the populations or on rates of N_2 fixation, reinforcing the supposition that diazotrophy in this system may be constrained by other factors (see also Chapter 16, Karl et al., this volume).

Table 4.2 Estimates of P Uptake and Turnover by *Trichodesmium* as Reported in Several Studies

Study	Location or isolate	Notes	V_{max}/µg chl *a* h Total	Intra	nmol P/col h Total	Intra	Ks µM	Td days
McCarthy and Carpenter, 1979	Sub-trop Atlantic		0.04[a]		0.0012		9	200
Sanudo-Wilhelmy et al., 2004	Tropical Atlantic	P replete	0.19[b]	0.089	0.007[c]	0.0032	0.32	0.28
		P limited	0.72[b]	0.3	0.026[c]	0.011	0.39	0.22
Fu et al., 2005	IMS 101	P replete	0.39		2.95[d]		0.34	
	GBRTRLI101	P replete	0.80		6[d]		0.68	
	IMS 101	P limited	2.27		17[d]		0.42	
	GBRTRLI101	P limited	4.73		35.5[d]		0.64	
Moutin et al., 2005	Trop SW Pacific						0.63	0.7–7
Sohm and Capone, 2006	Trop-NW Atlantic	Sta 5	5.5		0.104		0.85	
	Trop-NW Atlantic	Sta 6	10.8		0.09		0.38	
	Trop-NW Atlantic	Sta 6	14.8		0.12		1.1	
Sohm et al. in press	Trop-NW Atlantic	8 sta	9.6 (2.9)				0.22 (0.05)	
	Trop-N Pacific	7 sta	1.1 (0.3)				0.12 (0.04)	
	Coastal N, Aust.	5 sta	1.7 (0.7)				0.13 (0.05)	

[a] Assumed 34 ng chl/colony, from Carpenter, 1983.
[b] Assumed 180 pg chl *a* and 100 cells per trichome.
[c] Assumed 100 cells per trichome and 200 trichomes per colony.
[d] Assumed 100 cells and 50 trichomes per colony.

In contrast, an open ocean phosphorus enrichment experiment (CYCLOPS) conducted in the eastern Mediterranean did find evidence for P limitation of N_2 fixation in bulk water (Rees *et al.*, 2006).

An exciting, recent development is the detection of the potential for *Trichodesmium* to exploit phosphonate compounds as another source of P (Dyhrman *et al.*, 2006). More research is needed to clarify the importance of phosphonates as P source for this organism in nature. This pathway was predicted after the sequencing and analysis of the *Trichodesmium* genome (http://genome.jgi–psf.org/draft_microbes/trier/trier.home.html).

In the benthic realm, working in the framework of the ENCORE coral reef research program in Australia, Koop *et al.* (2001) reported stimulation of lagoon sediment N_2 fixation by additions of phosphate suggesting sediment N_2 fixation in carbonate systems may also be limited by phosphorus as earlier observed for seagrasses (Short *et al.*, 1990).

4.7.2. Combined nitrogen

Only a limited number of studies have considered the interaction of combined N sources with oceanic N_2 fixers. Some early work suggested that *Trichodesmium* only had a weak capacity for inorganic N uptake (Carpenter and McCarthy, 1975; Glibert and Banahan, 1988). However, more recent studies indicate that *Trichodesmium* is capable of exploiting some inorganic and organic N pools. Both Carpenter *et al.* (1992) and Capone *et al.* (1994) reported the capacity of *Trichodesmium* to take up some amino acids. Mulholland and colleagues undertook a comprehensive series of studies examining the uptake capacity for several combined N sources by *Trichodesmium* in culture and in the field (see Mulholland and Capone, 2000, for a general overview). With the NIBB1067 culture, they reported high affinity and capacity for ammonium, urea and glutamate but relatively low capacity for nitrate during growth on N free media, but that nitrate and urea supplied the bulk of daily N demand when they were available in excess (Mulholland *et al.*, 1999). In cultures without added N, ammonium uptake and release was dynamic and accounted for the bulk of N turnover while N_2 fixation represented only about 16–18% of total N turnover but 100% of the demand for net growth. The high turnover of N may support a high level of gross production accounting for some of the mismatch between C based and N based growth in the literature (Carpenter, 1983a,b; Lipschultz and Owens, 1996). Further studies note similar results in another culture of *Trichodesmium*, IMS101 and field populations (Mulholland and Capone, 1999). In another study of *Trichodesmium* IMS101 where similar levels of ammonium turnover were also documented, Mulholland and Capone (2001) were able to mass-balance C and N accumulation in biomass. They found that whereas C_2H_2 reduction estimates of N_2 fixation closely predicted net particulate organic nitrogen (PON) accumulation, short term (1 h) $^{13}CO_2$ uptake estimates during the light period overestimated particulate organic carbon (POC) accumulation in mid to late exponential phase. Again, this may be a result of the high ammonium turnover. In a subsequent study, Mulholland *et al.* (2004) also tracked the flux into dissolved organic nitrogen (DON), finding that DON release could account for the difference between $^{15}N_2$-based and C_2H_2 reduction based estimates of N_2 fixation.

Relatively few studies have considered the influence of combined N on nitrogenase activity in oceanic marine diazotrophs, perhaps because of the often low concentrations encountered in the upper ocean of oligotrophic areas (see Capone, 1988) for a summary of such work in benthic systems). Capone *et al.* (1990) used relatively high doses (100 μM) of ammonium to experimentally shut off nitrogenase synthesis, which typically resulted in a decrease in activity within 2 h. Mulholland *et al.* (2001) saw little short term effect of low (1 μM) additions of glutamate, glutamine, ammonium, nitrate and urea on nitrogenase activity by natural and cultured populations of *Trichodesmium*. However, 10 μM concentrations of ammonium and nitrate did reduce nitrogenase activity within 2–4 h. In contrast, Fu and Bell (2003a,b,c) reported little inhibition at this level for short term (first generation, 3 h) exposures to ammonium, nitrate or urea, although by the fifth generation, inhibition became evident.

Most recently, Holl and Montoya (2005) examined the interactions of nitrate with nitrogenase activity in *Trichodesmium* IMS 101 in continuous culture. Interestingly, maximum inhibition of nitrogenase activity occurred at 10 μM nitrate, but was not complete, with 30% of the activity remaining. This is in contrast to some earlier studies (Ohki *et al.*, 1991) where inhibition was complete. This may indicate fundamental differences in response between the continuous culture approach, relative to the batch cultures used in all earlier studies. At high concentrations, nitrate could account for most of the N demand, and more than made up for the decrease in N_2 fixation.

4.7.3. Inorganic carbon

Because of the high concentrations of inorganic carbon in surface waters of the ocean, it is rarely thought about as a potential limiting factor for plankton growth. However, several recent studies taking confluent research tracks have shown elevated DIC stimulates both growth and N_2 fixation in *Trichodesmium*.

Levitan *et al.* (2006) noted enhanced N_2 fixation, C:N ratios, filament length and biomass of *Trichodesmium* isolate IMS 101 at pCO_2 of 900 ppmv relative to 400 ppmv or 250 ppmv. Photosynthesis and respiration did not elicit a strong response to increasing pCO_2. Similarly, Hutchins *et al.* (2007) found a 35–100% increase in N_2 fixation and a 15–128% in CO_2 fixation rates in 2 isolates of *Trichodesmium* (IMS 101, and another strain isolated from the Great Barrier Reef) at 750 ppm relative to 380 ppm. The response occurred under both P replete and P depleted conditions. No growth was recorded at 150 ppm and a 4°C temperature increase only had minimal affects.

The model of Barcelos e Ramos *et al.* (2007) predicts both N_2 fixation rates and cell division rates will double at year 2100 from the projected increases in CO_2 levels, and would potentially result in lower C, N and P cell content as well as reduced cell size and steady C:N content (the last observations in contrast to Levitan *et al.*, 2006).

4.7.4 . Global patterns of nutrient limitation

As noted above, there is an emergent view that oceanic diazotroph populations may be limited by different nutritional factors in different ocean basins. In contrast to the general perception that Fe is the predominant limiting factor for oceanic N_2 fixation, Wu *et al.* (2000) noted that diazotrophs in the subtropical North Atlantic are more likely stressed for P in comparison to populations in the sub-tropical North Pacific. They suggested that this occurs because more Fe reaches

the subtropical North Atlantic than the subtropical North Pacific via aeolian dust deposition (Prospero and Lamb, 2003). They posited that the available Fe in the North Atlantic increases N_2 fixation rates, leading to a draw-down of all available P. Indeed, surface ocean concentrations of Fe are often relatively high in the North Atlantic (Wu *et al.*, 2001) and this input may drive the North Atlantic Ocean to P limitation, at least for N_2 fixation (Wu *et al.*, 2000). As noted, several lines of evidence have provided corroboration for this supposition (Sañudo-Wilhelmy *et al.*, 2001; Dyhrman *et al.*, 2002; Krauk *et al.*, 2006; Sohm and Capone, 2006).

In the Central North Pacific Ocean (CNPO) it had been hypothesized that P availability limits N_2 fixation and primary production (Karl *et al.*, 2001b; see also Karl *et al.*, Chapter 16, this volume). Karl *et al.* (2001a) suggested that since the mid 1970s there has been enhanced stratification in the CNPO and decreased inorganic nutrient availability, which has selected for diazotrophs and has caused a shift from an N-limited phytoplankton population to one that is P limited. However, except for the recent bioassay experiment, Zehr *et al.* (2007) noted above which found no increase with P addition in the transcription of *nifH* genes or in rates of activity over a control for samples from station ALOHA. experimental evidence for P limitation of N_2 fixation is currently lacking. Indeed, recent P★ analyses by Deutsch *et al.* (2007), find large excesses of P, relative to N, in surface waters of the eastern Pacific associated with the selective removal of N by denitrification and anammox in OMZ. They further note that this excess decreases in the absence of surface nitrate as waters advect offshore from sites of upwelling and associate this removal with nitrogen fixation. Again, these observations reinforce the suggestion by Wu *et al.* (2001) that iron limitation of diazotrophs is more likely in this system.

Field surveys using classical microscopic methods in limited areas of the ocean have reported high densities of *Trichodemium* at times in the southwest Sargasso Sea, in the Caribbean and tropical N. Atlantic, and China Sea/Kurishio (see Carpenter, 1983) for early work, also Carpenter *et al.* (2004)). There is some evidence for a bimodal distribution in nitrogen fixation zonally across the tropical Atlantic, with some shift in the dominant diazotrophs from *Trichodesmium* in the western basin and coccoids in the eastern basin (Montoya *et al.*, 2007; Langlois *et al.*, 2008), Tyrrell *et al.* (2003) noted a distinct drop in *Trichodesmium* densities in the S. Atlantic compared to the N. Atlantic. New molecular techniques have provided valuable tools to address some of the inherent limitations of traditional microscopic assessments of diazotrophic abundance and species composition, which cannot address variations on large spatial and/or temporal scales. Specifically, several recent papers have provided quantitative information on large scale distributions of several diazotrophs using qPCR methods. These data provide some corroboration for the perception that diazotrophic populations may vary both quantitatively and qualitatively among different basins. Foster *et al.* (2007), working in and around the plume of the Amazon River noted high abundances of *Richelia* at many stations. *Richelia* associations with diatoms accounted for about 60% of the *nifH* genes in surface waters, and were dominant at 12 of 22 stations. *Trichodesmium* dominated at 4 of the stations whereas Type A and type B cyanobacteria were dominant at only 1 station each. Type C cyanobacteria dominated at 4 stations. Langlois *et al.* (2008) reported results from 4 major cruises and about 76 discrete stations which sampled over a broad swath of the temperate to tropical N. Atlantic Ocean. They found filamentous

non-hetercyctous cyanobacteria to account for 53% of *nifH* sequences retreived in all samples, followed by Group A coccoids which accounted for 34% of all samples. Group B coccoids accounted for 4.3% of the total phylotypes.

In contrast, a recent study by Church *et al.* (2008) provided data from 7 stations in the Pacific Subtropical Gyre, 3 in the North Equatorial Counter Current and 3 stations in the North Pacific Transition Zone which indicated that filamentous cyanobacteria were not dominant at any of the stations whereas Type A coccoid cyanobacteria did account for the majority of *nifH* sequences (10 of 13 stations). Heterocystous forms dominated at 3 stations. Hence, quantitative field data suggest fundamental differences in the assemblages of diazotrophs between the N. Pacific and N. Atlantic subtropical gyres reinforcing the suggestion of basin-scale differences in factors limiting diazotrophs and structuring their populations.

Recent modeling efforts explicitly representing oceanic N_2 fixation using physiological growth requirements also have come to the conclusion that there are distinct oceanic zones with regard to predominant limiting factors for diazotrophs (Coles and Hood, 2006; Krishnamurthy *et al.*, 2007).

5. Biogeochemical Significance of Marine Nitrogen Fixation

In 1982, we had estimated annual global rate of N_2 fixation in the sea was 0.34×10^{12} mol N among the near surface plankton (Carpenter, 1983a,b) and 1.1×10^{12} mol N in sediments (coral reefs, sea grass beds, estuarine sediments, salt marshes and mangroves)(Capone and Carpenter, 1982a,b; Capone, 1983; Carpenter, 1983a,b) (Table 4.3). Our estimates were based on the relatively limited direct field data that had accumulated to that point and, for the plankton, historical data sets on the density of *Trichodesmium* in the world's oceans. We concluded at the time that this appeared to be a relatively minor input to the sea (Capone and Carpenter, 1982a,b).

Over the last several decades, independent lines of indirect geochemical evidence have strongly indicated that N_2 fixation rates are of greater quantitative significance than this early estimate (Mahaffey *et al.*, 2005) (Table 4.3). During the late 1970s and 1980s, N stable isotope distributions of suspended particulate matter first suggested that N_2 fixation was substantial in tropical waters (Altabet, 1988; Saino and Hattori, 1980; Wada and Hattori, 1976) as did subsequent observation of anomalously high DON concentrations in tropical surface waters (Hansell and Feely, 2000; Vidal *et al.*, 1999) and CO_2 drawdown in the absence of combined N at the Bermuda Atlantic Time Series (BATS) station (Michaels *et al.*, 1994). Inferences and rate estimates on basin scales have shown the global importance of N_2 fixation. For example there have been many reports of environment where substantial deviations from the Redfield ratio in N and P regenerated in some sub-euphotic zone waters occur (Deutsch *et al.*, 2001; Gruber and Sarmiento, 1997; Michaels *et al.*, 1996) (see Chapter 1 by Gruber, and Chaper by 13 by Hansell, this volume) and results from stable N isotope studies in the water column suggestive of N_2 fixation (Karl *et al.*, 1997; Montoya *et al.*, 2002). More recently, inorganic carbon budgets (Gruber *et al.*, 1998; Lee *et al.*, 2002) and ecosystem models in which physiological characteristics of diazotrophs are explicitly represented (Coles *et al.*, 2004; Hood *et al.*, 2004), (see Chapter 33 by Hood and

Table 4.3 Some Recent Extrapolations of Oceanic Nitrogen fixation

System	N_2 Fixation Tmol/y	System	Source
N. Atlantic	0.09	*Trichodesmium*, scaled	Carpenter, 1983a,b
	3.7–6.4	N⋆, residence time	Michaels *et al.*, 1996
	2 (3.2)[a]	Integrated N⋆, N:P	Gruber and Sarmiento, 1997
	4.5	C_t inventory	Gruber and Sarmiento, 1998
	2.4 (1.35)	C_t inventory	Lee *et al.*, 2002 (see Mahaffey *et al.*, 2005)
	1.1	C_t inventory	Anderson and Pondaven, 2003
	0.15–0.46 (0.9–2.8)[a,b]	Integrated Nxs, N:P	Hansell *et al.*, 2004, 2007
	5.5	Isotope Budget	Capone *et al.*, 2005
	1.6	*Trichodesmium*, scaled	Capone *et al.*, 2005
	1.4	P⋆	Deutsch *et al.*, 2007
N. Pacific	2.5	N⋆/Mass balance	Deutsch *et al.*, 2001
	1.5	*Trichodesmium*, scaled	Mahaffey *et al.*, 2005
	6.8	P⋆	Deutsch *et al.*, 2007
Global Marine	0.36	*Trichodesmium*, scaled	Carpenter, 1983a,b
	0.71	*Trichodesmium*, scaled	Carpenter and Capone, 1993
	10	C_t inventory	Lee *et al.*, 2002
	>>7.1	Isotope Budget	Brandes and Devol, 2002
	6.2–11	Extrap/Geochem	Galloway *et al.*, 2004
	9.8	P⋆	Deutsch *et al.*, 2007
	4.0	Modeled	Moore *et al.*, 2004

[a] Assuming an N:P ratio of 45 for diazotrophs rather than 125 as originally computed by Gruber and Sarmiento (1997).
[b] Assuming a domain area of 27.8 k m^2×10^6 (Gruber and Sarmiento, 1997).

Christian, this volume) have also concluded that N_2 fixation is of greater quantitative significance. Factors which led to the relatively low early estimates have been summarized by Capone and Carpenter (1999) and were largely a result of (1) scaling cell specific rates to global distributions of *Trichodesmium* based on historical surveys which systematically under-sampled their population densities and (2) ignoring the input of other diazotrophs (e.g., unicellulars and diatom–diazotroph associations) which have subsequently been discovered and shown to be of quantitative significance as well. Current estimates of oceanic N_2 fixation based on direct observations (largely to date of *Trichodesmium* alone) are much greater, but substantial differences still exist among independent geochemical estimates of N_2 fixation, and with direct biological estimates (Capone *et al.*, 2005; Mahaffey *et al.*, 2005).

5.1. Directly assessing nitrogen fixation

Today, with comprehensive data from more spatially extensive surveys, the direct estimate for open ocean planktonic N_2 fixation far exceeds that in sediments and rivals terrestrial biological rates. For instance, a recent accounting of N_2 fixation at 154 stations from 6 cruises in the tropical Atlantic yielded a conservative annual input of at least 1.6×10^{12} mol N (22×10^{12} g) by *Trichodesmium* alone (Capone *et al.*, 2005) (Table 4.3).

Numerous other potential agents of N_2 fixation have been recognized (see above). However, the quantification of their input has been a greater challenge and is an ongoing endeavor. While the isolation of diazotrophic heterotrophic bacteria and coccoid cyanobacteria led to early speculation that these components of the oligotrophic food web might also contribute to aggregate N_2 fixation (Carpenter, 1983a,b), initial attempts to quantify activity through sample concentration were unsuccessful (Carpenter, Capone and Zehr, unpublished data), perhaps because of the rarified densities of these organisms and the possibility of sample concentration artifacts. The detection of *nifH* transcripts in the small (<10 μm) fraction (Zehr *et al.*, 2001) has prompted renewed interest in assessing the quantitative significance of microbial diazotrophs. Improved methods with higher sensitivities in isotopic analyses have made quantification of activity in this smaller size fraction more amenable to study (Montoya *et al.*, 1996).

Several reports in varied locations have now provided quantification of N input in the small size fraction of the plankton. Montoya *et al.* (2004) found highly variable but at times, extremely high, rates of N_2 fixation in studies in the sub-tropical N. Pacific and along the north coast of Australia (Tables 4.4 and 4.5). Average rates (excluding one extreme value) for 10 stations on the North Pacific transect north of the Hawaiian Islands were about 520 μmol N $m^{-2}day^{-1}$. Curiously, rates were considerably and consistently lower at station ALOHA in the vicinity of the Hawaiian Islands. This was also concordant with an earlier study (Dore *et al.*, 2002) and another recent effort that employed plankton concentrates (Falcón *et al.*, 2004).

Falcón *et al.* (2004) also reported rates of N_2 fixation by plankton concentrates for the tropical Atlantic, which were modest. Garcia *et al.* (2006) have undertaken a size fractionation study in waters near New Caladonia in the southwest Pacific (Tables 4.4 and 4.5). They found that while the contribution in the larger size fraction (>10 μm– associated with *Trichodesmium*) often dominated the input, it was highly variable relative to a steadier background in the <10 μm fraction, which tended to dominate in the absence of macro-diazotrophs. They also examined the <3 μm fraction, which occasionally showed low levels of enrichment from the $^{15}N_2$ isotopic tracer. However, it was not clear that this was endogenous N_2 fixation in this size fraction or a result of trophic transfer. Holl *et al.* (2007), in studies of cold and warm core eddies in the Leeuwin current off the west coast of Australia found the bulk of N_2 fixation in the <10 μm fraction. In any event, there are far too few observations at present to be able to generalize the importance of N_2 fixation in the smaller size fractions of the plankton.

As mentioned, diazotrophic diatom associations (DDAs) can, at times, be of quantitative significance. A bloom of the endosymbiont *Richelia intracellularis* within

Table 4.4 Range of Reported Values for N$_2$ Fixation by in Unconcentrated Water Samples of Plankton with and without Endpoint Size Fractionation

Source	Location	Notes	Average nmol L^{-1}h^{-1}			Low (above 50 m) nmol L^{-1}h^{-1}	High nmol L^{-1}h^{-1}	Source
Bulk water	Tropical N Atlantic	surface				0	23	Goering et al., 1966
	Tropical N. Pacific					0	0.09	Dore et al., 2002
	Tropical N Atlantic					??	3.1	Voss et al., 2004
	Eastern Mediterranean		5.4	0.37		??	8.2	Rees et al., 2006
	Trop. SW Pacific		NA			0.08	1.8	Garcia et al., 2007
	NW Pacific		0.25	0.5	24			Needoba et al., 2007
	Western Med					0.08	0.7	Garcia et al., 2006
	Western Indian Ocean	WCR	0.017	0.016	9			Holl et al., 2007
		CCR	0.043	0.016	10			Holl et al., 2007
	Southeast Pacific	HNL	BD					Bonnet et al., 2008
		GYR	BD					Bonnet et al., 2008
		EGY	BD					Bonnet et al., 2008
	Gulf of California					0	1	White et al., 2007
Large (>10 µm)	Trop. SW Pacific					0.8	1.5	Garcia et al., 2007
Nano (<10 µm)	Sub-tropical N. Pacific	Aloha				0.01	0.016	Zehr et al., 2001
	Tropical N. Pacific					0.005	0.023	Dore et al., 2002
	Sub-tropical N. Pacific	Aloha				0.01	0.15	Montoya et al., 2004
	Sub-tropical N. Pacific	~30 N	0.72	0.2	11	0.05	1.85	Montoya et al., 2004
	N Aust Coast					0.02	60	Montoya et al., 2004
	Trop. SW Pacific	NA				<0.4	0.75	Garcia et al., 2007
	Trop. SW Pacific, lagoon	NA				0.33	0.7	Garcia et al., 2007
	Sub-tropical N. Pacific	Aloha				0.014	0.095	Zehr et al., 2007
	Kane'ohe Bay	Surface				0.029	0.048	Zehr et al., 2007
Pico (<3 µm)	Trop. SW Pacific	NA				<0.08	0.17	Garcia et al., 2007

nr – not reported; BD – below detectio.
WCR – warm core ring; CCR – cold core ring.
HNL – high nutrient low chlorophyll station; GYR – central gyre station; EGY – eastern gyre station.

Table 4.5 Summary of Direct Estimates of Areal Rates of N_2 Fixation for Various Diazotrophic Systems in Diverse Locations either as Compiled in Mahaffey *et al.*, 2005 or more recently reported

Sample Type	Location	Average μmol N m⁻² d⁻¹	se	n	Low μmol N m⁻² d⁻¹	se	n	High μmol N m⁻² d⁻¹	se	n	Number of Studies	Total number of stations	Weighted mean, μmol N m⁻² d⁻¹	Source
Trichodesmium colonies	Subtropical sites				0.6	0.27	5	41	nr	36	6	68	25	1,2,3,4,5
	Tropical N Atlantic				41	7.6	19	898	234	18	9	201	197	3,6,7
	Tropical N. Pacific				53	14	14	134	nr	2	4	75	55	8,9,10,11
	Arabian Sea	36	10	12							1	12	36	12
	N Aust Coast	353	164	19							1	19	353	11
Trichodesmium free trichomes	Tropical N. Pacific				89	26	11	126	nr	32	2	43	157	9,11,13
Trichodesmium blooms	Tropical N Atlantic				231		1	879		2	2	3	663	6,7
	Tropical N. Pacific	319									1	1	319	8
	Arabian Sea				129	23	7				1	7	129	12
	N Aust Coast	691	12	12							1	12	691	11
Richelia blooms	Tropical N Atlantic				1309	412	22	3500		1	3	37	2050	11,14
Pico (<3 μm)	Trop. SW Pacific				43	22	4	87			1			19
Nano (<10 μm)	Tropical N Atlantic				6	2	8	47	nr	2		25	22	11,15
	Tropical N. Pacific				2.2	nr	nr	66	19	7	3	22	37	15,16,17

	Sub-tropical N. Pacific				520	160	10	2800		1	1	9	727	17
	N Aust Coast				126	47	7	3955		2	1	9	977	11,17
	Trop. SW Pacific				160	62	4	300		1	1		?	19
	Trop. SW Pacific, lagoon	214	?	7							1			19
Large (>10 μm)	Trop. SW Pacific				171	89	4	474		1	1		?	19
Bulk water	Tropical N. Pacific				19		1	38			1			16
	WTNA	24	18		3.7			?			1			18
	ETNA	140	78		?			255			1			18
	Tropical N Atlantic				16	10	7	271	89	9	4	26	139	6,18
	Trop. SW Pacific				301	104	5	703		1	1			19
	N.W. Pacific (34°N, 129°W)	15									1			20
	Gulf of California				20			250				4		21

nr = not reported

1 (Mague, 1977); 2 (Carpenter & McCarthy, 1975); 3 (Carpenter & Price, 1977); 4 (McCarthy & Carpenter, 1979); 5 (Orcutt *et al*, 2001); 6 (Goering *et al.*, 1966); 7 (Capone *et al.*, 2005);
8 (Gunderson *et al.*, 1976); 9 (Saino, 1977); 10 (Karl *et al.*, 1997); 11 (Capone *et al.*, 2005); 12 (Capone *et al.*, 1998); 13 (Letelier & Karl, 1996); 14 (Carpenter *et al.*, 1999); 15 (Falcon *et al.*, 2004);
16 (Dore *et al.*, 2002); 17 (Montoya *et al.*, 2004); 18 (Voss *et al.*, 2004); 19 (Garcia *et al.*, 2007); 20 (Needoba *et al.*, 2007); 21 (White *et al.*, 2007)

the diatom *Hemiaulus hauckii* encountered off the NE coast of South America in 1996 had areal rates of N_2 fixation rate that exceeded that by *Trichodesmium* spp. in the same region by tenfold or more (Carpenter *et al.*, 1999). These associations may be particularly important in sequestering C to the ocean's interior (Scharek *et al.*, 1999a,b; Subramaniam *et al.*, 2008). Factoring these episodic events into basin scale budgets remains a challenge.

Several studies have examined unconcentrated bulk water using a $^{15}N_2$ tracer approach. Goering *et al.* (1966) first reported on unconcentrated, bulk water $^{15}N_2$ uptake studies off the northeast coast of South America. Their results could account for rates of about 86 and 271 μmol N_2 m^{-2}day^{-1} for 2 observations and 9 observations in the fall of 1966 and spring of 1967, respectively. They reported appreciable *Trichodesmium* in most of their samples. Voss *et al.* (2004) recently found relatively high rates on the eastern side of the basin off NW Africa, with somewhat lower rates on the western boundary, and activity was below detection limits in the central portion of the basin (Tables 4.4 and 4.5). Rees *et al.* (2006) observed very high rates of N_2 fixation in the eastern Mediterranean which were apparently limited by phosphorus availability (Table 4.4).

We recently assayed bulk (unconcentrated) water $^{15}N_2$ uptake during a cruise off the NE coast of South America during a period of relatively high Amazon River flow and abundant populations of the diatom *Hemiaulus* with the symbiotic cyanobacterium, *Richelia*, at many stations. Areal rates of N_2 fixation exceeded 1000 μmol N_2 m^{-2}day^{-1} (Subramaniam *et al.*, 2008). The earlier cruise to the same area mentioned above in which we encountered an extensive monospecific bloom of *Hemiaulus/Richelia* yielded comparable rates using gently concentrated surface water (Carpenter *et al.*, 1999). White *et al.* (2007) have also found *Richelia*/diatom associations to be an important source of N_2 in the Gulf of California.

Bonnet *et al.* (2008) recently reported on a transect through the southeast Pacific from the high nutrient (HNLC) region in the northwest of the gyre through its core and on to the eastern edge of the gyre. Despite the extreme oligotrophy, rates of N_2 fixation were below detection limits and could not be stimulated with additions of either iron or phosphate.

N_2 fixation may also proceed in waters at low rates at higher latitudes with lower temperatures and higher combined nitrogen concentrations (e.g. Needoba *et al.*, 2007; Holl *et al.*, 2007) or perhaps even deeper in the water column (Hewson *et al.*, 2007).

5.1.1. Diazotroph densities

As is evident from the data, areal rates of activity can be highly variable for both *Trichodesmium* and microbial diazotrophs with extremes in the mmol N m^{-2} day^{-1} range, with a greater spread of values overlapping in the 10–100 μmol N m^{-2} day^{-1} (Mahaffey *et al.*, 2005) (Tables 4.4 and 4.5). With the help of epifluorescent microscopy, quantification of macrodiazotrophic organism such as *Trichodesmium* and *Richelia* symbiotic in diatoms has been relatively straightforward.

However, determining the *in situ* density of micro-diazotrophs is more problematic. While coccoid cyanobacteria such as *Synechococcus* are reported to occur at densities of about 10^4 ml^{-1} in oligotrophic waters, presumptively diazotrophic

coccoids appear to occur at much lower densities of 10s to 100s of cells per ml (Falcón *et al.*, 2004; Montoya *et al.*, 2004).

With the recognition of several distinct groups of coccoid cyanobacteria based on recovered *nifH* sequences (Table 4.1), specific primers and probes have been developed to directly evaluate the abundance of target phylotypes using quantitative PCR (qPCR). Short *et al.* (2004) first applied this method to determine the abundance, and spatial and temporal patterns of 2 diazotrophic phylotypes in Chesapeake Bay. Densities of up to 140 and 340 copies ml^{-1} were found for one phylotype (being associated with higher salinity) and the other most abundant phylotype was detected at mesohaline stations. Church *et al.* (2005) used a similar qPCR approach examining the *nifH* gene copy densities of *Trichodesmium*, two coccoid cyanobacterial diazotrophs (Groups A and B), one heterocytous group (het-1, later recognized as *Richelia* associated with *Rhizosolenia* (Foster and Zehr, 2006)) and a group III phylotype at Station ALOHA. They reported that on a gene copy basis, Group A cyanobacteria were dominant (Table 4.6). In a second study by Church *et al.* (2005), they demonstrated that *nifH* gene copy expression for Group A occurred during the day, as did *Trichodesmium*.

Recently, Foster *et al.* (2007) reported on densities of seven groups of diazotrophic cyanobacteria in the plume of the Amazon River. In mesohaline waters, the highest densities were found for *Richelia* associated with *Hemiaulus*, while in more marine waters, *Trichodesmium* dominated *nifH* copy density. The three coccoid groups (Groups A, B, and C) were generally in lower in abundance than the two of the larger diazotrophs (het-1, *Trichodesmium*) and showed higher abundances at the more saline stations. Group B *nifH* copy abundances co-varied with *nifH* copies of *Trichodesmium*.

Less information is currently available on the density of diazotrophic bacterioplankton in marine ecosystems. Church *et al.* (2005) used a primer and probe set designed to target a Group III phylotype and found relatively high densities ($\sim$70 copies ml^{-1}) in the near surface, which declined with depth. However, this phylotype became predominant over the cyanobacteria below the euphotic zone. Hewson *et al.* (2007) recently reported their analysis of diazotrophic populations in the Sargasso Sea and elsewhere, including deep waters, a few *nifH* phylotypes and transcripts from deeper depths below the euphotic (up to about 6000 m) were amplified and quantified (Table 4.6). Microarray fingerprints of diazotroph diversity patterns from samples obtained in the upper water column in samplings two years apart were remarkably similar.

Two recent studies first mentioned above have provided the most comprehensive coverage to date for the N. Pacific Gyre (Church *et al.*, 2008) and tropical and subtropical N. Atlantic (Langlois *et al.*, 2008). No diazotrophs were detected at stations in the Pacific Sub-Artic Gyre. As mentioned above, Type A and B coccoid were dominant at most stations both within the NPSG and in transitional zones to the north and south. Within the NPSG they were most abundant between 14 and 29° N where they could occur in densities exceeding 100 and 10 cells per ml, respectively (Table 4.6). At several stations, heterocyctous forms were found to dominate while non-heterocyctous filamentous forms were rarely dominant. In contrast, Langlois *et al.* (2008) reported that in many of the warmer (28–30°C) regions of the N. Atlantic, particularly areas subject to high dust inputs, non-heterocystous filamentous forms were numerically more abundant. Type A coccoids also occurred in substantial numbers, although their greatest densities occurred in somewhat cooler (22–23°C) waters.

Table 4.6 Densities of Microbial Diazotrophs in the Water Column as Derived from qPCR Studies

Location	Clone or Isolate	Phylotype	Average copies/ml	Maximum copies/ml	Salinity	Notes	References
Chesapeake Bay	907h22	Delta		143	Meso	Apr max	Short *et al.*, 2004
	912h4	Alpha-beta		340	Marine		
Station ALOHA	Tricho	Cyano	4	7	Marine		Church *et al.*, 2004
(mixed layer)	Grp A	Cyano	200		Marine		
	Grp B	Cyano	2		Marine		
	Grp III		70		Marine		
Arabian Sea transect	Coccoid	Cyano		380	Marine		Mazard *et al.*, 2004
Tropical N. Atl. &	Tricho	Cyano	4.5	2000	Marine		Foster *et al.*, 2007
Amazon plume	Grp A	Cyano	9.5[a]	260	Marine		
	Grp B	Cyano		1300	Marine		
	Grp C	Cyano		71	Marine		
	Rich-H	Cyano	130	17000	Meso		
	Rich-R	Cyano	0.3	62	Meso		
N. Atl, Hydrostation S	BT5667A01	Grp III (beta)		0.043	Marine	1000 m	Hewson *et al.*, 2007
N. Atl, Hydrostation S	BT19215A01	Gamma		0.002	Marine	5948 m	
Eq. Pacific	EP19212A01	Gamma		0.002	Marine	1389 m	
N. Pacific (34°N, 129°W)	Grp A	Cyano	7.0 +/− 1.7	34	Marine	Top 60 m	Needoba *et al.*, 2007
	Grp B	Cyano		7.6	Marine		
	Grp C	Cyano		4.3	Marine		
Sub-tropical & tropical	Tricho		2.5[b]	−1000	Marine		Langlois *et al.*, 2008
N. Atlantic	Grp A		1.3[b]	−1000	Marine		
	Grp B			−100	Marine		
	Grp C			−100	Marine		
	Gam A			−50	Marine		
	Gam P			−10	Marine		
	Clll			−1	Marine		
N. Pacific-NPSG	Tricho			1	Marine	10m	Church *et al.*, 2008
	Grp A			>100	Marine	10m	
	Grp B			−10	Marine	10m	
	Het-1			100	Marine	10m	
	Het-2			100	Marine	10m	

[a] Average for all 3 groups.
[b] Weighted average from Fig. 3, for 3 regions in the central tropical N. Atlantic.

5.1.2. Diazotroph biovolume

As has been pointed out by Carpenter *et al.* (2004), while *Trichodesmium* cells may be relatively rare compared to *Synechoccocus* and *Prochlorococus*, they are also very large cells (up to 10 µm in diameter). Even a single colony of 200 trichomes in a liter of surface seawater would represent about 15,000–20,000 cells and could account for more than 40% of all prokaryotic autotrophic biovolume and carbon (assuming typical densities of *Synechoccus* and *Prochlorococcus*) (Table 4.7). Given the presumed lower densities of diazotrophic coccoid cyanobacteria, a colony of *Trichodesmium* per liter would also presumably dominate diazotroph biovolume (assuming a cell diameter for the coccoids of about 5 µm) (Table 4.7). Also, maximum cell specific rates of N_2 fixation appear to be higher in *Trichodesmium* (10–30 fmol N cell^{-1} h^{-1}, (Capone *et al.*, 2005) than those reported for diazotrophic coccoid cyanobacteria ($\sim$ 4 fmol N cell^{-1} h^{-1}; see Gallon and Stall, 1992; Grobbelaar and Huang, 1992; Mitsui *et al.*, 1987; Reddy *et al.*, 1993).

5.1.3. Final thoughts on activity and density of diazotrophs

Thus, diverse results from the field on both the density and activity of *Trichodesmium*, micro-diazotrophs and DDAs, the relative intensity of N_2 fixation is highly variable and heterogeneous. On a local basis, each component may make substantial contributions. A critical evaluation of the relative global importance of each awaits more extensive and robust estimates of the N input by symbiotic associations and the smaller microbial diazotrophs at diverse locations.

Galloway *et al.* (2004) recently estimated global oceanic N_2 fixation to be in the range of $100–200 \times 10^{12}$ g year^{-1}, roughly equal to the estimate for terrestrial N_2 fixation in the absence of human activities. It now appears that upwards of 50% of the nitrogen supporting new production of some tropical and sub-tropical marine upper water column ecosystems could be derived from N_2 fixation (Capone *et al.*, 2005; Karl *et al.*, 1997; Mahaffey *et al.*, 2005) (see Below).

5.2. Comparison of geochemical vs. direct estimates

Current global or basin-scale geochemical estimates, be they based on N excesses or isotopic mass balances, represent net fluxes integrated over relatively large spatial and temporal scales. Direct estimates, on the other hand, are snap shots and will reflect natural variability occurring over shorter (e.g., daily, seasonal & interannual) time scales. Furthermore, depending on the nature of the direct estimate, they may only capture specific components of the diazotrophic flora (see above). In order to accurately quantify N_2 fixation by direct or geochemical methods, an understanding of the temporal variation in N_2 fixation, residence time of the tracer, and ventilation timescales of the water column is vital.

At present, geochemical estimates are available for the North Atlantic (Gruber and Sarmiento, 1997), *cf.* (Hansell *et al.*, 2004, 2007) and Pacific (Deutsch *et al.*, 2001), while the greatest density of direct observations are for *Trichodesmium* in the tropical North Atlantic and North Pacific (Table 4.3). We have recently scaled globally averaged rates of *Trichodesmium* N_2 fixation to seasonally averaged waters greater than or equal to 25°C as a proxy for highly oligotrophic waters

Table 4.7 Relative Biovolume and C Content of Phytoplankton and *Trichodesmium* in 1L assuming 1 Colony per Liter and a Cell Diameter of *Trichodesmium* of 10 μm. Adapted from Carpenter *et al.*, 2004

		Diam		Cell vol		% of Total	% of Prok total	fg C/cell	fg C/μm3	cell C ng/L	% of Total	% of Prok total
		μm	μm^3	cells/L	μm^3/L							
Prochloro	Sphere	0.68	0.165	30000000	4939087	1%	13%	54	325[a]	1605	1%	12%
Synecho	Sphere	0.87	0.345	10000000	3447914	1%	9%	112	325	1121	1%	8%
Type A	Sphere	5	65.4	200000	13089971	2%	2%	21271	325	4254	4%	4%
Tricho	Cylinder	10	785	20000	15707965	3%	42%	333333[b]	424	6660	6%	49%
Eukaryotes	Sphere	10	524	1000000	523598833	93%	NA	98143	187	98143	88%	NA

[a] From Durand *et al.*, 2001.
[b] Assumes 5 μg C and 150 trichomes per colony and 100 cells per trichome (Carpenter 1983; Carpenter *et al.*, 2004).

(Galloway *et al.*, 2004). Given the basin-specific averages weighted for the extent of each study, *Trichodesmium* N_2 fixation scaled to waters of 25°C and above can explain about 40% and 59% of some of the geochemically inferred N_2 fixation in the North Atlantic and North Pacific, respectively (Mahaffey *et al.*, 2005).

We believe this is remarkable in explaining a substantial fraction of the geochemically derived estimate. The difference is likely a function of input from *Trichodesmium* blooms (Carpenter and Capone, 1992; Capone *et al.*, 1996), microbial diazotrophs (Montoya *et al.*, 2004) and symbiotic associations (Carpenter *et al.*, 1999). As noted, all three can contribute intense amounts of nitrogen, although the broader inputs over larger time and space scales are not currently well constrained.

Recent research has found low, but measurable rates of N_2 fixation in cooler surface waters (Table 4.6) (Needoba *et al.*, 2007). If this is a general observation in colder waters, it would further close the gap. Similarly, while they predominate in surface waters (Church *et al.*, 2008), diazotrophs can also be found below the euphotic zone (Hewson *et al.*, 2007), and do exhibit low levels of activity (Hamersley, Capone, Montoya et al. in prep).

An ongoing debate concerns the extent that the current N cycle is close to steady state (see Chapter 1, Gruber, this volume). Recent estimates of denitrification considerably exceed those of N_2 fixation (Codispoti *et al.*, 2001), perhaps because of our lack of understanding of the quantitative importance of other sources of N_2 fixation such as that occurring in the nano- and pico-plankton and associated with symbionts. Indeed, isotopic constraints suggest that the marine N Cycle is much closer to steady state, and that N_2 fixation and denitrification are tightly coupled and in fact provide a N homeostat (Deutsch *et al.*, 2004; Gruber, 2004).

As detailed above, there appears to be spatial patterns in the predominance of different diazotrophs in different oceanic regions, both within and among major basins, as well in the nature of nutrient limitation of these populations. These trends are likely related to the nature of external nutrient input in these regions. There is also an ongoing reevealuation with regard to the oceanic distribution of N_2 fixation itself (Fig. 4.5). Substantial research has documented the density and intensity of N_2 fixation by *Trichodesmium* in the tropical N. Atlantic (e.g. Carpenter *et al.*, 2004; Capone *et al.*, 2005; Montoya *et al.*, 2007; Langlois *et al.*, 2008) along with geochemical identification of regions of excess nitrogen in the thermocline (Gruber and Sarmiento, 1997). In contrast, reanalysis of the nitrate and phosphate field of the N. Atlantic suggest far less N excess than previously suggested (Hansell *et al.*, 2004, 2007) while isotopic mass balance studies at BATS, including the DON pool, suggest minor inputs by N_2 fixation (Knapp *et al.*, 2005). Several modeling studies point to other areas of the global ocean (e.g. the Pacific and Arabian Sea) as more significant in net N_2 fixation (Deutsch *et al.*, 2007; Moore *et al.*, 2004) (Fig. 4.5). Thus, there is a need to reconcile these divergent observations.

6. Summary and Future Directions

Recent research on oceanic diazotrophs has shown remarkable diversity, with numerous species in free-living genera (*Trichodesmium* spp., *Katagnymene* spp., *Crocosphera* spp. and *Anabaena gerdii*) as well as coccoid symbionts with dinoflagellate and
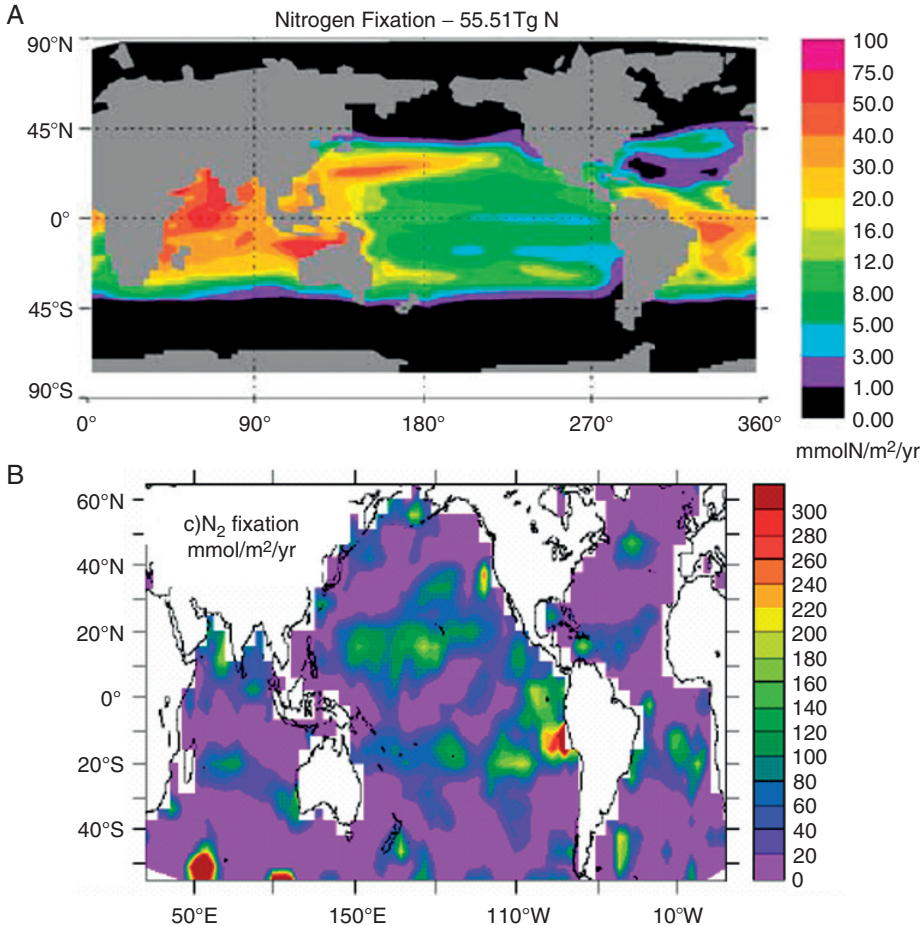
Figure 4.5 Modeled distributions of nitrogen fixation in the worlds oceans. (A) From Moore *et al.* (2004) with permission. (B) From Deutsch *et al.* (2007) based on P★ draw-down with permission.

diatom hosts and the heterocystous *Richelia intracellularis* and *Calothrix rhizosoleniae* living symbiotically with several diatom genera. The fact that a significant fraction of *nifH* sequences from tropical pelagic waters cluster with proteobacteria suggests that these organisms might also be important in nitrogen transformations, and recent research indicates that oxygen minimum zones may also promote N_2 fixation. More research is clearly needed to quantify rates of N_2 fixation and the factors that control growth rates in all these organisms.

In benthic environments, ranging from the rhizosphere of shallow water macro-phyte communities such as *Zostera, Thalassia* and *Spartina* hundreds of different diazotrophic strains have been isolated, and these are typically microaerophyllic or anaerobic, and often are sulfate respiring bacteria. These diazotrophs make signifi-cant contributions to the nitrogen economy of their respective plant communities.

Similarly, in coral reef communities, N_2 fixation in carbonate sands are a major source of N_2 fixation, and the recent discovery of diazotrophic coccoid cyanobacteria living within the tissues of *Montastrea* corals suggests that there may well be more diazotrophic symbioses with corals. Many sponges harbor cyanobacterial symbioses, and these may also be important sources of fixed nitrogen.

In the pelagic zone, iron and phosphorus availability is a major constraint on N_2 fixation. In the north Atlantic, positive $N\star$ anomalies in the sub euphotic zone, resulting from Aeolian dust deposition of Fe, corresponds to areas in which P limits growth of diazotrophs. Genomics research indicates the capacity of *Trichodesmium* to utilize phosphonates as a source of P, however research is needed to determine how significant this source might be. In the Pacific, Fe availability more severely limits diazotrophic activity, but P is in short supply as well. Iron requirements of diazotrophs are about 10 fold greater than non–diazotrophic phytoplankton, considerably less than previous estimates. While aeolian dust addition studies have shown increases in diazotrophic activity of tropical plankton, there is a need by climate modelers for more quantitative data on the effects of dust on overall primary productivity, N_2 fixation rate, plus a better understanding of the chemistry of dust dissolution.

There is precious little information on the pathways of fixed N from pelagic diazotrophs to other members of the plankton and nekton. Viral lysis may be a major pathway as is the "leakage" of fixed N from healthy *Trichodesmium* cells. Research on $\delta^{15}N$ of zooplankton in broad areas of the north Atlantic suggests that fixed N is readily incorporated. Clearly a better understanding of grazers, particularly salps, and grazing rates on *Trichodesmium* and other planktonic diazotrophs, is needed.

Recent estimates of global oceanic N_2 fixation are in the range of 100–200 $\times$ 10^{12} g year^{-1}, roughly equal to the estimate of terrestrial N_2 fixation in the absence of human activities. Thus it appears that about 50% of the N supporting new production in some tropical and sub-tropical ecosystems could come from N_2 fixation. The distribution of diazotrophs globally is very patchy both vertically and horizontally, and it is very labor intensive to obtain quality biomass data and N_2 fixation rates. The authors have spent many hours identifying and quantifying diazotrophs and assessing nitrogenase activity. The development of better remote sensing algorithms and sensors can help in assessing near surface populations of diazotrophs such as *Trichodesmium*. Better indirect methods such as those employing stable isotopes, $N\star$ or modeling efforts can also help, but since fixed N is such an important commodity in the ecology of marine organisms, it is important that we have a better assessment of rates in the marine environment.

Several conundrums remain to be resolved including 1) have we greatly underestimated marine N_2 fixation, 2) what are the quantitative contributions of the newly identified diazotrophs, 3) what is the spatial distribution of N_2 fixation and 4) to what extent does the nature of nutrient limitation control the composition of diazotrophic populations locally and over broader scales.

More novel insights into marine nitrogen may be just around the corner with the influx of new and improved methodologies and analytical capabilities. Stable isotope probing has been used with enriched C isotopes to identify directly active

components of microbial populations by isolation of nucleic acids and specific biomarkers (Boschker and Middelburg, 2002; Radajewski et al., 2000). A similar approach should be feasible for N isotopes as well. The identification of biomarkers which may be specific for discrete groups of diazotrophs would foster this area (e.g., Carpenter et al., 1997). Another technology which holds great promise is nanoscale secondary ion mass spectrometry (NanoSIMS) which allows analysis of intracellular distributions of elements, including discrete isotopes (Kuypers and Jørgensen, 2007; Popa et al., 2007).

As we come to fully appreciate the implications of increasing atmospheric carbon dioxide concentrations on upper ocean chemistry and physical structure, it is important that we evaluate these impacts on major biogeochemical processes. N_2 fixation is an obvious ecological function that requires such evaluation. Preliminary assessment of the effect of temperature shifts on the distribution of Trichodesmium populations in the upper ocean have been made (Breitbarth et al., 2006). The physiological effects of increasing CO_2 have only recently been realized (Levitan et al., 2006; Barcelos e Ramos et al., 2007; Hutchins et al., 2007). These studies necessarily need to be extended to other relevant diazotrophs as well as to the effects of pH.

Other indirect effects of climate change may bear on marine N_2 fixation rates. Increasing atmospheric deposition of combined nitrogen is occurring and will continue to increase into the future, with direct implications to marine diazotrophy (Duce et al., 2008). Initial modelling efforts predict an negative effect on marine N_2 fixation (Krishnamurthy et al., 2007).

For many areas of the ocean, dust input is held to be an important source of nutrients for N_2 fixation. Changes of patterns of dust inputs are therefore expected to affect this key process (Moore et al., 2006). Lastly, expansion of oceanic OMZs is predicted (Stramma et al., 2008). We have discussed above the potential interactions of OMZs and marine N_2 fixation.

Research on marine N_2 fixation promises to continue to be a dynamic area with surprises around each corner.

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NITRIFICATION IN MARINE SYSTEMS

Bess B. Ward

Contents

1. INTRODUCTION

Nitrification is the process whereby ammonium (NH_4^+) is oxidized to nitrite (NO_2^-) and then to nitrate (NO_3^-). It thus links the most oxidized and most reduced components of the nitrogen (N) redox cycle and helps determine the overall distributions of these important nutrients. Ammonium rarely occurs at significant concentrations in oxygenated habitats. It is recycled rapidly between heterotrophic and N_2 fixing organisms (which excrete NH_4^+ directly or release organic N that is microbially degraded to NH_4^+) and many heterotrophic and photosynthetic plankton (which utilize NH_4^+ as a N source) in the surface ocean.

Similarly, NO_2^- rarely accumulates in oxygenated habitats (see below for exceptions), although NO_2^- is an essential intermediate in several oxidation and reduction processes in the N cycle. Nitrate, the end product of nitrification, however, accumulates in the deep ocean, and seasonally in the deep water of lakes, where there is no demand for inorganic N by phytoplankton.

The oxidation of NH_3 to NO_2^- and of NO_2^- to NO_3^-, although thermodynamically favorable when linked to reduction of oxygen, has long been considered a biological process, with only minor known abiotic contributions. For example, photochemical oxidation of NH_4^+ to NO_2^- was debated in the 1930s (Rakestraw and Hollaender, 1936; ZoBell, 1933). It was eventually demonstrated that NO_2^- that results from high doses of UV radiation actually comes from reduction of NO_3^- (Armstrong and Tibbitts, 1968). Photochemical oxidation likely occurs in the surface ocean, but is a minor flux. Oxidation of NH_3 and amino-level N in organic compounds to N_2 gas linked to reduction of manganese oxide has been demonstrated in sediments (Hulth *et al.*, 1999; Luther *et al.*, 1997) and this pathway may have significant ramifications for the N cycle of sediments. Whether the Mn^{++} reduction pathway represents abiotic nitrification or anaerobic biological nitrification (see below) remains to be resolved.

Ammonium often accumulates to quite high concentrations in anoxic sediments and in stratified waters where oxygen concentrations are very low, which is consistent with the obligate aerobic physiology of conventional nitrifying bacteria. Recently, however, a novel process referred to as anammox (for anaerobic NH_4^+ oxidation) has been elucidated and shown to be capable of oxidizing NH_4^+ to N_2 under anoxic conditions (Mulder *et al.*, 1995; van de Graaf *et al.*, 1995). This process is in fact a form of denitrification, because its function is to remove fixed N form the system. Perhaps we should refer this process as oxidative denitrification or NH_4^+ denitrification, to distinguish it from conventional nitrification, which does not result directly in the loss of fixed N from the system. Anammox is elaborately discussed in the Chapter on denitrification by Devol, this volume.

The most important organisms in aerobic nitrification are the so-called nitrifying bacteria and the NH_3 oxidizing archaea (AOA). Until very recently, the known nitrifiers were restricted to the Proteobacterial phylum. The Proteobacterial genera are not all closely related to each other, but appear to have arisen from a photosynthetic ancestor, diverging before the ability to nitrify was developed in various groups (Teske *et al.*, 1994). There are two functionally distinct groups of nitrifiers: those that oxidize NH_4^+ to NO_2^- (NH_3-oxidizing bacteria and archaea, AOB and AOA) and those that oxidize nitrite to NO_3^- (NO_2^--oxidizing bacteria, NOB). No organism is known to carry out both reactions. These unique metabolic traits are not without costs; the chemolithoautotrophic lifestyle enables nitrifiers to exploit a unique niche in natural systems, but also confers constraints such as slow growth and inflexible nutritional requirements.

A new group of aerobic NH_3-oxidizing nitrifiers was recently discovered, first by detection of NH_3 oxidizing genes of apparent Archaeal origin in environmental metagenomic libraries (Schleper *et al.*, 2005) and subsequently verified with the cultivation of a strain of Archaea (NH_3-oxidizing archaea, AOA) that oxidizes NH_3 to NO_2^-, apparently using a pathway very much like that known in AOB

(Konnecke *et al.*, 2005). It now seems likely that AOA are more abundant than AOB in marine systems (Wuchter *et al.*, 2006), and are also prevalent in soils (Leininger *et al.*, 2006).

Several other useful reviews are available (Hagopian and Riley, 1998; Kaplan, 1983; Ward, 1986, 2000, 2002; Ward and O'Mullan, 2005). The 2002 review contains expanded information on the molecular ecology of nitrifiers, the 2005 chapter contains detailed information on methods for both rate measurement and molecular ecological investigations of AOB, and the present chapter contains new information on the NH_3-oxidizing Archaea, which were unknown at the times of the earlier reviews.

2. Nitrifying Microorganisms

2.1. Aerobic nitrification

2.1.1. Autotrophic nitrification

Most of the aerobic nitrification that occurs in natural habitats is thought to be performed by obligately autotrophic, or in a few cases, mixotrophic, bacteria and archaea. The classical autotrophs are best known and have been assumed to be responsible for the major fluxes in this pathway, so we shall consider them first.

Most of our knowledge of autotrophic nitrifiers derives from studies on cultivated strains, and the best known of those are the NH_3-oxidizing genus *Nitrosomonas* and the NO_2^--oxidizing genus, *Nitrobacter*. The cultured autotrophic nitrifiers, both NH_3 oxidizers and NO_3^- oxidizers, depend on CO_2 as their major carbon source and fixation is carried out via the Calvin cycle. It is estimated that CO_2 fixation accounts for about 80% of the energy budget of an autotroph (Forrest and Walker, 1971; Kelly, 1978). In addition, the use of NH_4^+ or NO_2^-, respectively, as the sole source of reducing power for this autotrophic growth is relatively inefficient: about 35 mol of NH_4^+ or 100 mol of NO_3^- must be oxidized to support fixation of a single mole of CO_2 (Baas Becking and Parks, 1927). This "perverse insistence" on fixing their own CO_2, utilizing a unique but low yield energy source (Wood, 1986), accounts for their well deserved reputation for slow growth, even under optimal conditions in the laboratory. The commonly cultivated strains of *Nitrosomonas* and *Nitrobacter* have minimal generation times of 7–13 h (Bock *et al.*, 1989).

The only cultivated AOA, *Nitrospumilus maritimus*, depends on CO_2 as its only carbon source and the presence of even low levels of organic carbon were inhibitory to growth. The pathway of CO_2 fixation is, however, unknown. Hyperthermophilic Crenarchaeota generally utilize a 3-hydroxypropionate pathway or a reductive TCA cycle for autotrophic carbon fixation. Another cultivated marine Crenarchaeota strain, *Cenarchaeum symbiosum*, a sponge symbiont, appears to use the 3-hydroxypropionate pathway. It cannot be concluded on this basis which pathway is used by the AOA, but it very likely that is not the Calvin cycle. *N. maritimus* had a minimal generation time of 21 h, longer but roughly on the same scale as AOB.

The overall reaction for NH_3 oxidation (Eq. (5.4)) shows that the process consumes molecular oxygen and produces hydrogen ions, in addition to NO_2^-. A requirement for molecular oxygen occurs in the first step of the oxidation (Eq. (5.1)), which is catalyzed by a monooxygenase (NH_3 monooxygenase, AMO). The uncharged gaseous NH_3 is the actual substrate for AMO, as demonstrated by the pH dependence of the reaction rate (Suzuki et al., 1974; Ward, 1987a). Synthesis and activity of AMO in N. europaea, a terrestrial model organism, respond directly to NH_3 concentration (Stein et al., 1997), albeit at levels much greater than observed in the marine water column. Some AOB accumulate very high levels of NH_4^+ internally, however (Schmidt et al., 2004a), so the effective concentrations might not be those detected in the environment. Ammonia monooxygenase has never been completely purified and assayed in cell free conditions, although its gene sequence has been derived for both Nitrosomonas and Nitrosococcus type AOBs (Alzerreca et al., 1999; Klotz and Norton, 1995; Sayavedra-Soto et al., 1998). AMO contains copper and probably also iron in its active form (Zahn et al., 1996).

The immediate product of AMO is hydroxylamine, which is further oxidized by hydroxylamine oxidoreductase (HAO) to NO_2^- (Eq. (5.2)). AOA apparently do not possess the hydroxylamine reductase gene, so the pathway of ammonia oxidation in these organisms must be quite different from that outlined here for AOB. Oxygen is also consumed by the terminal oxidase (Eq. (5.3)), as a result of electron transport generating ATP for cellular metabolism.

$$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O \, (\text{ammonia monooxygenase})$$
$$(5.1)$$

$$NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^- \, (\text{hydroxylamine oxidoreductase})$$
$$(5.2)$$

$$2H^+ + 0.5O_2 + 2e^- \rightarrow H_2O \, (\text{cytochrome oxidase}) \qquad (5.3)$$

$$NH_3 + 1.5O_2 \rightarrow NO_2^- + H_2O + H^+ \qquad (5.4)$$
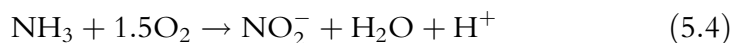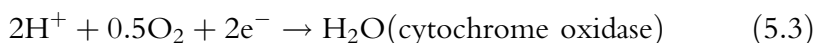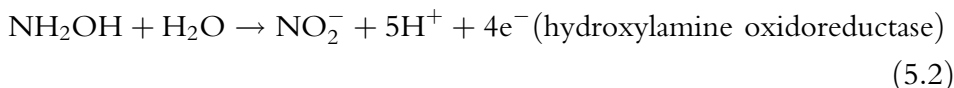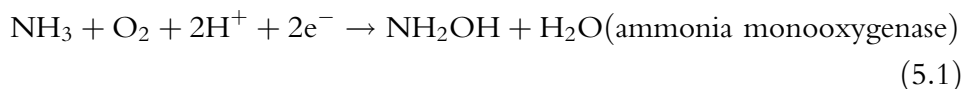
The overall reaction is energy yielding, and allows sufficient ATP production to support reverse electron transport for CO_2 fixation. However, the first step, oxidation of NH_3 to hydroxylamine, requires the input of reducing power. The second step, hydroxylamine oxidation, yields four electrons. These join the electron transport chain at the level of ubiquinone, from which two are shunted back to AMO for activation of NH_3. The N oxidation and electron transport pathways in Nitrosomonas are linked in the cytoplasmic membrane and periplasmic space; detailed information from the N. europaea genome (Chain et al., 2003) is consistent with the previous biochemical characterizations of the system (Whittaker et al., 2000). Depending on conditions (and enhanced at low oxygen concentrations), nitric oxide (NO), nitrous oxide (N_2O) and even dinitrogen gas (N_2) have been reported as secondary products

in autotrophic NH_3 oxidation by both marine and terrestrial strains (Schmidt et al., 2004b; Zart and Bock, 1998). Although N_2O and NO can be produced in vitro by HAO from hydroxylamine (Hooper and Terry, 1979), reduction of NO_2^- appears to be the dominant pathway in whole cells (Hooper et al., 1997; Poth and Focht, 1985; Remde and Conrad, 1990). Nitrite reductase activity has been demonstrated in Nitrosomonas europaea (DiSpirito et al., 1985; Miller and Wood, 1983) and in several marine AOB (Casciotti and Ward, 2001). Genes with homology for the copper type nitrite reductase and the nitric oxide reductase of heterotrophic denitrifiers have been detected in a group of marine Nitrosomonas isolates (Casciotti and Ward, 2001, 2005). Thus it appears that the marine AOB produce N_2O by a pathway that is homologous with that in denitrifiers (''nitrifier denitrification''). The pathway for nitrous oxide production in AOB remains controversial, however, because N. europaea mutants with nonfunctional NO_2^- reductase genes can also produce N_2O (Beaumont et al., 2002), implying that an alternative pathway is present in some strains. It remains to be verified whether marine nitrifiers exhibit the same phenomenon. There is evidence from the genomes of AOB that N. europaea, N. marina and N. oceani contain different suites of electron transport molecules and quite different NO_2^- reductase genes (Chain et al., 2003; Klotz et al., 2006), which may imply they also have different mechanisms for N_2O production. It remains to be determined whether AOA have similar pathways or capabilities.

The biochemistry of NO_2^- oxidation is simpler than NH_3 oxidation because it is only a two electron transfer and involves no intermediates. The additional oxygen atom in NO_3^- is derived from water (Eq. 5.5), and the molecular oxygen that is involved in the net reaction (Eq. 5.7) results from electron transport involving cytochrome oxidase (Eq. 5.6).

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ \text{(nitrite oxidoreductase)} \qquad (5.5)$$

$$2H^+ + 0.5O_2 \rightarrow H_2O \text{(cytochrome oxidase)} \qquad (5.6)$$

$$NO_2 + 0.5O_2 \rightarrow NO_3^- \qquad (5.7)$$
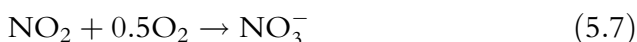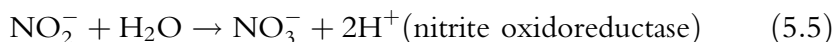
The energy yield of NO_2^- oxidation is even less that that of NH_3 oxidation, necessitating the oxidation of vast amounts of NO_2^- in order to fix small amounts of CO_2. For both processes, therefore, the biogeochemical impact is greater on the N cycle than on the C cycle.

The biomass produced by primary fixation of CO_2 by nitrifiers can be significant in some habitats. Under sea ice in Antarctica (Horrigan, 1981), an anchialine cave in the Yucatan Peninsula, Mexico (Pohlman et al., 1997) and the Mississippi River Plume in Gulf of Mexico (Pakulski et al., 2000b) are examples. In these cases, the significance of autotrophic nitrification as an in situ source of organic carbon is enhanced relative to the production of organic matter by photosynthesis. Photoautotrophic carbon production is minimal due to the absence of light caused by ice cover, the geometry of the cave itself, and in the Mississippi River system, high levels of suspended matter, respectively. Chemoautotrophy might also be a significant source of in situ production on sinking

particles (Karl *et al.*, 1984) and in hydrothermal plumes where elevated NH_4^+ (and perhaps methane) concentrations could support NH_3-oxidizing bacteria (Cowen *et al.*, 1998; Lam *et al.*, 2004). It now appears that AOA comprise a significant fraction of the prokaryotic cells in seawater (Wuchter *et al.*, 2006), but it is not known to what extent these cells are obligate autotrophs. If they possess a wider metabolic repertoire than the typical bacterial nitrifiers, i.e., if they predominantly access carbon and energy sources other than CO_2 and NH_4^+, they may make a very important contribution to microbial biomass that is not linked to nitrification.

a. **Phylogeny of bacteria and archaea involved in nitrification:** The history of the study of nitrifying bacteria has been described elsewhere (Ward, 2002; Watson *et al.*, 1989) and only the current status of their diversity and evolutionary relationships will be summarized here. The classic species distinctions were based on cell shape and the arrangements of internal membranes (Koops and Moller, 1992; Watson *et al.*, 1989). These have been largely superceded by the evolutionary relationships deduced from 16S rRNA sequencing.

The phylogeny of bacterial nitrifiers (Teske *et al.*, 1994) shows that most of them are descendents of a common ancestor that was photosynthetic, rather than descending from a common ancestral nitrifier. Extensive phylogenetic trees depicting the relationships among cultivated nitrifiers, and the relationships of sequences obtained without cultivation from environmental samples, are available in the publications cited in this section and are not reproduced here.

The NH_3-oxidizing bacteria (AOB), including the marine AOB, fall into two major phyla in the Proteobacteria (Koops *et al.*, 2003; Purkhold *et al.*, 2003). The β subdivision species, containing genera known as *Nitrosospira* and *Nitrosomonas*, have been the subject of many recent studies. The main tool for investigating them in both culture and environmental samples is polymerase chain reaction (PCR) followed by cloning and DNA sequencing. On the basis of 16S rRNA sequence analysis, several clusters and a large amount of microdiversity within clusters has been detected in a wide variety of environments, both terrestrial and aquatic. Stephen *et al.* (1996) surveyed enrichment cultures and gene libraries of soils and marine sediments and found that *Nitrosomonas* sequences were more often associated with enrichment cultures and *Nitrosospira* with libraries. Smith *et al.* (2001) compared the phylogenetic affiliations and diversity of AOB 16S rRNA clone libraries and enrichment cultures obtained from the same marine sediment samples from a seawater loch being used for salmon farming. Only one (a *Nitrosomonas* type) of 18 enrichment cultures was represented in the 40 AOB clones retrieved without culturing. *Nitrosospira*-like sequences are often dominant in NH_3-oxidizer sequence clone libraries retrieved from the oceanic environments (Bano and Hollibaugh, 2000; O'Mullan and Ward, 2005).

A few generalities linking species or strains to environmental conditions have emerged. For example, McCaig *et al.* (1999) investigated the community structure of β-subdivision AOB in marine sediments underlying fish farms, and found a novel *Nitrosomonas* group whose distribution correlated with elevated NH_4^+ concentrations. Purkhold *et al.* (2000) summarized the ecological characteristics of the known cultivated strains and the major clusters defined by environmental

sequences for the betaproteobacterial AOB. None of the major clades are restricted to seawater. There are two major clades, one in the *Nitrosospira* cluster and one in the *Nitrosomonas* cluster, which have been reported primarily from marine environments for which no cultivated representatives are known. These findings suggest that the *Nitrosomonas* lineages are less common in natural samples than *Nitrosospira*s, and raise concerns about biases introduced by relying on pure culture techniques.

The γ subdivision NH_3 oxidizers are represented by a single genus containing two species, *Nitrosococcus oceani* and *N. halophilus*, which have only been reported from marine or saline environments. Several different strains of *N. oceani* exist in culture, derived from various locations in the Atlantic and Pacific Oceans, but they all appear to be closely related (Ward and O'Mullan, 2002). *N. oceani* has been detected by immunofluorescence and by PCR in saline lakes in Antarctica (Voytek *et al.*, 1999) and in various marine systems (O'Mullan and Ward, 2005; Ward and Carlucci, 1985; Ward and O'Mullan, 2002; Ward *et al.*, 1989a) and by immuno-fluorescence in the Mediterranean Sea (Zaccone *et al.*, 1996). Two strains of *N. halophilus* were isolated from a salt lake and a salt lagoon, and both have NaCl optima of about 700 mM, compared to 500 mM for *N. oceani* (Koops *et al.*, 1990). Compared with the large amount of data available for the betaproteobacterial AOB, there are few reports of *Nitrosococcus*-like sequences obtained from the marine environment and none from freshwater environments, and no reports of finding *N.halophilus*-like sequences. This lack of information on *Nitrosococcus* may be a matter of PCR primer specificity and limited research effort at present, but it just as likely implies that *Nitrosococcus* is a minor component of the AOB assemblage.

The 16S rRNA sequence on only one AOA is currently available, and that sequence places *Nitrosopulilus maritmus* within the low-temperature marine Crenarch-aeota, a group abundant in seawater and distinct from low temperature Crnarchaeota in soils (Konnecke *et al.*, 2005). Phylogenetic analysis of several hundred AOA partial *amoA* sequences identified major groups that clustered by environment; water column sequences clustered together whether from the Black Sea, Monterey Bay or the eastern tropical North Pacific, while sediment sequences clustered together with other sedi-ment and soil sequences (Francis *et al.*, 2005). It is assumed that these are all derived from Crenarchaeota, but this is a large and more diverse group that previously appreciated, so the phylogeny of AOA remains largely unexplored.

The new phylogeny of NO_2^- oxidizing bacteria (Bock and Wagner, 2003), based on 16S rRNA sequences, shows that the best known autotrophic NO_2^- oxidizer, *Nitrobacter*, comprises a coherent genus in the α subdivision of the Proteo-bacteria. This genus is most closely related to non nitrifying genera that contain autotrophic members and strains capable of denitrification (Teske *et al.*, 1994). Like *Nitrosococcus oceani*, *Nitrococcus mobilis* (Watson and Waterbury, 1971) belongs to the γ subdivision of the Proteobacteria, the only example of both NH_3- and NO_2^--oxidizing phenotypes occurring in the same subdivision. *Nitrospina gracilis*, the only species in this genus, represented by two isolates (Watson and Waterbury, 1971), is assigned to the δ subdivision of the Proteobacteria. Possibly the most unusual nitrifier is the genus *Nitrospira*, which is represented by only two isolates, and does not share a common lineage with the other nitrifiers. A novel *Nitrospira* strain,

N. moscoviensis, isolated from a heating system in Moscow, Russia, was assigned to a new genus outside of the Proteobacteria (Ehrich *et al.*, 1995). These authors reanalyzed the *Nitrospira marina* sequence, which Teske *et al.* (1994) had placed in the δ Proteobacteria, and concluded that *Nitrospira* belonged outside the Proteobacteria, in a deeply branching cluster related to *Leptospirilla*. Sequences closely related to this group have now been reported from biofilms and freshwater sediments (Daims *et al.*, 2001), but the only report so far of *Nitrospira*-like sequences from marine clone libraries is from a sediment sample at 2339 m (Li *et al.*, 1999). In stream sediments (Altmann *et al.*, 2004), *Nitrospira* cells (enumerated by fluorescence *in situ* hybridization) were 5–9 times more abundant than *Nitrobacter* cells. The marine isolate (Watson *et al.*, 1986) was obtained from the Gulf of Maine and the authors reported that similar cells were present in many enrichments, suggesting it is a common member of the marine nitrifier assemblage.

Except for *Nitrospira* and *Nitrospina*, all of the bacterial nitrifiers cluster in phyla that are characterized by photoautotrophic ancestry and are themselves characterized by the possession complex intracytoplasmic membranes. These membranes are thought to be the site of the redox proteins involved in N oxidation, and therefore homologous with the photosynthetic membranes of photosynthetic bacteria and cyanobacteria. Nevertheless, the bacterial nitrifiers are polyphyletic, and the phenotype has apparently arisen independently numerous times. The homology of the functional genes (*amo, hao*) involved in their physiology implies gene transfer events, however, rather than independent evolution of these enzymes. The fact that the *amoA* genes from AOB and AOA are homologous raises the question of the ultimate origin of the NH_3 oxidizing phenotype. If the ancestral Crenarchaeota were thermophiles, it is possible that NH_3 oxidation originally arose in thermophiles and spread from the archaea to the bacteria. Internal compartmentalization was not observed in *N. maritimus*, but whether some of the AOA possess internal cytoplasmic membranes has not been established.

An understanding of the phylogeny of nitrifying bacteria is relevant to the study of nitrification in aquatic habitats because it has implications for detection and quantification methods. Although the bacterial nitrifiers are polyphyletic, they are not so diverse as to be unmanageable; their affiliation within a small group of lineages makes them amenable to identification and detection using a relatively small suite of molecular probes. This approach forms the basis of much current knowledge on the diversity and distribution of autotrophic nitrifying bacteria, and has already made important contributions to the study of nitrifying archaea as well.

The autotrophic bacterial NH_3 oxidizers show significant metabolic and morphological similarities with another group of autotrophic bacteria, the methane oxidizers. They are also closely related phylogenetically to the methane oxidizers, in both the γ and β subdivisions (Teske *et al.*, 1994). Prior to the availability of ribosomal RNA sequence data for determination of phylogenetic relationships, it had been reported that NH_3-oxidizing nitrifiers were capable of methane oxidation and vice versa (Dalton, 1977; Jones and Morita, 1983; Ward, 1987a). Thus it was an interesting verification of the metabolic studies when it was reported that the central genes in the two pathways, genes encoding NH_3 monooxygenase in the nitrifiers and methane monooxygenase in the methanotrophs, were evolutionarily related

(Holmes *et al.*, 1995). It has not been possible to determine unequivocally which process is the natural or predominant one for some nitrifiers and methanotrophs in nature (see later text).

2.1.2. Heterotrophic nitrification

The autotrophic NH_3 oxidizers are considered to be obligate chemolithoautotrophs with no source of energy other than NH_3 and no net source of cellular carbon other than CO_2. Obligate autotrophy had been attributed to the absence of one or more enzymes in the tricarboxylic acid cycle, but inspection of the complete genome for *Nitrosomonas europaea* shows that a complete TCA cycle is present (Chain *et al.*, 2003). *N. europaea* has limited genetic capability for transport and metabolism of organic molecules, but the basis for its apparent obligate dependence upon NH_4 and CO_2 is still not entirely clear.

The autotrophic NO_2^- oxidizers have been reported to exhibit various degrees of heterotrophy, although generation times and time required for adaptation to new substrates is on the order of weeks (Bock, 1976). Recently completed genomes of terrestrial nitrite oxidizers indicate that they too possess a complete TCA cycle, but do not have the complete pathways for utilization of carbon substrates larger than 3 carbons (Starkenburg *et al.*, 2006). The NOB also have very limited organic transport capabilities (Starkenburg *et al.*, 2008). Marine strains for AOB and NOB have not been thoroughly investigated for their heterotrophic capabilities, and the potential for them to exhibit this metabolism in the ocean is unknown. The only cultivated AOA is apparently an obligate chemoautotroph, but uncultivated marine Crenarchaeota are apparently capable of amino acid assimilation (Ouverney and Fuhrman, 2000), so the extent of the AOA metabolic repertoire remains an open question.

Heterotrophic nitrification has been reported for bacterial genera including *Bacillus*, *Paracoccus*, *Pseudomonas*, *Thermus*, *Azoarcus*, and the fungus, *Aspergillus*. Heterotrophic nitrification usually refers to the production of NO_2^- or NO_3^- either from NH_4^+ or from organic substrates by heterotrophic bacteria, and is best studied in a few denitrifiers where it is linked to aerobic denitrification (Stouthamer *et al.*, 1997). The enyzmology of the process is unknown (Nemergut and Schmidt, 2002) and its physiological role is not understood, as it usually cannot support growth and in fact, reduces growth yield compared to denitrification alone.

Liberation of NO_3^- from organic intermediates has also been reported for green algae (Spiller *et al.*, 1976) and N-fixing legumes (Hipkin *et al.*, 2004) but the relevance of these processes in the marine environment is unknown. Although the organisms performing them are autotrophic, the processes might be considered heterotrophic because the released N is derived from an organic intermediate.

It has been argued that heterotrophic nitrification involves enzyme systems that are quite different from those of the autotrophs (Wehrfritz *et al.*, 1993) and that heterotrophic nitrification cannot serve as an energy generating mechanism (Castignetti, 1990), as the autotrophic process does. In the aerobic denitrifiers, which are also capable of nitrification, the initial enzyme, AMO, appears to be quite similar to the enzyme in autotrophic nitrifiers. However, HAO differs significantly and in the heterotrophs, is a smaller, simpler enzyme that performs a two electron transfer (instead of the four electron transfer of the autotrophic HAO) and

releases nitroxyl as the product, rather than NO_2^- (Richardson *et al.*, 1998). Thus, no electrons are available for reverse electron transport in the heterotrophic system and the purpose of the process appears to be a means of disposing of excess reductant in times of redox stress, rather than harvesting that reductant for cellular energy.

In aquatic systems, the most important heterotrophic nitrifiers are thought to be a class of denitrifiers which are capable of aerobic denitrification, and which can also oxidize NH_3. The organism in which these physiologies were first described is now known as *Paracoccus pantatrophus* (formerly known as *Thiosphaera pantotropha*). *P. pantotrophus* oxidizes NH_3 to NO_2^- using an enzyme that exhibits important similarities and differences compared to AMO from autotrohpic nitrifiers (Moir *et al.*, 1996). The NO_2^- so generated can be released into the medium or denitrified to N_2. Denitrification of NO_2^- or NO_3^- can occur under atmospheric levels of oxygen (Robertson *et al.*, 1995). Aerobic denitrifiers have mostly been isolated from wastewater treatment systems, and their prevalence and ecological significance is unknown in natural aquatic systems.

Because autotrophic nitrification is such a hard way to make a living, it might seem curious that heterotrophic nitrification is not more common. The amounts of NO_2^-or NO_3^- formed are usually quite small compared to autotrophic nitrification, however, and the energetics are apparently even less favorable than for the autotrophic nitrifiers (Stouthamer *et al.*, 1997). There are no substantive reports of heterotrophic nitrification in the marine environment, despite its mention in anecdotal reports and suggestion of it as a possible explanation for curious observations (Hovanec and DeLong, 1996).

Thermophilic heterotrophic nitrifiers, capable of oxidizing NH_3 to NO_2^-, were isolated from several hydrothermal vent habitats (Mevel and Prieur, 1998). Most of the isolates were NO_3^- reducers or denitrifiers and exhibited a wide diversity related to the genera *Thermus* and *Bacillus*. Because they are able to ammonify, nitrify and reduce NO_3^-, they would seem to possess most of the physiologies of importance in the N cycle. Such organisms may be common in mesophilic aquatic habitats, but have not been quantitatively investigated.

Heterotrophic strains capable of oxidizing NO_2^- have also been reported. Sakai *et al.* (1996) described several classes of heterotrophic strains that oxidized NO_2^- to NO_3^- with variable amounts of NO_3^- accumulation, depending partly on the denitrification capabilities of the strains. Strains which were capable of both oxidation and reduction of N oxides were shown to switch between the two directions of conversion depending on the oxygen tension of the culture (Sakai *et al.*, 1997).

Heterotrophic nitrification has been studied in terrestrial systems, especially acid forest soils, where it has been difficult to document autotrophic nitrification. Experiments using isotopes to differentiate production of NO_3^- from inorganic and organic substrates in a forest system found that heterotrophic nitrification accounted for less than 10% of the total nitrification rate (Barraclough and Puri, 1995). No information of this sort is available on the occurrence or significance of heterotrophic nitrification in aquatic systems. The potential for NH_3 and NO_2^- oxidation by heterotrophic bacteria in aquatic systems warrants further exploration, and the capability may be present in many strains already in culture. If heterotrophic nitrification is common in nature, then a focus on autotrophic nitrification as the

model system and the basis for estimation of rates is too narrow and unrealistic. On a per cell basis, heterotrophic nitrification is much slower than is autotrophic nitrification, and the relative impact on N turnover must be greater by autotrophs due to their total dependence on N oxidation for energy. Nevertheless, widespread abundance of more numerous heterotrophic nitrifiers could compete in importance with the smaller number of slow growing autotrophs. Facultative nitrifiers that grow heterotrophically most of the time would seem to be an advantageous compromise, but mixotrophy is surprisingly uncommon in the microbial world (Whittenbury and Kelly, 1977). It is intriguing to wonder whether AOA will be an exception to this generalization. If heterotrophic nitrification were significant, it might not be detectable in the net stoichiometry of organic matter remineralization.

2.2. Anaerobic nitrification

The interest in anaerobic nitrification and aerobic denitrification arises mainly from the necessity to treat large volumes of wastewater to reduce N loading before release into natural waters. Classical nitrification and denitrification are environmentally incompatible processes, the first being obligately aerobic and the second induced only under conditions of anoxia. It is therefore usual to involve two steps in wastewater treatment, an aerobic step to convert NH_3 to NO_3^- and a subsequent anaerobic step, to convert the NO_3^- to N_2. If the two steps could be combined in one organism under either aerobic or anaerobic conditions, much time and money would be saved in wastewater treatment. And clearly, if a single organism is capable of combined nitrification and denitrification in a bioreactor or water treatment plant, such an organism could be of considerable importance in the natural environment as well.

The heterotrophic nitrifier mentioned earlier, *P. pantatrophus*, carries out at least part of both processes under aerobic conditions. *P. pantatrophus* was originally isolated from wastewater and its ability to denitrify aerobically, as well as this ability in several other conventional heterotrophic denitrifiers, has been confirmed (Robertson *et al.*, 1995).

Autotrophic nitrifying bacteria exhibit some abilities for anaerobic metabolism as well. Enrichment cultures under chemolithotrophic conditions and very low oxygen concentrations catalyzed the net removal of NH_4^+ as N_2 (Muller *et al.*, 1995). Bock and coworkers have shown that *Nitrosomonas eutropha* produces gaseous products, mainly NO and N_2, during growth on nitrogen dioxide gas (NO_2) and NH_3 (Schmidt and Bock, 1997). The process proceeds at a slower rate than NH_3 oxidation in the presence of a normal air atmosphere and supports cell growth. Additions of NO_2 and NO enhanced the complete removal of N in the form of NH_3 and organic N without the addition of organic carbon substrates (Zart and Bock, 1998).

A completely novel process in which NH_3 and NO_2^- are converted anaerobically to N_2 was reported several years ago (Mulder *et al.*, 1995; van de Graaf *et al.*, 1995) and the process has been quantitatively described (Jetten *et al.*, 1998; Strous *et al.*, 1999). The organisms responsible for this novel metabolism have been identified as *Planctomyces* (Strous *et al.*, 1999), and the genome of one strain has

been almost completely sequenced (Strous *et al.*, 2006). Referred to as "anammox" (anaerobic NH_4^+ oxidation), the process in wastewater involves a consortium of the planctomycete organism and an autotrophic NH_3 oxidizer such as *Nitrosomonas europaea* or *N. eutropha*. Ammonia is oxidized to NO_2^- by the autotroph under microaerophilic conditions. The NO_2^- so produced is reduced to N_2 by the planctomycete. Both oxygen and NO_2^- concentrations are maintained at nearly undetectable levels by the metabolism of the members of the consortium, and while both organisms grow quite slowly (generation times for the planctomycete of ≥ 11 days are reported), the net removal of NH_4^+ occurs at a rate 25 times faster than that reported for N removal by anaerobic autotrophic nitrification alone (Jetten *et al.*, 1998).

The anammox process was discovered and characterized in anaerobic wastewater treatment systems, and 16S rRNA sequences identified as belonging to the plancto-mycete member have been detected in several such systems. Research into the occurrence of the process and presence of the organisms in natural aquatic systems has proceeded rapidly in the last few years. Anammox has now been documented in estuarine (Risgaard-Petersen *et al.*, 2004a; Tal *et al.*, 2005; Trimmer *et al.*, 2003; Trimmer *et al.*, 2005) and marine (Dalsgaard and Thamdrup, 2002; Thamdrup and Dalsgaard, 2002) sediments and in the water column of the Black Sea (Kuypers *et al.*, 2003) and Golfo Duce, an enclosed Bay on the west coast of South America (Dalsgaard *et al.*, 2003) and most recently in the shelf waters of the Benguela upwelling zone (Kuypers *et al.*, 2005), and the OMZs of the eastern tropical South Pacific (Thamdrup *et al.*, 2006; Hamersly *et al.*, submitted for publication). Anammox is an oxidative process and there appear to be some enzymatic similarities between anammox and aerobic NH_3 oxidation (Strous *et al.*, 2006), the ecological significance of anammox is the same as denitrification, i.e., the loss of fixed N in anoxic environments. Therefore, for a full treatment of anammox, please see Chapter 6, Denitrification by Devol, this volume.

Nitrite oxizers are also reported to possess an anaerobic metabolism and even to grow under denitrifying conditions (Freitag *et al.*, 1987). Subsequent work showed that ATP was not produced during NO_2^- reduction (Freitag and Bock, 1990). Very little work has been done in this area with marine NO_2^- oxidizers, but NO_2^- oxidation has been reported in environments where very little oxygen is present (see later text).

In addition to the unconventional activities of conventional nitrifiers and deni-trifiers and the discovery of novel N metabolisms in new organisms, it has also been recently proposed that a short circuit of the nitrification/denitrification couple can also be accomplished abiotically. In marine sediments, which typically contain relatively high manganese levels, N_2 can be produced by the oxidation of NH_4^+ and organic N by manganese dioxide in air. The reduced Mn^{++} thus formed can be reoxidized by oxygen to continue the oxidation of NH_4^+, or can reduce NO_3^- to N_2 (Luther *et al.*, 1997). While the free energy of these coupled reactions is shown to be favorable, it remains to be seen whether the abiotic process can be unequivocably identified in natural systems and the degree to which it may compete with the biologically catalyzed processes. Hulth *et al.* (1999) detected anoxic production of both NO_2^- and NO_3^- in marine sediments, concurrent with the production of

reduced manganese. The NO_3^- production was directly proportional to the initial manganese oxide content, and the NO_3^- was subsequently depleted, apparently by denitrification. A series of linked redox cycles in which anoxic nitrification, coupled to manganese reduction, was linked in series to anoxic organic matter oxidation through several biogeochemical reductants, including iron and hydrogen sulfide, was proposed as the mechanism (Hulth *et al.*, 1999). These authors suggested that the lithotrophic nitrification they observed is biologically mediated and potentially of significance in the N cycle at sites where oxidized metals are reworked into anoxic sediments. Other authors have failed to detect significant coupling between Mn^{++} and NH_4^+ in anoxic sediments (Thamdrup and Dalsgaard, 2000). ^{15}N tracer experiments, including the kind used to demonstrate anammox (e.g., Dalsgaard and Thamdrup, 2002) have also shown that the anaerobic oxidation of NH_4^+ via manganese is not a significant flux, at least in some sediments (Risgaard-Petersen *et al.*, 2004a; Thamdrup and Dalsgaard, 2000).

Anoxic NH_3 oxidation, whether it results directly in N_2 formation (as in anammox) or in NO_3^- production (when linked to manganese reduction), would introduce new links into the aquatic and sediment N cycle. Failure to account for anoxic NH_3 oxidation might lead to an underestimate of NH_4^+ removal, because the products do not accumulate; they are either lost to the atmosphere immediately, or rapidly reduced by the next step in the anaerobic cycling of organic matter.

3. ROLE OF NITRIFICATION IN THE MARINE NITROGEN CYCLE

Many forms of organic and inorganic N can be utilized by phytoplankton, and the transformation of NH_4^+ into NO_3^- by nitrification does not change the absolute inventory of N available for algal nutrition. In soils, the different ionic properties of NH_4^+ and NO_3^- are important in determining inorganic N availability in the soil solution, but in aquatic systems, the properties of these ions are less important to their distributions. Because of the different chemical properties and varying preferences, abilities, and metabolic costs of utilizing NH_4^+ versus NO_3^-, and the role of NO_3^- as a substrate for denitrification, however, this transformation is very important in marine systems.

Because NH_4^+ contains N at the oxidation level of proteins, it is readily assimilated by both phytoplankton and bacteria, and is a preferred N source. Ammonia oxidizers may be in competition for NH_4^+ with other planktonic organisms. The different physiological requirements of phytoplankton and nitrifiers probably play a role in determining exactly where in the water column NH_4^+ assimilation and NH_4^+ oxidation occur. As explained below, most nitrification occurs within or near the base of the euphotic zone in the upper 100 or so meters of the ocean. However, there is usually very little NO_3^- in the surface ocean, due to utilization by phytoplankton, except in "high nutrient low chlorophyll" regions and when supplied by episodic events such as regional upwelling. The NO_3^- in the deep water of the oceans has accumulated from nitrification because phytoplankton assimilation is essentially zero below the euphotic zone. It is because of nitrifiers

that N accumulation in the deep waters is in the form of NO_3^-, rather than NH_4^+. The deep NO_3^- reservoir can be made available to phytoplankton by mixing, upwelling, and seasonal overturn. These physical processes bring cold deep nitrate-rich water up to the surface where, in the presence of light, phytoplankton can assimilate the NO_3^-. Thus, although NO_3^- is not usually abundant in surface waters, it is a very important N source for phytoplankton.

The NO_3^- that is produced by nitrification serves as a substrate for denitrification in low oxygen environments in both the water column and sediments (Devol, this volume). Although denitrification involves several semi-independent steps that need not function together, it is common for denitrifiers to begin the sequence with NO_3^- and to produce varying amounts of the other products depending upon the environmental conditions. Thus, although denitrifiers appear to have little in common with nitrifiers, the former are in fact dependent upon the latter – other than lightning and fertilizers, nitrifiers are the only significant source of NO_3^-. Similarly, NO_2^- and NO_3^- are the oxidants in the anaerobic oxidation of NH_4^+. While NO_2^- can be produced by either nitrification or denitrification, the production of the oxidized forms ultimately depends on aerobic autotrophic nitrification. Thus the role of nitrifiers in the N cycle of marine systems is to link the oxidizing and reducing processes of the N cycle by converting NH_4^+ to NO_3^-.

Nitrification can also be an important sink for oxygen in aquatic environments. NO_3^- is released through the aerobic oxidation of organic matter and nitrification according to the classic Redfield stoichiometry (Redfield *et al.*, 1963):

$$(CH_2O)x(NH_3)\gamma(H_3PO_4) + (x + 2\gamma)O_2 \rightarrow xCO_2$$
$$+ \gamma HNO_3^- + (x + \gamma)H_2O^-$$
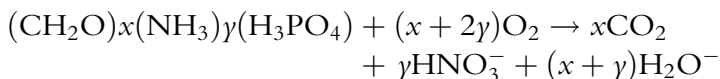
where $x = 106$ and $\gamma = 16$. In this formulation, oxygen consumption should be inversely related to NO_3^- production with a slope of $138/16 = 8.6$. In fully oxygenated sediments (i.e., carbon loading is not sufficient to exhaust the available oxygen), such relationships are often found. Grundmanis and Murray (1982) and Jahnke *et al.* (1982) reported ratios of 10.1 and 7 respectively, and both state that these are in reasonable agreement with the predicted stoichiometry once differential diffusivities are taken in to account. Redfield stoichiometry implies that nitrification is responsible for $32/138 = 23\%$ of the total oxygen consumption associated with organic matter diagenesis. In environments where denitrification and anammox occur, the net O_2/NO_3 stoichiometries may vary (Devol, this volume).

3.1. Distribution and abundance of nitrifiers in the marine environment

Although most strains of NH_3-oxidizing and NO_2^--oxidizing bacteria have characteristic intracytoplasmic membrane structures, which can be visualized by electron microsocopy, it is not possible to distinguish the otherwise nondescript cells from other bacteria and archaea in water samples using standard microscopic techniques for cell enumeration, e.g., epifluorescence microscopy with DNA fluorochromes.

Most probable number (MPN) methods relying on the appearance or disappearance of NO_2^- in dilution media have been used to estimate the abundance of NH_3 oxidizers and NO_2^- oxidizers, respectively, in aquatic environments. MPN approaches are widely used to estimate abundances of nitrifying bacterial populations in soils, but there are very few modern reports using this technique in marine systems. Examples of data on nitrifying bacterial abundanced are provided in Table 5.1. Bianchi et al. (1999) estimated the abundance of both NH_3 and NO_2^- oxidizers in the water column of the Mediterranean Sea in the Rhone River plume using MPN. Maximum abundances of both types were found in surface water of the stations closest to the river and reached levels of 3.5×10^4 cells ml^{-1} for NH_3 oxidizers and 1.2×10^4 for NO_2^- oxidizers. Ammonia plus NO_2^- oxidizing cells averaged less than 2% of the total microbial abundance. McCaig et al. (1999) used MPN to estimate the abundance of NH_3 and NO_2^- oxidizers in polluted sediments underlying marine fish farms. In the sample from directly underneath the fish cage, NO_2^- oxidizers were abundant and NH_3-oxidizers were not detected. Farther away from the cage, NH_3 oxidizers were detected, but NO_2^- oxidizers were not present above detection level at these stations. Hall (1986) estimated from data in a number of lake studies that the efficiency of recovery for MPN was 0.05–0.001% for NH_3 oxidizers. He also concluded that MPN abundances showed little correlation with observed nitrifying activities. This technique should, however, yield isolates of the most abundant cell type present, assuming it can grow under the enrichment culture conditions. Phillips et al. (2000) compared MPN and a competitive PCR method for enumeration of NH_3-oxidizing bacteria in estuarine sediments and detected 1–3 orders of magnitude higher abundances using cPCR (on the order of $10^5 = 10^6$ cells g^{-1}).

Immunofluorescence (IF) was first applied to the study of nitrifying bacteria in soil and lake systems by Schmidt and coworkers (e.g., Fliermans et al., 1974; Schmidt, 1978; Smorczewski and Schmidt, 1991; Stanley et al., 1979) and the method was subsequently used by Ward and coworkers to enumerate nitrifiers in seawater (e.g., Ward and Carlucci, 1985; Ward and Perry, 1980). In a small survey of marine and estuarine sites, Ward (1982) reported that Nitrosomonas serotypes were more abundant than Nitrosococcus and that total abundance of NH_3 oxidizers (Nitrosomonas plus Nitrosococcus serotypes) ranged from 10^4 cells ml^{-1} in Chesapeake Bay to 10^2 cells ml^{-1} in inshore ocean waters and $10–10^2$ cells ml^{-1} offshore. Maximum abundances (10^6 cells l^{-1} for both NH_3 and NO_2^- oxidizers) occurred near the bottom of the photic zone in the vicinity of the primary NO_2^- maximum in the Peru upwelling system (Ward et al., 1989a) but such characteristic patterns are not always detected. Nitrosococcus oceanus abundance, determined by IF, was positively correlated with temperature, particulate organic carbon and N and total bacterial abundance, and negatively correlated with dissolved oxygen, in brackish Mediterranian lagoons (Zaccone et al., 1996). There was no relationship between IF and MPN counts over the 20 month sampling period of this study.

Immunofluroescence requires that the target organisms be cultivated so that antibodies can be produced against the cells to be enumerated. The antibodies responsible for the cell-staining reaction are those that interact with components of the outer cell membrane, and the reaction can be very specific (Ward and Carlucci, 1985). This is both a strength and a disadvantage of the method. It allows

Table 5.1 Abundances of Nitrifying Bacteria Reported from Aquatic Systems

Location	Depth (m)	AOB cell abundance	NOB cell abundance	Method	Reference
Sediments					
Freshwater lake sediments	Surficial sediments	$8–37 \times 10^5$ cells g^{-1}	$21–67 \times 10^5$ cells g^{-1}	MPN	Smorczewski and Schmidt, 1991
Sediments under marine fish farm cages	0.5 cm core top	Up to 0.2×10^6 cells m^{-2}	Up to 25×10^6 cells m^{-2}	MPN	McCaig et al., 1999
Marine (saline) Water column environments		Cells ml^{-1}	Cells ml^{-1}		
Lake Bonney (Antarctica)	Oxic water column	Nitrosomonas 2×10^3		IF	Voytek et al., 1998
	Oxic water column	Nitrosococcus 0.8×10^3		IF	Voytek et al., 1998
Mediterranean lagoon	Oxic water column	Nitrosococcus $1–10^3$		IF	Zaccone et al., 1996
Peru upwelling	Ave over water column	Nitrosomonas + Nitrosococcus 0.3×10^3	Nitrobacter + Nitrococcus 0.2×10^3	IF	Ward et al., 1989a
Southern California Coastal waters	Ave over water column	Nitrosomonas + Nitrosococcus 0.3×10^3	Nitrobacter + Nitrococcus 0.3×10^3	IF	Ward and Carlucci, 1985
Chesapeake Bay	Ave over water column	Nitrosomonas+Nitrosococcus 10^7		IF	Ward, 1982

AOB – Ammonia-oxidizing bacteria; NOB – Nitrite-oxidizing bacteria; IF – Immunofluorescence; MPN – Most Probable Number

strain or species specific detection, so that distribution patterns can be discerned for individual types, but therefore does not allow an enumeration of total abundance for all similar physiological types. The necessity for cultivation of the antigenic strain also guarantees that most of the strains that are important in the ocean cannot be tested for cross reactivity and it seems likely that they are not detected by these assays.

The molecular methods that have been used to detect and investigate the diversity of NH_3 oxidizers in the ocean have not been widely adapted for their quantitative analysis and enumeration. This is in great contrast to the study of AOB and NOB in soils and freshwater systems, particularly wastewater bioreactor and biofilm environments (reviewed by Schramm, 2003), where competitive and quantitative PCR assays based on both 16S rRNA and *amoA* genes that are specific for nitrifiers have been used to enumerate cells of various subgroups of nitrifiers. In a study designed to test the adaptation of fluorescence *in situ* hybridization (FISH) identification of specific cells to automated enumeration by flow cytometry, Sekar *et al.* (2004) detected both *Nitrosospira* and *Nitrosomonas* sequences in their 16S rRNA clone libraries. They subsequently used an enhanced sensitivity method (catalyzed reported deposition [CARD-FISH) to enumerate cells of both types in water samples from the coastal North Sea. Probes for *Nitrosospira* and *Nitrosomonas* detected 3.4% and 4.0% of the total DAPI-stained cells in a sample from March 2003, but the same probes did not detect any cells from samples taken in August, September, and November 2002 at the same site (Sekar *et al.*, 2004). Lam *et al.* (2004) enumerated betaproteobacterial AOB using FISH in and near neutrally buoyant plumes emanating from hydrothermal vents in the Endeavor segment of the Juan de Fuca Ridge. Along with many other reduced substrates, the plumes often contain high NH_4^+ concentrations and thus might be expected to provide an enriched environment for NH_3 oxidizers. AOB reached maximal abundances of $1–1.4 \times 10^4$ cells ml^{-1} in the plume itself and were present at much lower levels (minimum of 0.04×10^4 cells ml^{-1}) in background (non-plume) samples. The cells were preferentially associated with particles, constituting up to 51% of the total cells enumerated by DAPI in the >3 μm particle fraction, whereas AOB constitued up to 4.9% of the free living cells enumerated by DAPI (Lam *et al.*, 2004). These numbers are 10-fold higher than previous AOB counts obtained by immunofluorescence from shallower depths in the open and coastal ocean (Ward *et al.*, 1982, 1989a) and comparable to those from nutrient rich environments such as Chesapeake Bay (Ward, 1982), suggesting an important role for nitrifiers in this environment. Depending on the specificity of oligonucleotide probes, genetic methods should detect higher numbers than IF, because of the specificity and culturability issues mentioned earlier.

Clone libraries may give some indication of the relative abundance of target organisms. In this case, it would be instructive to know the percentage of a total bacterial 16S rRNA clones that were identified as AOB or NOB- like sequences. AOB sequences are easily retrieved when targeted directly with AOB-specific primers, but are very rare in total 16S rRNA libraries.

The results from FISH and IF measurements show that nitrifying bacteria generally contribute a minor fraction of the total microbial assemblage in natural waters, with estimates ranging from 0.1% to 7.5% of the total cell number in the

water column. This generalization is consistent with the autotrophic nature of nitrifiers, which requires that individual cells process relatively large amounts of N for minimal return in terms of carbon assimilation. The abundances estimated for the hydrothermal vent plumes are quite high (Lam *et al.*, 2004), but consistent with environmental parameters that favor the autotrophic NH_3 oxidizing metabolism. Abundance data for NO_2^- oxidizers are almost non-existent and for NH_3 oxidizers very sparse. This is an area in which quantitative PCR and enhanced sensitivity single cell detection methods (e.g., CARD-FISH) could be very usefully applied. FISH and quantitative PCR assays for 16S rRNA and *amoA* genes detected up to 1000 fold greater abundances of Crenarchaeota than AOB in coastal North Sea water (Wuchter *et al.*, 2006), indicating that unlike AOB, the AOA can be a major contribution to the total microbial assemblage.

3.2. Methods for measuring nitrification rates in water and sediments

As with most biogeochemically important processes, there is no perfect method for direct measurement of nitrification rates. Potential artifacts arise from the use of incubations, but incubation free methods can also be problematic.

3.2.1. Changes in din concentration and specific inhibitors

The easiest experimental design might be simply to incubate samples and measure the concentrations of NO_2^- or NO_3^- over time. This approach provided some of the earliest evidence for the occurrence of biologically mediated nitrification (Rakestraw, 1936; von Brand *et al.*, 1937). Vaccaro (1962) estimated nitrification rates by measuring changes in NO_2^- and NO_3^- in incubated samples, from depths up to 800 m in the Sargasso Sea, in the presence of added NH_4^+ or mixed whole plankton. He reported that both additions stimulated the net production rate of NO_2^- or NO_3^-. In such an experiment, accumulation of NO_2^- or NO_3^- indicates net nitrification. A decrease in the concentration over time could be observed, however, even when nitrification is occurring, if consumption of NO_3^- or NO_2^- exceeds production in the incubation bottle. This is likely to be the case in surface waters where the low concentration of fixed N relative to high biological demand means that large fluxes can be obscured by tight coupling between production and consumption terms.

The simple nutrient measurement approach can be modified by the use of specific inhibitors. Chemicals that specifically inhibit either NH_4^+ oxidation (e.g., acetylene, allylthiourea, methyl fluoride, N–serve) or NO_2^- oxidation (chlorate) are added to replicate incubation bottles (Bianchi *et al.*, 1997; Billen, 1976). The bottles must be incubated in the dark to prevent assimilation of by phytoplankton. A decrease in the concentration of NO_2^- over time in the bottles in which NH_4^+ oxidation was inhibited provides an estimate of the NO_2^- oxidation rate. The rate of NO_2^- increase in the bottles to which NO_2^- -oxidation inhibitor was added approximates the rate of NH_4^+ oxidation. Aside from the overall limitations of

bottle incubations, the potential artifacts associated with the inhibitor approach relate to the conditions of the incubation:

(1) Preventing production by phytoplankton probably has cascading effects on the activities of other microbes in the bottle, such that the rate of NH_4^+ mineralization is reduced, therefore changing the source term for the nitrification substrate.

(2) Incubating in the dark may release the nitrifiers from light inhibition such that the measured rate exceeds the *in situ* rate.

(3) Incubations typically last 48 h, which is sufficient to overcome the lag induced by light inhibition, but is also long enough to create quite unnatural conditions.

The search for precisely specific inhibitor compounds has been extensive and has resulted in a plethora of potentially useful compounds. Many are problematic for reasons not directly related to nitrification. For example, acetylene inhibits both nitrifiers and denitrifiers (Balderston *et al.*, 1976; Berg *et al.*, 1982). Thus its use to measure one process will also inhibit the other, and when one depends on the other (as is the case when denitrification depends upon nitrification for NO_3^-), both rates are affected and the independent measurement of one is not possible. It is reported that the length of exposure to acetylene can be optimized to differentiate between its affects on nitrification and denitrification (Kester *et al.*, 1996). N–serve is a commercial preparation that specifically inhibits NH_3-oxidizing bacteria (Goring, 1962) and it serves as the basis for the sensitive $^{14}CO_2$ method for measurement of nitrification rates. Being chemolithoautotrophs, nitrifiers fix CO_2 while oxidizing N. The amount of CO_2 fixation due to nitrifiers can be computed by difference between incubations with and without addition of an inhibitor that specifically removes the contribution of nitrifiers (Billen, 1976; Dore and Karl, 1996; Somville, 1978). Then a conversion factor is used to translate the CO_2 fixation into NH_4^+ and NO_2^- oxidation rates. This conversion factor has been shown to vary in the case of NH_3 oxidizers by a factor of five in pure cultures (Billen, 1976; Glover, 1985), and thus its use introduces some uncertainty, since the factor can only be directly determined in field samples if the ^{15}N methods are performed in parallel (see below).

To complicate matters further, N–serve is not soluble in water, so its addition to samples requires that it be dissolved in an organic solvent. This solvent can affect the other members of the community; e.g., ethanol stimulates dark CO_2 incorporation by heterotrophic bacteria (Owens, 1986; Ward, 1986). Thus in systems where heterotrophs are a large part of the overall assemblage, the N–serve plus solvent approach may over estimate the dark CO_2 incorporation due to nitrifiers (Priscu and Downes, 1985; Viner, 1990; Ward, 1986).

Inhibitor approaches similar to those described earlier for water samples have been used in sediments (Henricksen *et al.*, 1981; Miller *et al.*, 1993). The methyl–fluoride and difluoromethane methods (Caffrey and Miller, 1995; Miller *et al.*, 1993) seem particularly promising because the gases can diffuse thoroughly into the core with minimal disturbance of microzones and gradients. These NH_4^+ oxidation inhibitors are added to cores and the accumulation of NH_4^+ over time is assumed to represent the net rate of nitrification. Other processes that consume NH_4^+ would lead to an underestimate of the rate. De Bie *et al.* (2002) found that both

acetylene and methyl fluoride stimulated dark $^{14}CO_2$ fixation in estuarine sediments. They concluded that methyl fluoride could not be used to estimate nitrification from this approach in natural samples although NO_2^- production was inhibited by 95% in pure cultures of *Nitrosomonas europaea* by both of these inhibitors. CO_2 fixation is an indirect estimate of nitrification and may reflect activities of many other kinds of metabolism. Measurement of NH_4^+ accumulation in conjunction with specific inhibitors is more direct, but still subject to interference from other processes that produce and consume NH_4^+ in natural samples.

To overcome the bias resulting from uneven dispersal of tracer or inhibitor, sediment rate measurements are often made in slurries, which destroy the gradient structure of sediments, which is essential to the *in situ* fluxes. Slurries may provide useful information on potential rates, but not *in situ* rates. Potential nitrification rates and rates measured in intact cores were not correlated in estuarine sediments (Caffrey *et al.*, 2003). The lack of correlation was explained by the inclusion of variable amounts of anoxic sediments in the slurries from which the potential rates were derived.

3.2.2. Isotopic incubation methods

The direct radioisotope tracer method, in which the accumulation of radiolabeled product from added radiolabeled substrate over time yields a rate estimate, is not very practical for measuring rates of nitrification in the environment. Capone *et al.* (1990) were able to quantify nitrification rates using ^{13}N, but the isotope is so short-lived (10 min half-life) that its use is usually impractical.

The other main approach to measuring nitrification rates directly is to use the stable isotope, ^{15}N, as a tracer (Olson, 1981a; Ward *et al.*, 1984). The signal of transfer of the tracer from substrate to product pool (e.g., $^{15}NH_4^+$ to $^{15}NO_2^-$) can be detected regardless of what other processes are occurring in the incubation (so *in situ* light conditions can be used) and no assumptions of steady state need be made. The major drawback of this method is the necessity to add a tracer, sometimes in excess of the natural concentration of substrate. In culture, AOB and NOB respond to increased substrate concentrations by increasing oxidation rates (Carlucci and Strickland, 1968; Suzuki *et al.*, 1976; Ward, 1987a), suggesting that addition of non-tracer levels of isotopically labeled substrate should artificially enhance measured oxidation rates. Such an enhancement is not generally observed in the case of NH_3 oxidation, however, suggesting that natural nitrifier assemblages are not substrate limited at *in situ* levels (Olson, 1981a, Ward, 1990).

The problem of excess substrate addition in tracer experiments has been largely overcome by the advent of more sensitive mass spectrometers, however, and estimates obtained under conditions approaching *in situ* are possible. Owing to the great sensitivity of isotope ratio mass spectrometry, much shorter incubations (compared to the inhibitor and inventory methods) are possible (a few hours to 24 h are commonly used). Although they have not yet been widely applied for this purpose, the recently introduced sensitive isotope methods for determination of ^{15}N content of NO_2^- and NO_3^- (McIlvin and Altabet, 2005; Sigman *et al.*, 2001) should make true tracer level incubations possible. Details on the most commonly used isotope tracer methods for

measurement of nitrification can be found in work by Ward and O'Mullan (2005) and in the Chapter by Lipschultz (this volume).

The ^{15}N approach is most useful in water samples because complete mixing of the tracer is possible. In sediments, rate measurements are constrained by the inhomogeneous nature of the sample and the dependence of rates on the structure of the environment. In this situation, fluxes between overlying water and sediment cores can be analyzed to obtain areal rates. In conjunction with ^{15}N tracer addition, estimates of nitrification rates can be obtained from the dilution of NO_2^- or NO_3^- in the overlying water due to its production in the sediments (Capone et al., 1992). The isotope pairing method for measurement of denitrification (Nielsen, 1992; Rysgaard et al., 1993) is essentially an isotope dilution approach from which both nitrification and denitrification rates can be calculated.

Even if rate measurements in sediments are made using whole core incubations, e.g., when the inhibitor is a gas, it is still difficult to obtain a depth distribution of the rate (usually, an areal rate is obtained). A sophisticated measurement and model based system that avoids direct rate measurements has been used to overcome this problem. Microelectrodes, which have very high vertical resolution, are used to measure the fine scale distribution of oxygen and NO_3^- in freshwater sediments. By assuming that the observed vertical gradients represent a steady state condition, reaction–diffusion models can then be used to estimate the rates of nitrification, denitrification and aerobic respiration and to compute the location of the rate processes in relation to the chemical profiles (e.g., Binnerup et al., 1992; Jensen et al., 1994; Meyer et al., 2001; Rysgaard et al., 1994). Recent advances and details of the microelectrode approach can be found in the Chapter by Joye and Anderson (this volume).

Comparison of incubation methods: The inhibitor methods using ^{14}C have advantages in higher sensitivity, smaller sample volume, easier analysis and therefore greater throughput than the ^{15}N tracer approaches. Therefore, in spite of the potential artifacts mentioned above, the inhibitor approach remains attractive to some investigators. Incubations of 24 h up to 12 days were used to compare the ^{14}C inhibitor, $^{15}NH_4^+$ oxidation and $^{15}NO_3^-$ dilution methods to estimate nitrification rates in the water column of the Baltic Sea (Enoksson, 1986). Artifacts associated with elevated substrate additions and long incubations were identified. The $^{15}NO_3^-$ dilution method was not recommended due to sensitivity problems. Andersson et al. (2006) suggested a formal distinction between nitrification activity as measured by ^{15}N and nitrifier growth as measured by specific inhibition and $^{14}CO_2$ incorporation. The ratio of the two rates was found to vary as a function of temperature and oxygen concentration, and the necessity to exclude phytoplankton $^{14}CO_2$ uptake by performing incubations in the dark was seen as unacceptably artifactual (Andersson et al., 2006). Gundersen (1966) had long ago reported that the ratio of N oxidized to C fixed decreased at low oxygen concentrations relative to air.

Incubation methods, whether enclosing water in a bottle or bag or sediment in a coring tube, may introduce unavoidable artifacts. Even the act of sampling introduces artifacts; in the case of water sampling, larger sinking particles are usually not included in bottle incubations, so the metabolism associated with such particles is not represented in incubations. Approaches that avoid incubations altogether are therefore attractive, but they are largely limited to measuring changes in chemical

concentrations over time, and therefore can detect net transformation rates only. The compromise made by most investigators is to maximize the size and minimize the length of incubations in order to minimize artifacts associated with wall growth or preferential inclusion or exclusion of grazers.

3.2.3. Geochemical constraints to estimate nitrification rates

Stable isotope signatures and distributions in constituents of the N cycle can be used to identify rate processes and potentially to constrain their rates. Because nitrification is often closely coupled to the processes that produce and consume its substrates and products, nitrification would not be expected to leave a clear signature in many environments. Ammonia oxidizing bacteria in culture, however, exhibit relatively large enrichment factors (Casciotti *et al.*, 2003; Delwiche and Steyn, 1970; Mariotti *et al.*, 1981), leaving behind isotopically enriched NH_4^+ and producing isotopically depleted NO_2^-. The fractionation associated with NO_2^- oxidation to NO_3^- has not been directly determined in culture, due to the difficulty of separating NO_2^- and NO_3^- for independent isotopic analysis (Delwiche *et al.*, 1970). It is assumed, however, that the ^{15}N of the NO_3^- produced is depleted relative to the NH_4^+ from which it derived, as is the oxygen in NO_3^- relative to that in NO_2^- and water from which it is derived. Because NO_2^- rarely accumulates, most of the fractionation due to nitrification probably happens at the rate limiting NH_3 oxidation step (Kendall, 1998). Isotopically light NO_3^- in the surface water of the eastern tropical North Pacific was attributed to nitrification, and direct measurements of nitrification using ^{15}N tracer techniques substantiated the distribution of the process in the euphotic zone (Sutka *et al.*, 2004).

Sutka *et al.* (2006) investigated the potential for intramolecular isotopic (isotopomer) distributions to identify the source of nitrous oxide in the environment. Ammonia-oxidizing bacteria, methane-oxidizing bacteria (which can also oxidize NH_4^+ and hydroxylamine) and denitrifiers can all produce N_2O in the laboratory and are all potential sources of N_2O in the marine environment. Methanotrophs and AOB had indistinguishable site preferences for ^{15}N in N_2O produced from NH_3 or hydroxylamine (Sutka *et al.*, 2003, 2006). AOB can produce N_2O by two pathways; one by oxidation of hydroxylamine and one by reduction of NO_2^-. The NO_2^- reduction pathway in nitrifiers – nitrifier denitrification – is homologous with that of denitrifiers (Casciotti and Ward, 2001, 2005) and showed the same site preference in N_2O as found in denitrifiers (Sutka *et al.*, 2006). Thus the isotopomer approach cannot distinguish between the oxidative production of N_2O by methanotrophs vs. AOB, nor can it distinguish between canonical heterotrophic denitrification and nitrifier denitrification. However, the oxidative and reductive pathways to N_2O production did have significantly different isotopomer signatures. In order to use isotopomers to identify the source of N_2O in the ocean, we need to know which of the two possible pathways AOB use to produce N_2O. It may be important that the isotopomer studies mentioned above did not test marine nitrifiers – the observed isotopic fractionation and functional gene sequences indicate that there may be biochemical differences among the genera of AOB, and of course the most abundant AOB and AOA have not been cultured or tested for these pathways. The capacity for nitrifier denitrification may be ubiquitous among AOB (Casciotti and Ward, 2005;

Shaw *et al.*, 2006) and even some of the genome of the crenarchaeal relative of the cultivated AOA possesses the genes that encode the pathway (Hallam *et al.*, 2006).

On a basin or areal scale, the expected rates of nitrification can be estimated from other biogeochemical distributions and rates. Dore and Karl (1996) compared nitrification rates measured using the ^{14}C inhibitor approach to the estimated fluxes of N_2O, assuming a very high N_2O yield from nitrification. Although they reported broad agreement, both rate estimates are very poorly constrained. Ward and Zafiriou (1988) measured NH_3 oxidation rates as deep as 3000 m using the [15]NH_4^+ oxidation method and noticed that the rate decayed rapidly with depth (Fig. 5.3). They compared the integrated nitrification rate in the eastern tropical North Pacific to the predicted N mineralization rate derived from sediment trap fluxes in the same area (Martin *et al.*, 1987). The integrated trap flux and nitrification rates differed by about a factor of 2, suggesting broad agreement and implying that nitrification is coupled to mineralization of sinking particles, but that the nitrification process occurs not on the particles themselves, but in the water captured in the incubation.

Berelson used the equation of Martin *et al.* (1987) to compare mineralization rates at the 17 stations from the regional JGOFS studies and found that the stations varied by only a factor of two in the magnitude of the fitting parameter, b (Berelson, 2001). The greater the value of b, the more rapid the decrease in flux with increasing depth, implying greater mineralization activity at shallower depths. The curvature was correlated with the magnitude of the flux, which varied by a factor of $\sim$20 among the 17 stations. The linear relationship between b and POC flux can be used to constrain mineralization, and thus nitrification, rates, assuming most mineralization occurs in the water column and that the NH_4^+ released is completely nitrified. For POC fluxes ranging from 1.3 mmol C m^{-2} day^{-1} (the lowest equatorial Pacific station) to 24.9 mmol C m^{-2} day^{-1} (the North Atlantic Bloom Experiment), PON fluxes of 0.133–.167 to 2.9–3.6 mmol N m^{-2} day^{-1} can be computed (assuming a C/N ratio of the particles of 8 or 10, respectively; Antia, 2005). The integrated nitrification rates reported by Ward and Zafiriou (1988) for the eastern tropical North Pacific are about the only data suitable for comparison over this vertical extent, and they ranged from 1.1–2.7 mmol N m^{-2} day^{-1}.

The fundamental agreement between these estimates of integrated PON flux (i.e., mineralization rates due to PON loss) and observed nitrification rates has important biogeochemcal implications. Firstly, nitrification is coupled to mineralization and the rate decreases dramatically with increasing depth. This is consistent with incubation rate measurements that usually detect highest rates within the top 100 m of the water column. Most of the nitrification required to balance mineralization over the water column occurs in the planktonic phase or on particles that can be captured in bottles, rather than on rapidly sinking particles, suggesting that mineralization leads to the break up of particles and the release of dissolved NH_4^+ that is subsequently nitrified. Such rate estimates are an important constraint on inferences of microbial metabolic activities: regardless of the phylogenetic identify of the nitrifiers, they can't be growing or nitrifying very rapidly over most of the deep ocean. Even if 40% of the microbial cells in the deep ocean are chrenarchaeota with the capacity for NH_3 oxidation, their livelihood, whether it be NH_3 oxidation or organoheterotrophy, is fundamentally constrained by substrate supply, which decreases greatly with depth.

The distribution of nitrous oxide may provide a further geochemical constraint on nitrification rates and distributions. Using a global dataset of N_2O distributions, Nevison *et al.* (2003) derived a parameterization for the instantaneous production by nitrification of N_2O per mole of O_2 consumed as a function of O_2 concentration and depth. Assuming that NH_3 oxidizers produce N_2O in increasing proportion to NO_2^- as O_2 concentration decreases (as previously reported; Goreau *et al.*, 1980), an annual N_2O production rate of $\sim 5.8 \pm 2$ Tg N year^{-1} was estimated (Nevison *et al.*, 2003). It is not a simple calculation to derive a total nitrification rate from this result because of the nonlinear relationship between O_2 concentration, nitrification rate and the proportion of NH_4^+ that is lost as N_2O. Nevertheless, it might be possible to estimate a global nitrification rate with vertical flux data as discussed above, which would also be constrained by the global ocean N_2O flux calculations.

3.3. Distribution of nitrification in water and sediments

When Kaplan reviewed the status of nitrification in the marine environment in 1983, direct measurements of nitrification rates in water column and sediment environments were few and far between. The proliferation of inhibitor approaches, plus increased accessibility and sensitivity of mass and emission spectrometers has allowed great progress in this area. In 1983, Kaplan was unable to make many generalizations about major patterns in the rate and distribution of nitrification. Since that time, advances in experimental data and conceptual frameworks have elucidated the following patterns:

1. Nitrification rates in oxygenated water columns show typical depth distributions with maximum rates near the bottom of the euphotic zone and a rapidly declining rate with increasing depth below that.
2. Nitrification in the water column is tightly coupled with NH_4^+ regeneration, such that NO_3^-, not NH_4^+, accumulates in the deep sea.
3. Nitrification rates are often low in the well lit surface waters of the ocean, probably due to light inhibition and competition for NH_4^+ with phytoplankton and heterotrophic bacteria.
4. Nitrification is ubiquitous in surface sediments and is often tightly coupled to denitrification in the sediment redox gradient.

3.3.1. Nitrification in the water column

The major N product of organic matter decomposition in seawater is NH_4^+, but NH_4^+ is present at trace or undetectable levels in the huge volume of the deep ocean. Rather, deep water contains NO_3^- at 20–40 μM concentrations, which would seem to imply that nitrification occurs mainly in the deep ocean. Nitrate concentrations in the surface ocean are usually maintained at low levels because phytoplankton assimilate NO_3^- more rapidly than it can be supplied by mixing or diffusion from the deep NO_3^- reservoir. Ammonium, which is produced in the photic zone by heterotrophic processes, is also usually immediately assimilated by phytoplankton and heterotrophic bacteria before it can be nitrified. The important physical and biological differences in the source functions of NH_4^+ and NO_3^- are

the basis of the new production paradigm (Dugdale and Goering, 1967; Eppley and Peterson, 1979) as a framework to understand phytoplankton N demand and growth in the surface ocean, and the subsequent flux of N to the deep sea and ocean floor (Fig. 5.1A).

Ammonium is considered a ''regenerated'' source of N, because it is produced *in situ*, in the euphotic zone, from ammonification of organic matter. The N in
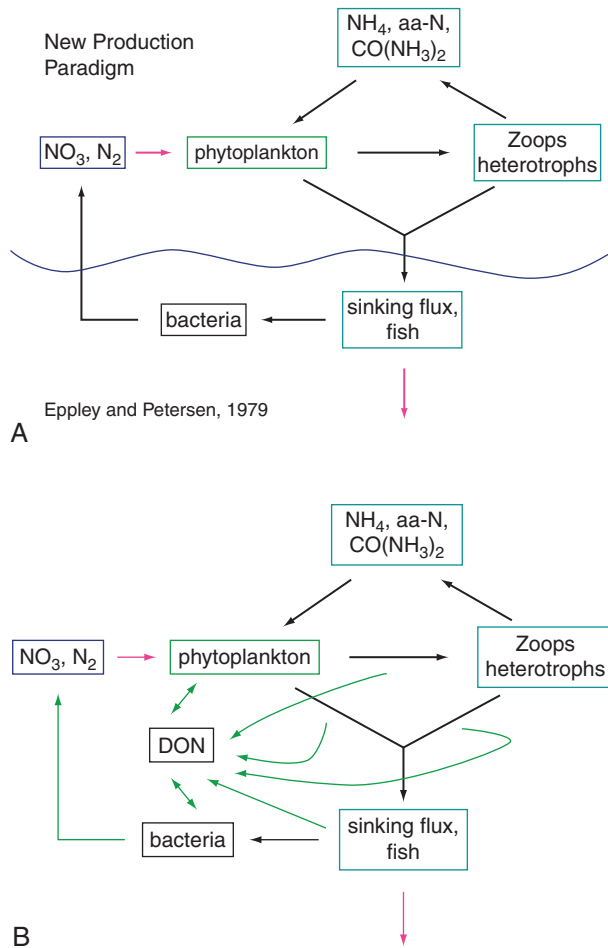
Figure 5.1 (A) The New Production Paradigm, redrawn from Eppley and Petersen, 1979, representing nitrogen transformations in the surface layer of the ocean. N assimilation by phytoplankton is derived from either "new" (NO_3, N_2) or regenerated (NH4, amino acids, urea) nitrogen. In the first panel is the original view of Eppley and Petersen, in which nitrification occurs only below the euphotic zone such that nitrate is a "new" nutrient. The two red arrows are equivalent because "new production" is balanced by the export flux from the euphotic zone. (B) In the second panel, nitrification, as well as various production and consumption terms involving dissolved inorganic nitrogen (DON), occurs in the euphotic zone. Nitrate is thus at least partially a "regenerated" nutrient, and the flux through the labile portion of the DON pool can be rapid and linked to other euphotic zone processes.

NH_4^+ is recycled rapidly and repeatedly between living biomass (phytoplankton, the zooplankton that graze on them, protozoans, bacteria and archaea) and the inorganic nutrient form, which is released from heterotrophic metabolism and grazing. Nitrate, on the other hand, is "new" N because it is virtually absent from the euphotic zone most of the time and must be transported into the system by physical means—mixing or upwelling from deep waters or falling in rain – in order for phytoplankton to use it. The rate of NO_3^- supply can be equated with the steady state rate of export production, primary production based on NO_3^- as a N source (Eppley and Peterson, 1979). This equality between supply and export makes it possible to measure "new production," and by inference, the sinking flux of N, by measuring the assimilation of NO_3^- in incubated samples. In this scenario, nitrification is responsible for the production of NO_3^- in the deep water, from whence it is transported to the surface layer; nitrification is explicitly absent from the euphotic zone itself.

Depth distribution of nitrification rates: Actual measurements of the depth distribution and rate of nitrification show, however, that NO_3^- production and consumption are not in fact so completely or conveniently separated in space. The highest nitrification rates, both NH_4^+ oxidation and NO_2^- oxidation, occur not in the deep ocean, but in a region near the bottom of the euphotic zone. In this depth interval, the light intensity is very reduced and phytoplankton are light limited, and their rates of nutrient assimilation are therefore reduced. It is in this interval that nitrifying bacteria can compete with phytoplankton for NH_4^+; a sharp peak is often observed in the nitrification rate at a depth in the water column where the light intensity is 5–10% of surface light intensity or in the vicinity of the deep chlorophyll maximum, when it occurs (Lipschultz *et al.*, 1990; Sutka *et al.*, 2004; Ward, 1987b; Ward *et al.*, 1984) (Fig. 5.2).

Rates of nitrification reported for the open ocean are in the range of a few to a few hundred nanomolar per day, and have been detected as deep as 3000 m (Ward and Zafiriou, 1988). Most of the data discussed here are NH_4^+ oxidation rates. Nitrite oxidation rate methods are more prone to experimental artifacts; many fewer results have been published and many of the high rates reported are probably overestimates. Rates of nitrification reported in various aquatic environments are presented in Table 5.2. Where profiles extending to a depth of several hundred to a few thousand meters are available, the main pattern that emerges is the association of the highest rates of NH_3 oxidation with the lower region of the euphotic zone (Fig. 5.3). In the eastern tropical North Pacific, NH_3 oxidation rates at the maximum were no more than 20 nM day^{-1} (Ward and Zafiriou, 1988). In the Peru upwelling region, a maximum rate of 747 nM day^{-1} was reported (Lipschultz *et al.*, 1990). In the temperate eastern Pacific Ocean off western North America, maximum rates of 45 nM day^{-1} were reported (Ward, 1987b). Nitrite oxidation shows a less predictable distribution with depth, probably due to methodological problems; in the Peru upwelling system, maximum rates of 600 nM day^{-1} were observed near the lower boundary of the euphotic zone, but high rates (e.g., nearly 300 nM day^{-1}) were observed within the oxygen minimum zone (Lipschultz *et al.*, 1990).

Several studies of nitrification rates have focused on the primary NO_2^- maximum, rather than attempting complete depth profiles. Dore and Karl (1996) reported a few
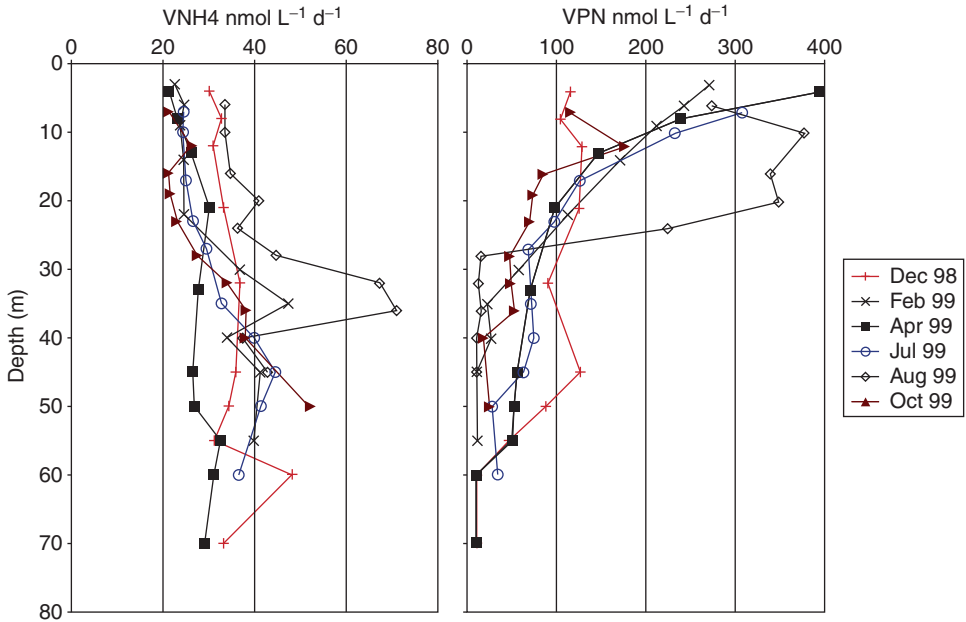
Figure 5.2 Depth distribution of ammonia oxidation rate (VNH4) and ammonium assimilation rate (VPN) in the surface layer in Monterey Bay, CA. Data were obtained from ^{15}N-NH$_4$ tracer incubations at simulated in situ light intensities. (From Ward 2005)

rate measurements based on inhibitor experiments from the central Pacific Ocean and calculated rates up to 137.4 and 138 nM day^{-1} for NH$_4^+$ and NO$_2^-$ oxidation, respectively. Rates 10-fold lower were reported for the same station using ^{15}N tracer methods (Sutka *et al.*, 2004). Ammonium and NO$_2^-$ oxidation rates were usually comparable, and were maximal just below the primary NO$_2^-$ maximum. Bianchi *et al.* (1994) reported nitrification rates up to 1–2 μM day^{-1} in the Rhone River Plume, with rates decreasing to the usual oceanic levels with increasing distance from shore in the Mediterranean Sea. In a recent compilation of nitrification rate measurements, Yool *et al.* (2007), a great range of rates in the upper 250 m of open ocean environments. Using a specific nitrification rate (d-1) they did not detect consistent depth patterns, but did document nitrification within the photic zone. Clearly the simple model that distinguishes NO$_3^-$ as a new nutrient should be modified (Fig. 5.1B); nitrate produced from nitrification in the eupotic zone is often sufficient to meet phytoplankton demand in the same depth interval.

The rate of nitrification in deep ocean water is minimal, due to the decreasing flux of NH$_4^+$ from organic matter decomposition with increasing depth. Thus in many parts of the world ocean, the typical depth distribution of nitrification shows a subsurface maximum which occurs near the bottom of the euphotic zone, and very low rates persisting to great depths. The great accumulation of NO$_3^-$ in the deep sea is therefore due to a small production term and a lack of any significant consumption terms. An exception to this deep sea condition is found in the vicinity of

Table 5.2 Nitrification Rates Reported from Aquatic Systems

Location	Depth (m)	NH_4^+ Ox Rate $(nmol\, l^{-1} day^{-1})$	NO_2^- Ox Rate $(nmol\, l^{-1} day^{-1})$	Method	Reference
Water column					
Sagami Bay, Japan	Photic zone ave, June light	50,000		^{15}N tracer	Miyazeki *et al.*, 1973
Sagami Bay, Japan	Photic zone ave, June dark	26,000		^{15}N tracer	Miyazeki *et al.*, 1973
Sagami Bay, Japan	Photic zone ave, October, light	38,000		^{15}N tracer	Miyazeki *et al.*, 1973
Sagami Bay, Japan	Photic zone ave, October, dark	19,000		^{15}N tracer	Miyazeki *et al.*, 1973
Mangrove forest, Arabian Sea coast (<3 m depth)	Surface, seasonal study	2.4–2321		^{15}N tracer	Dham *et al.*, 2002
Western North Pacific	10–20 m	4.8–29		^{15}N tracer	Miyazeki *et al.*, 1975
Western North Pacific	100–125 m	4.1–8.9		^{15}N tracer	Miyazeki *et al.*, 1975
Southern California Bight	0 m	0–0.7		^{15}N tracer	Olson, 1981a
Southern California Bight	Subsurface max (approx 70 m)	up to 140		^{15}N tracer	Olson, 1981a
Central North Pacific Gyre	109–148 m	2.24–7.3		^{15}N tracer	Olson, 1981a
North Pacific, coastal	0 m	0.2–0.7		^{15}N tracer	Ward *et al.*, 1984
North Pacific, coastal	30–60 m	20–30		^{15}N tracer	Ward *et al.*, 1984

Southern California Bight	100 m	2–40	25–50	[15]N tracer	Ward, 1987b
Southern California Bight	400 m	1–5	10–100	[15]N tracer	Ward, 1987b
Eastern Tropical South Pacific	Subsurface maximum (50–100 m)	20–100	100–600	[15]N tracer	Lipschultz *et al.*, 1990
Eastern Tropical South Pacific	400 m	0–2	0–20	[15]N tracer	Lipschultz *et al.*, 1990
Eastern Tropical North Pacific	Subsurface maximum (80–100 m)	4–58		[15]N tracer	Ward and Zafiriou, 1988
Eastern Tropical North Pacific	1000 m	0–2		[15]N tracer	Ward and Zafiriou, 1988
Eastern Tropical North Pacific	2000 m	0–2		[15]N tracer	Ward and Zafiriou, 1988
Monterey Bay, North Pacific	0–20 m	20–30		[15]N tracer	Ward, 2005
Monterey Bay, North Pacific	30–50 m	Up to 80		[15]N tracer	Ward, 2005
Cariaco Trench	0 m	0		[15]N tracer	Ward and Kilpatrick, 1991
Cariaco Trench	150 m	24–50		[15]N tracer	Ward and Kilpatrick, 1991
Cariaco Trench	>250 m	0		[15]N tracer	Ward and Kilpatrick, 1991
Black Sea	30	4.8	48	[15]N tracer	Ward and Kilpatrick, 1991
Black Sea	60–80 m	60	240	[15]N tracer	Ward and Kilpatrick, 1991

(*Continued*)

Table 5.2 Nitrification Rates Reported from Aquatic Systems (*continued*)

Location	Depth (m)	NH$_4^+$ Ox Rate (nmol l^{-1}day^{-1})	NO$_2^-$ Ox Rate (nmol l^{-1}day^{-1})	Method	Reference
Central North Pacific	Approx 160 M	Up to 120	Up to 125	Inhibitor, ^{14}C	Dore *et al.*, 1996
Central North Pacific	20–200 m	Up to 12		^{15}N tracer	Sutka *et al.*, 2004
Equatorial Pacific	0–140 m	0–40		^{15}N tracer	Raimbault *et al.*, 1999
Central Atlantic transect	0–150 m	1–10	1–30	^{15}N tracer	Clark *et al.*, 2008
Rhone River Plume NW Mediterranean	Surface plume summer	3408–4200	1536–2304	Inhibitor	Bianchi *et al.*, 1999
Rhone River Plume NW Mediterranean	Surface plume winter	120–720	120–720	Inhibitor	Bianchi *et al.*, 1999
Rhone River Plume NW Mediterranean	Nepheloid layer	720 ± 192	528 ± 144	Inhibitor	Bianchi *et al.*, 1999
NW Mediterranean	Nitrite max, 40–60 m	120–270	50–160	Inhibitor	Bianchi *et al.*, 1999
Southern Ocean	0 m	24	14.4–36	Inhibitor	Bianchi *et al.*, 1997
Southern Ocean	50 m	24–43.2	28.8–48	Inhibitor	Bianchi *et al.*, 1997
Southern Ocean	100 m	24–84	9.6–72	Inhibitor	Bianchi *et al.*, 1997
Gulf of Mexico	70 m		4800–12,000	Δ [NO$_3^-$], drogue	French *et al.*, 1983
Hydrothermal Plume	1750–2200 m	Up to 91		Inhibitor	Lam *et al.*, 2004
Marine Sediments		Areal or Mass Rate	Areal or Mass Rate		
Coral Reef Sediments, Australia	Integrated		Up to 1.6 mmol N m^{-2} day^{-1}	^{15}N dilution	Capone *et al.*, 1992

Coral Reef, Enewetak Atoll	Surface, integrated		85–1008 mmol N m^{-2} day^{-1}	DIN flux, N-serve inhibition	Webb and Weibe, 1975
Sponges, Caribbean Sea	Surface, integrated		Up to 413 mmol N m^{-2} day^{-1}	DIN flux	Diaz et al., 1997
Sponges, Caribbean Sea	Surface, per g		Up to 620 nmol N g^{-1} wwt h^{-1}	DIN flux	Corredor et al., 1988
Sponges, Caribbean Sea	Chondrilla nucula		11.52 mmol N m^{-2} day^{-1}	DIN flux	Corredor et al., 1988
Estuarine sediments, sandy	Surface, per g	Up to 40 nmol N g^{-1} h^{-1}		DIN flux, difluoromethane inhibition	Magalhaes et al., 2005
Estuarine sediment, rocky biofilm	Surface, per g	up to 190 nmol N g^{-1}h^{-1}		DIN flux, difluoromethane inhibition	Magalhaes et al., 2005
Marine Loch cores	0–2 cm	1600 µmol l^{-1} day^{-1}		^{15}N tracer	Mortimer et al., 2004
Marine Loch cores	6–8 cm	83.8 µmol l^{-1} day^{-1}		^{15}N tracer	Mortimer et al., 2004
Marine Loch cores	Anoxic layers	0.005– 1.55 µmol l^{-1} day^{-1}		^{15}N tracer	Mortimer et al., 2004
Muddy sediment	Surface, integrated		120–432 mmol N m^{-2} day^{-1}	DIN flux	Gilbert et al., 2003
Mid-Atlantic Bight continental shelf	Surface, integrated		0.64–2.82 mmol N m^{-2} day^{-1}	N$_2$ and NO$_3{}^{-}$ flux	Laursen and Seitzinger, 2002
Svalbard shelf	Surface, integrated		16–112 nmol N g^{-1} wwt h^{-1}	DIN flux	Thamdrup and Fleischer, 1998
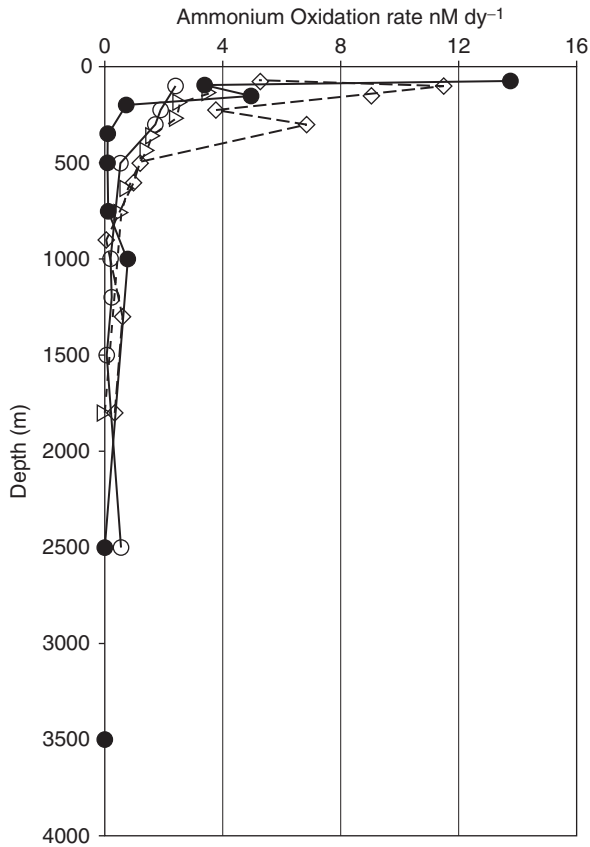
Figure 5.3 Depth distribution of ammonia oxidation rate from four stations in the Eastern Tropical North Pacific. Data obtained from $\pm$N-NH_4 tracer incubations at simulated in situ light intensities. (From Ward and Zafiriou 1988)

hydrothermal vent plumes, where NH_4^+ concentrations are elevated to hundreds of nM (Lilley *et al.*, 1993) and oxidation rates (up to 91 nM day^{-1}) on the same order as those detected in surface water have been reported (Lam *et al.*, 2004).

Based partly on anecdotal evidence from culture work, observations in waste water treatment systems with very high particulate loads, the tendency of nitrifiers to grow in aggregates in bioreactor biofilms, and the prevalence of small particles in natural waters, it has been suggested that nitrification occurs mainly on particles and is mediated by particle–attached bacteria (Hagopian and Riley, 1998). Nitrifier sequences were found both associated with particles and in the bulk seawater phase in the northwestern Mediterranean Sea. In the clone library of 16S rRNA sequences, *Nitrosomonas*-like sequences were preferentially associated with particles and *Nitrosospira*-like sequences dominated in clones from the planktonic phase (Phillips *et al.*, 1999). This may indicate niche preference by the different groups on the basis of attachment to particles, substrate concentration or other physical/

chemical parameters of particle surfaces (Prosser and Embley, 2002). Karl *et al.* (1984) reported high numbers of AOB associated with particles caught in sediment traps and attributed high rates of chemoautotrophy to the particle-associated assemblages. The relative contribution to nitrification rates by particle-associated vs. truly planktonic nitrifying bacteria or those associated with smaller suspended particles is difficult to assess. The distribution of rates as a function of depth indicates that rapidly sinking, i.e., large, particles cannot be the main site of the process (see above, geochemical constraints); nitrification associated with suspended small particles could be consistent with the typical subsurface rate maximum and depth profile that is characteristic of oceanic waters. Nitrifiers appear to escape predation by protozoans by aggregating into particles that are too large to be grazed (Lavrentyev *et al.*, 1997). Thus a trophic effect on nitrification is implied, which also has ramifications for the planktonic vs. particle-associated question.

3.3.2. Nitrification in sediments

The magnitude of nitrification rates in sediments can be much higher, and is certainly more variable, than that reported from water column measurements (Table 5.2). The variability arises not only from the small scale heterogeneity inherent in sediments (partly due also to bioturbation and association of nitrification with the walls of faunal tubes in the sediments), but from the wide range in the level of organic matter input to sediments in shallow water. Very high NO_2^- and NO_3^- fluxes to the water column from sediments, including coral reefs (Capone *et al.*, 1990; Webb and Weibe, 1975) and sponge dominated reef surfaces (Diaz and Ward, 1997), have been attributed to nitrification associated with invertebrates and biotic sediments. Welsh and Castadelli (2004) showed that nitrification activity was associated with the tissues, as well as the external shell surfaces, of filter feeding bivalves. They suggested that nitrifying bacteria may colonize invertebrates and establish specific biological relationships with them (Welsh and Castadelli, 2004), as was suggested by the high nitrification rates observed in sponges (Diaz and Ward, 1997) and the detection of AOB sequences in sponge tissues. High rates have also been reported from the shallow waters of sediment dominated systems such as mangrove forests (Dham *et al.*, 2002) Nitrification in the shallow water column of the mangrove forest was highly seasonal and strongly coupled with NH_4^+ regeneration and NO_2^- assimilation rates (Dham *et al.*, 2002). In contrast, nitrification rates were very low in the sediments of a coastal lagoon where uptake of DIN by seagrass dominated the N cycle (Rysgaard *et al.*, 1996).

Irrigation by worm tubes and sediment infauna has been shown to increase nitrification rates by increasing oxygen availability (Pelegri *et al.*, 1994; Pelegri and Blackburn, 1996; Svensson, 1998). The presence of worm tubes, which are actively irrigated by the invertebrate inhabitants, introduces small scale spatial variability into the oxygen distribution in sediments. Nielsen *et al.* (2004) used microelectrodes to measure small scale distributions of oxygen and NO_3^- around individual worm tubes and found that nitrification and NO_3^- reduction (denitrification plus NO_3^- reduction to NH_4^+) were spatially separated: nitrification was restricted to the oxic zone (up to 1.5 mm) around the tubes, while NO_3^- reduction occurred in the adjacent anoxic zone. Both processes were stimulated by the addition of NH_4^+ indicating that NO_3^- produced by nitrification limited the rate of denitrification. Webb and Eyre

(2004) found that the presence of burrowing shrimp (*Trypaea australiensis*) increased denitrification rates by fivefold compared to unoccupied sediments, while oxygen consumption increased by 81%. Most of the increased oxygen demand was attributed to microbial respiration and oxidation processes (rather than to respiration by the shrimp itself), implying that the increased denitrification was coupled to increased nitrification resulting from increased organic matter decomposition (Webb and Eyre, 2004). Gilbert *et al.* (2003) investigated the relationship between coupled nitrification/denitrification rates and the density of macrofaunal burrow spacing using simulated burrows and reaction-diffusion models. Nitrification rates were maximal at an intermediate tube density, and denitrification varied in direct proportion to nitrification, indicating the importance of the coupling of the two processes through *in situ* NO_3^- production. The main controls on nitrification rate were the supply of oxygen and NH_4^+, and competition for oxygen by other reductants. Dollhopf *et al.* (2005) found that coupled potential rates of nitrification and denitrification in salt marsh sediments were positively correlated with each other and with macrofaunal burrow abundance and attributed this effect to enhanced oxygen availability due to bioirrigation. Nitrification potential, measured using chlorate inhibition, was positively correlated with the occurrence of macrofaunal structures in estuarine sediments (Mayer *et al.*, 1995). Local sediment conditions are likely to be important in determining the degree of coupling and net rates of nitrification and denitrification. In a bay in southern Australia, denitrification rates were not related to bioirrigation and it was suggested that high irrigation rates removed NH_4^+ that was regenerated in anoxic regions of the sediments too fast for it to be nitrified and recycled (Berelson *et al.*, 1998). Any variability among nitrifier community composition between aerated and non-aerated sites would introduce complexity into the community response, perhaps shifting the optimum to slightly different length and packing scales, but probably not changing the nature of the relationships and the coupling between nitrification and denitrification.

Nitrifiers living in marine sediments that are periodically or persistently exposed to anoxic conditions would need to be able to survive periods of inactivity or even serious inhibition in order to recover when conditions improved. The physiological basis of this survival or tolerance of anoxic conditions is unknown, and the degree to which nitrifiers can recover from serious anoxia is questionable. In microcosm studies with estuarine sediments, nitrification was almost completely inhibited by sulfide, the end product of bacterial sulfate reduction that occurs widely in anoxic marine sediments (Joye and Hollibaugh, 1995). A pulse of sulfide, which was detectable in the sediments for only a few hours, inhibited nitrification for at least 24 h. Thus in marine sediments where sulfate reduction occurs, the ability of nitrifiers to respond to daily oxygen fluctuations may be impeded. Even when the oxygen-sulfide interface deepens during the day, nitrifiers may be unable to recover from the sulfide poisoning. In that case, both nitrification and denitrification (which is partially dependent upon NO_3^- supply from nitrification) may occur at slower rates than would be predicted for similar environments in freshwater sediments (where sulfate, and therefore sulfide release from sulfate reduction, is much less prevalent). In contrast, Jensen *et al.* (1993) reported that nitrification could be stimulated essentially instantaneously by addition of oxygen to sulfidic freshwater sediments. Using microelectrodes to measure oxygen and NO_3^-

profiles in a sediment core, Jensen *et al.* (1993) detected an almost immediate increase in nitrification rate when anaerobic sediments were aerated, implying that nitrifiers are inhibited but not poisoned by sulfide. When sediments were allowed to equilibrate with overlying water of differing oxygen concentrations, the zones of nitrification and denitrification stabilized at greater depths with increasing oxygen concentration, reflecting oxygen penetration into the core (Fig. 6, Jensen *et al.*, 1994). This difference may reflect the different community composition of freshwater and marine nitrifying bacteria, or it may be related somehow to salinity and its effect on nitrifiers.

In both deep and shallow sediments, nitrification can be one of the main sinks for oxygen (Blackburn and Blackburn, 1993; Grundmanis and Murray, 1977). Nitrification accounted for up to 50% of the oxygen demand in the Mississippi River plume at intermediate salinities (Pakulski *et al.*, 1995) but was much less important in the nearby Atchafalaya River, which is dominated by heterotrophic processes (Pakulski *et al.*, 2000). In continental shelf sediments, nitrification and denitrification are often closely linked. Coupled nitrification/denitrification is invoked to explain the observation that the rate of N_2 flux out of sediments can greatly exceed the diffusive flux of NO_3^- into the sediments (Devol and Christensen, 1993). Ammonium, produced during aerobic and anaerobic organic matter mineralization, is oxidized to NO_3^- and subsequently reduced to N_2. Anaerobic oxidation of the regenerated NH_4^+ could also be a factor in the otherwise lack balance between NO_3^- supply and consumption. Nitrification can supply up to 100% of the NO_3^- consumed by denitrification (Laursen and Seitzinger, 2002; Lehmann *et al.*, 2004). In deep sea sediments, denitrification may not be detected in the upper 30–50 cm, and NO_3^- accumulates in near Redfield stoichiometry due to nitrification of regenerated NH_4^+ during organic matter diagenesis (Grundmanis and Murray, 1982).

Although oxygen and NH_4^+ conditions likely differ between planktonic and sediment environments, there is no clear evidence from clone libraries that water column and sediment nitrifying communities are significantly different in composition and regulation. The most extensive comparisons are possible on the basis of *amoA* clone libraries from Chesapeake Bay sediments (Francis *et al.*, 2003) and the Monterey Bay water column (O'Mullan and Ward, 2005). Although unique sequences were found in both environments, it was more common to find the sequences from different environments clustering together, and with representatives from both planktonic and sediment sequences from other studies. Thus on the basis of functional genes alone, it is not possible to distinguish major sediment vs planktonic strains. 16S rRNA sequences that were 96–98% identical with known betaproteobacterial nitrifying isolates were obtained in clone libraries from anoxic sediments in a marine Loch (Freitag and Prosser, 2003). The sedimentation rate at this site implied that the sediments from which the sequences originated had been buried for about 100 years, implying the long term viability of nitrifying bacteria in anoxic environments. The same authors did not detect gammaproteobacterial or anammox type planctomycetes-like sequences, and concluded that some groups of betaproteobacterial AOB might be capable of growing and perhaps nitrifying under anoxic conditions. Mortimer *et al.* (2002, 2004)

detected a sharp peak of NO_3^- in pore water profiles from the sediment environment, and were able to detect low NH_3 oxidation rates at the depth (20 cm) of the NO_3^- peak, and also documented the presence of both *Nitrosospira-* and *Nitrosomonas*-like 16S rRNA sequences in the sediments. It was concluded that the buried NO_3^- peak was due to slumping of the coastal sediments and represented a non steady state feature, rather than a long term result of anoxic NH_3 oxidation. The ability of AOB to persist for long periods in unfavorable conditions is well known, but the continued activity of conventional aerobic AOB in anoxic sediments is doubtful.

4. Environmental Variables Affecting Nitrification Rates and Distributions

It is clear from the discussion of the depth distribution of nitrification rates that variables such as light intensity and substrate concentration are important determinants of the magnitude and location of nitrification in the water column, as they are for much of the biogeochemical cycling in the environment. Their effects have been studied in both laboratory culture experiments and in field samples, using incubations and measurements of natural assemblages. Culture studies have so far been restricted to bacterial nitrifiers, so regulation inferred from studies of AOB may not apply to archaeal ammonia oxidizers. On the other hand, rate measurements from environmental samples reflect the ecological properties of the natural assemblages, regardless of their phylogentic composition. Thus environmental effects inferred from field samples may provide insight into the physiology of uncultivated organisms involved in the processes.

4.1. Temperature

The effect of temperature is of potential importance in wastewater systems where nitrifying bacteria are cultured under artificial conditions, or in terrestrial environments or shallow marine systems where temperature changes on short time scales. Temperature was a significant regulator of nitrification rates in the shallow water column of mangrove forests (Dham *et al.*, 2002), although oxygen and NH_4^+ concentrations were more important. Different temperature optima are reported for strains isolated from different kinds of terrestrial environments (Golovacheva, 1976; Jiang and Bakken, 1999). A marine betaproteobacterial AOB species with a temperature tolerance between $-5°C$ and nearly $29°C$ was isolated from Alaskan shelf sediments (Jones *et al.*, 1988). In the oceanic environment, however, temperature is not generally considered to be an important variable for nitrification because bacterial populations are generally adapted to the temperature of their environments. Thus, one can demonstrate a classical dependence of the rate of nitrification on temperature in any particular environment, but temperature is not generally the limiting factor *in situ*. Nitrifiers adapted to low temperature can nitrify under low temperature conditions at rates comparable to the rates attained by nitrifiers adapted to higher

temperatures living under high temperature conditions. Nitrification rate was highly adapted to temperature in arctic sediments, with temperature optima ranging from 14 to 40°C, depending on the temperature of the environment (Thamdrup and Fleischer, 1998). While temperature is an important master variable for biological processes, nitrification is if anything less sensitive to regulation by temperature than other processes, and is usually regulated in the environment by some other variable.

4.2. Salinity

Because salinity in the open ocean does not vary on scales that are important to physiological response in bacteria, sensitivity to salinity changes is not an important variable in determining nitrification rates or nitrifying bacterial community composition in the ocean. It appears, however, that salinity is a very important, perhaps dominant, variable in regulating nitrification in riverine and estuarine environments. Highest nitrification rates were associated with low to moderate salinities in the Mississippi (7 psu) and Atchafalaya (8 psu) River plumes (Pakulski et al., 2000), but turbidity and competition with phytoplankton and other bacteria also played a role in determining the distribution of nitrification. An earlier study in the Mississippi River plume had detected highest nitrification rates at higher salinities (27 psu) (Pakulski et al., 1995).

Using the N-serve inhibition method with $^{14}CO_2$ uptake to estimate nitrification, Somville (1984) showed that salinity was an important variable in determining nitrification rates in the Schelde estuary in The Netherlands. She concluded that nitrifier populations were adapted to their in situ salinities and suggested that community composition – and salt tolerance – varied along the estuarine gradient. Large shifts in the composition of 16S rRNA clone libraries representing AOB communities were subsequently found along the salinity gradient from 0 to 15 psu in the Schelde estuary (Bollman and Laanbroek, 2002; de Bie et al., 2001). The Schelde libraries were dominated by Nitrosomonas-like sequences, but two different groups switched dominance in the clone libraries between about 3 and 5 psu. Nitrosospira-like sequences were detected only rarely and mostly at the highest salinity station. Sequences representing both the uncultivated marine Nitrosospira clade and the marine Nitrososmonas clade were found in clone libraries from Chesapeake Bay sediments (Francis et al., 2003). The level of amoA sequence diversity within samples from Chesapeake Bay was compared to the pairwise differences in environmental parameters (e.g., salinity, oxygen, temperature, NO_3^- and NH_4^+ concentrations) between stations. Difference in salinity was the dominant variable and accounted for 62% of the variation in pairwise sequence dissimilarity between stations. Ammonia concentration, the next most important variable, explained only an additional 2.7%. The greatest diversity in AOB clone libraries occurred in the freshwater end member stations, and these libraries were also most different in composition from all the other samples, indicating that different organisms perform the nitrification function in different parts of the bay, depending on salinity. Members of the uncultivated Nitrosopira clade dominated the clone libraries from higher salinity stations. In a study that included three estuarine stations with salinities ranging from 8.7 to 31.7, different and temporally stable

AOB communities were detected by terminal restriction fragment analysis of *amoA* genes (Bernhard *et al.*, 2005). *Nitrosospira*-like sequences were also identified as the seawater dominants in this environment.

Using sediments collected from the middle of the estuarine salinity gradient in the Douro estuary (Portugal), manipulation of salinity in the overlying water was used to test the effect of salinity on nitrification and denitrification (Magalhaes *et al.*, 2005). Denitrification rates did not vary in response to salinities of 0 and 35 psu, but nitrification was optimal at 15 psu. This may indicate the presence of a halotolerant denitrifier assemblage, while the nitrifier assemblage was adapted to its *in situ* salinity and was inhibited by both lower and higher salinity. In the Rhone river estuary, highest nitrification rates were found in the surface low salinity layer, but both NH_3 and NO_2^- oxidation rates were correlated primarily with NH_4^+ concentration, and influenced by temperature, rather than salinity directly (Bianchi *et al.*, 1999).

Rysgaard *et al.* (1999) tested the effect of salinity on nitrification in a Danish estuary to determine whether the increased desorption of NH_4^+ from sediments was responsible for the decreasing nitrification rates at high salinity. They concluded that salinity influenced nitrification rates independently of NH_4^+ concentrations and suggested that some physiological factor must be involved.

Many authors have described the salinity tolerances of nitrifying bacterial cultures (Macfarlane and Herbert, 1984; Stehr *et al.*, 1995) and it is clear that halotolerance is not the rule among AOB. Seven strains of *Nitrosomonas* isolated from different regions of the Elbe showed different ranges of salinity tolerance (Stehr *et al.*, 1995). *Nitrosococcus halophilus*, one of two known species in the gammaproteobacterial AOB, has a higher salinity optimum and upper limit than does *N. oceani*, the strain detected widely in the open ocean (Koops *et al.*, 1990). The salt requirements of cultivated AOB are summarized by Koops *et al.* (2003). Salinity may restrict the distribution of nitrifying strains and help determine nitrifier community composition, but as with temperature, appropriately adapted communities minimize the variability in nitrification rates as a function of salinity. The apparent dominance of AOA over AOB abundance in seawater is consistent with strong selection by salinity in favor of AOA. AOA have also been detected in terrestrial (Leininger *et al.*, 2006) and wastewater (Park *et al.*, 2006) environments, however, and salinity as a variable in the overall ecology of AOA remains to be investigated.

4.3. Inhibitory compounds

Both NH_3 oxidizers and NO_2^- oxidizers, but especially the former, are susceptible to inhibition by a wide range of compounds, and several different modes of action have been documented (Bedard and Knowles, 1989). The two most common modes of inhibition are: (1) interference with the active site of the primary enzyme (i.e., NH_3 monooxygenase in AOB; NO_2^- oxidoreductase in NOB) by compounds that share structural homology with NH_3 or NO_2^- and (2) metal binding compounds, which interfere with the action or availability of copper in the NH_3-oxidizing enzymes. In both NH_3- and NO_2^--oxidizers, the susceptibility to inhibitors by key enzymes forms the basis of some methods used to measure the rate of nitrification in the environment (see above).

In terrestrial systems, the presence of certain organic compounds (e.g., mono-terpenes produced by plants) has been proposed to limit the rate of nitrification and the inhibition of nitrification in acid soils has long been of concern. The potential of naturally occurring organic compounds to inhibit nitrification in seawater has not been considered very much. Inhibition by organosulfur compounds has been demonstrated in cultured marine NH_3 oxidizers (Ly et al., 1993) and the inhibitory effect of sulfide on nitrification is thought to limit nitrification and coupled nitrifi-cation/denitrification in marine sediments (see below; Joye and Hollibaugh, 1995). While naturally occurring organic compounds have not been investigated as poten-tial inhibitors in seawater, the product of their photodecomposition, carbon monoxide, has been implicated (Jones and Morita, 1984). While CO, like methane, acts as a substrate analog or a suicide inhibitor for NH_3 oxidizers, the direct inhibitory effect of light on nitrifiers (see below) is considered to outweigh the potential effect of CO inhibition in surface waters. As a substrate analog, CO can be oxidized by NH_3–oxidizing bacteria, and Jones (1992) proposed to exploit this capability as a method for quantification of NH_3 oxidation rates in natural systems. Like methane oxidation (Ward, 1987b), however, the rate of CO oxidation by nitrifiers depends on the relative concentration of alternative substrates, which may cause artifacts in relating overall rates of CO oxidation to nitrification. The susceptibility of AOA to the known suite of nitrification inhibitors remains to be investigated.

4.4. Light

The inhibitory effect of light was reported by German researchers in the 1960s (Muller-Neugluck and Engel, 1961; Muller-Nugluck and Engle, 1961; Schon and Engel, 1962), verified in enrichment cultures in seawater (Horrigan et al., 1981) and later described in more detail (e.g., Vanzella et al., 1989). Even in enrichment cultures of nitrifiers derived from the sea surface film, nitrification was severely inhibited by light, such that periods of more than 12 h darkness were necessary to allow net nitrification to occur over a 24 h period (Horrigan et al., 1981). Vanzella et al. (1989) found evidence that NO_2^- oxidizers were more sensitive to sunlight than were NH_3 oxidizers, based on single culture studies, but Guerrero and Jones (1996a) showed that species specific responses may obscure any generalizations among major groups. Light at near UV wavelengths of 300–375 nm, as well as in the visible range (<500 nm), was inhibitory for the cultures tested, and sunlight caused almost complete inhibition within 2 hr of the AOB tested, and 67% and 17% inhibition for the two NOB tested (Guerrero and Jones, 1996a). Many biological molecules such as DNA and proteins, quinones, flavins, NADH and other molecules involved in electron transport activity absorb primarily in the near UV wavelengths. Bock (1965) showed that light caused the oxidation of cytochrome c in both AOB and NOB, and suggested that photoinhibition resulted from damage to the electron transport system. The specific phototargets involved in light inhibition have not been thoroughly investigated, but photosensitivity is consistent with the potential for photodamage of these essential molecules in microbes living in near surface waters. Horrigan and Springer (1990) reported that oceanic strains of NH_3 oxidizers were generally more sensitive to light inhibition than were estuarine strains. Degree

of inhibition was inversely correlated with NH_3 oxidation rate, but the mechanism of this relationship could not be determined. Horrigan and Springer (1990) argued that the greater sensitivity of oceanic NH_3 oxidizers might be responsible for the generally lower rates of NH_3 oxidation observed in oceanic vs. river or estuarine environments, but the complex of other factors such as overall rates of material processing could not be independently evaluated.

There is abundant evidence from culture studies that both AOB and NOB are photosensitive. It is a high priority to investigate the photosensitivity of AOA. Even if all nitrifiers exhibit photoinhibiton in some form, however, the direct and indirect ecological implications of this physiology for the rates and distributions of nitrification in the environment are not easily predicted. Dissolved organic matter in seawater, as well as turbidity due to sediments or phytoplankton, might all provide photoprotection in surface waters, and regulation of nitrification by other factors discussed in this section may be much more important in many environments.

Ammonia and NO_2^- oxidation rates are often reported to be highly coupled and to occur at about the same rate. This coupling would be consistent with the observation that neither NH_4^+ nor NO_2^- accumulates to high levels in most natural waters. For NO_2^-, the exceptions to this rule are the primary NO_2^- maximum of near surface waters and the secondary NO_2^- maximum characteristic of oxygen minimum zones. The surface water feature has been attributed to two main processes. Because the primary NO_2^- maximum is usually associated with low light intensities at the bottom of the euphotic zone, some interaction between biological processes and light is suspected. In one scenario, phytoplankton are responsible: assimilation of NO_3^- (via reduction to NO_2^- and then to NH_4^+) requires energy, especially the step at which NO_2^- is reduced to NH_4^+. Under low light intensity, phytoplankton might not have enough energy to reduce the NO_3^- completely, and some of the intermediate NO_2^- is allowed to leak out of the cell. In the alternative scenario (Olson, 1981b), nitrifying bacteria are responsible: on the basis of information then available (Bock, 1965; Muller-Neugluck and Engel, 1961; Schon and Engel, 1962), Olson hypothesized that NO_2^- oxidizers are more sensitive to light inhibition than are NH_3 oxidizers. Therefore, NH_3 oxidizers are active at slightly shallower depths in the water column than are NO_2^- oxidizers. This leads to an accumulation of NO_2^- in the interval between the depths at which NH_3- and NO_2^--oxidizers are released from light inhibition. Guerrero and Jones (1996a) had reported that AOB were more sensitive to light inhibition than were NOB, but found that NOB required longer recovery times than AOB (Guerrero and Jones, 1996b). Thus differential recovery time, rather than differential light inhibition, could explain the formation of the primary NO_2^- maximum by nitrification.

Several studies of nitrification rates in surface seawaters from various geographical regions show profiles that are consistent with light inhibition of both NH_3 and NO_2^- oxidation (Lipschultz et al., 1990; Ward and Carlucci, 1985; Ward and Kilpatrick, 1991; Ward, 1987a; Ward et al., 1984). Simulated in situ rate measurements (i.e., measured under realistic light conditions) show a clear negative relationship with ambient light intensity (Ward et al., 1984). Nitrifiers may be somewhat protected from light inhibition in surface waters by the presence of absorbant organic compounds in seawater and by mixing, which prevents long intense

exposure at the surface. However, the data from simulated *in situ* rate measurements are consistent with light inhibition in surface waters, and the well verified sensitivity of nitrifiers in culture strongly suggests an influence of light in the environment. In two year study of NH_4^+ oxidation in the surface layer the highest rates were usually associated with lowest light intensity, but low rates occurred at all intensities (Fig. 5.4), implying a role for additional factors. Dore and Karl (1996) did not directly assess the effect of light on nitrification rates (all rates were measured in the dark) but they attributed two distinct features within the primary NO_2^- maximum to phytoplanton (the upper primary NO_2^- maximum) and nitrifiers (the lower primary NO_2^- maximum). After a comprehensive review of previous models for the formation of the primary NO_2^- maximum, and the distributions of chlorophyll, NO_2^- and NO_3^- in the Sargasso Sea, Lomas and Lipschultz (2006) concluded that phytoplankton NO_2^- release was the dominant factor in creating and maintaining the primary NO_2^- maximum, with a secondary role in some seasons for nitrification.

Lipschultz *et al.* (1985) documented the light inhibition of NH_3 oxidation in the Delaware River and concluded that this effect influenced the spatial distribution of nitrification in the estuary. Depending on their depth, light is not usually a problem for nitrification in sediments. In shallow sediments, light may have an indirect positive effect on nitrification rates by increasing photosynthesis, and thus increasing oxygen supply to the sediments (Lorenzen *et al.*, 1998).

The fact that nitrification rates are often maximal in the vicinity of the bottom of the euphotic zone has important implications for our understanding of N supply for primary production. The important distinction made in the new production paradigm (see above), between NH_4^+, which is the "regenerated" nutrient and NO_3^-, the "new" nutrient, is the basis of ^{15}N-tracer methods to assess new and regenerated primary production. However, if nitrification occurs in the same depth interval where nitrate assimilation occurs, then nitrate too is a "regenerated" nutrient.
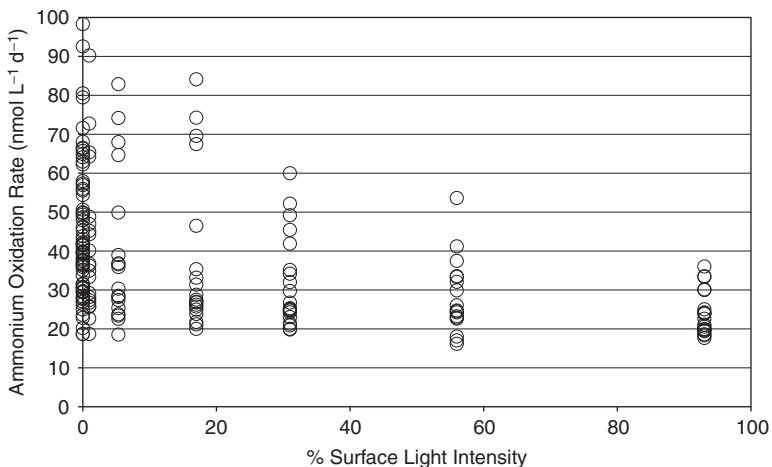
Figure 5.4 Relationship between ammonia oxidation rate and simulated in situ incubation light intensity. Data are shown for approximately 220 ^{15}N-NH_4 tracer incubations performed over the course of 2 years in Monterey Bay, CA. (From Ward, 2005)

Then, total NO_3^- assimilation would depend on a combination of NO_3^- supplied by nitrification, plus that supplied by mixing from the deep reservoir. Several studies have addressed the question of whether significant nitrification occurs in the euphotic zone, and have concluded that *in situ* nitrification could supply 100% or more of the phytoplankton NO_3^- demand (Bianchi *et al.*, 1997; Dore and Karl, 1996; Ward *et al.*, 1989b). A large role for *in situ* nitrification to supply the phytoplankton NO_3^- demand is also supported by biogeochemical models (Mongin *et al.*, 2003). Estimated vertical fluxes of NO_3^- via eddy pumping were also not sufficient to support the observed primary production in the Sargasso Sea (Martin and Pondhaven, 2003). Thus nitrification and NO_3^- assimilation by phytoplankton can be closely coupled, even though the two processes are favored by quite different environmental conditions. Nitrate must be considered a regenerated nutrient in some cases, and estimates of new primary production in the euphotic zone on the assumption that NO_3^- is supplied only as a new nutrient from outside the system is not valid (Yool *et al.*, 2007).

Even in coastal systems where allocthanous nutrient supply can be very large, nitrification and be an important source of NO_3^-. Dham *et al.* (2002) measured very high nitrification rates in the summer in the shallow water of mangrove forests. The rates were highly correlated with both NH_4^+ and NO_3^- concentrations and could supply the entire phytoplankton NO_3^- demand in summer.

4.5. Substrate concentration

The influence of light may be compounded by the necessity for NH_3 oxidizers to utilize NH_4^+, which is in short supply in those depth intervals in the water column where light is most intense. When NH_4^+ assimilation and NH_4^+ oxidation are measured in the same incubation, it is seen that assimilation is more important in the upper portion of the euphotic zone and nitrification in the lower portion (Fig. 5.3 and Ward, 1987a). This pattern suggests that in the well lit upper waters, phytoplankton are able to assimilate NH_4^+ but nitrifiers are either unable to compete for NH_4^+ in the presence of phytoplankton or else, light inhibition prevents them from utilizing NH_4^+ in that environment. In the lower portion of the euphotic zone, phytoplankton may be light limited and less able to assimilate NH_4^+, whereas nitrifiers are released from light inhibition and better able to utilize the NH_4^+ being released by mineralization or N_2 fixation in that interval. There is no direct evidence that nitrifiers are poor competitors with phytoplankton in the lower euphotic zone, but direct tests of this hypothesis in shallow sediments confirm that benthic algae are superior competitors for NH_4^+ relative to benthic AOB communities (Risgaard-Petersen *et al.*, 2004b). The analogous situation in the surface ocean is certainly consistent with observed rate and biomass distributions. Incubation experiments under various conditions of light and NH_4^+ additions could be used to differentiate the effects of light and substrate competition. It would be especially important to impose true tracer level substrate additions and very sensitive isotope determinations to avoid even minor perturbations caused by additions of substrate and product.

In cultures of nitrifying bacteria, the dependence of nitrification rate on substrate concentration is first order at low concentrations (10–100 μM; these

concentrations are low for culture conditions and some sediments, but very high compared to surface seawater). The instantaneous rate of NH_4^+ oxidation or NO_2^- oxidation increases predictably with increasing NH_4^+ or NO_2^- concentration until some, often very high, inhibitory level is reached. However, researchers often have been unable to demonstrate consistent substrate dependence for NH_3 oxidizers in natural assemblages. A general relationship between measured NH_4^+ oxidation rate and ambient NH_4^+ concentration supports the importance of substrate concentration. Early studies (Vaccaro, 1962) showed that net NO_2^- and NO_3^- accumulation in samples from the Gulf of Mexico was stimulated by the addition of NH_4^+. In ^{15}N tracer experiments on water samples when substrate additions are made at the micromolar level representative of the environment, NH_4^+ oxidation is usually independent of substrate concentration. Lack of response to substrate perturbation (e.g., Olson, 1981a; Ward and Kilpatrick, 1990) has led to speculation that the affinity of NH_3 -oxidizers for NH_4^+ may be so great that current experimental methods cannot detect a response to enhanced substrate concentrations. In contrast to NH_3 oxidation, NO_2^- oxidation in natural samples usually shows a more conventional response to added substrate (e.g., Olson, 1981a), but this second step in the nitrification process has received less attention. If AOA dominate the NH_3 oxidizing assemblage of seawater, then the substrate affinity arguments above apply to AOA, but their substrate affinity and kinetics in culture have not been studied.

Nitrification in sandy sediments and in biofilms on rocky substrates in a Portuguese estuary was stimulated by increased NH_4^+ concentration, up to thresholds greater than 100 μM, above which higher concentrations became inhibitory (Magalhaes et al., 2005). Considering measurements from several marine and estuarine environments, Caffrey et al. (2003) found that potential nitrification rates were positively correlated with ambient NH_4^+ concentration when oxygen was available. Very high NH_4^+ concentrations were associated with anoxic sediments, however, where no nitrification occurred. Availability of NH_4^+ in sediments is expected to be influenced by ionic strength, because NH_4^+ ions are displaced from sediment adsorption sites by other cations at high salinity. The idea that the salinity effect on nitrification could be explained by the interaction with NH_4^+ availability (Gardner et al., 1991; Seitzinger et al., 1991) does not appear to be the case (see salinity section above).

4.6. Oxygen concentration

Nitrifying bacteria are traditionally considered to be obligate aerobes; they require molecular oxygen for reactions in the N oxidation pathways and for respiration. They are reputed to be microaerophiles, however, who thrive best under relatively low oxygen conditions. Microaerophily may be important in interface environments such as the sediment water interface and in the oxygen minimum zones of the ocean. The role of oxygen in sedimentary nitrification and coupled nitrification/denitrification is discussed above in the section on nitrification in sediments.

While net nitrification and growth at the expense of inorganic N occurs only under aerobic conditions in autotrophic nitrifiers, both NH_3- and NO_2^--oxidizing

nitrifiers are apparently capable of partial or even complete denitrification. Loss of fixed N has been observed in cultures of nitrifying bacteria growing at reduced oxygen tension. *Nitrosomonas* sp. marine in a lithotrophic medium (no added organics) grew best under an oxygen concentration of 1% in the headspace (compared to 20% in air) (Goreau *et al.*, 1980) and they also produced the greatest amount of nitrous oxide relative to NO_2^- under those conditions. Production of nitrous oxide, nitric oxide and N_2 was reported for *Nitrosomonas* growing in the presence of organic compounds in the absence of oxygen (Stuven *et al.*, 1992). *Nitrosomonas* could also grow using hydrogen as an electron donor and NO_2^- as its electron acceptor (Bock *et al.*, 1995). NO_2^- oxidizers can grow via dissimilatory NO_3^- reduction in the presence of organic matter and the absence of oxygen (Freitag *et al.*, 1987).

The potential ecological impact of this physiological versatility in nitrifying bacteria has not been widely investigated in natural systems. The anaerobic capabilities of nitrifiers have received much more attention in connection with sewage and wastewater treatment, where there is economic incentive to enhance the conversion of NH_4^+ to N_2 under totally anaerobic or totally aerobic conditions. The conditions that are conducive to denitrification by nitrifying bacteria are the same ones that induce denitrification in classical denitrifying bacteria. It is conceivable that both metabolic types are involved in the production and consumption of trace gases in the same environment. Both N_2O and NO are involved in important atmospheric processes; they contribute to greenhouse warming and to catalytic destruction of stratospheric ozone. Thus, understanding which processes are responsible for their production could prove to be important for understanding or potentially regulating their fluxes.

A significant positive correlation between apparent oxygen utilization (AOU) and N_2O accumulation is often observed in marine systems (Cohen and Gordon, 1978; Nevison *et al.*, 2003; Yoshinari, 1976). The relationship implies that nitrification is responsible for N_2O accumulation in oxic waters where it is released as a byproduct of mineralization going to completion via nitrification. The relationship breaks down at very low oxygen concentrations ($\sim 6\ \mu M$, Nevison *et al.*, 2003), where N_2O is usually below atmospheric saturation due to consumption by denitrification. The lack of correlation between N_2O accumulation and N-star (an indicator of N cycle stoichiometry; Deutsch *et al.*, 2001) is additional support for the conclusion that most of the N_2O in the ocean is derived from nitrification rather than denitrification (Nevison *et al.*, 2003). In a few special places in the open ocean, oxygen concentration is depleted to a level low enough to allow net denitrification to occur in the water column. These regions, referred to as oxygen deficient zones, occur in the eastern tropical South Pacific (off the coast of Peru), in the Arabian Sea and the eastern tropical North Pacific Ocean (off the west coast of Mexico). The coupling between nitrification and denitrification has also been studied in these systems, which are essentially analogous to the sediment environments described above, except that the oxygen and NO_3^- gradients extend over tens to hundreds of meters. Suboxic and anoxic waters and sediments tend to have large fluxes, and sometimes large accumulations, of the gaseous intermediates of nitrification and denitrification. This is probably due to the sensitivity of the various organisms and enzymes involved in their production and consumption to oxygen concentration in the local environment of the microorganism.

In studies of N cycling in oxygen deficient zones, nitrification and denitrification appear to be linked, as might be expected from analogy with sediment systems. Ammonium oxidation typically is maximal near the bottom of the euphotic zone close to the upper boundary of the oxygen minimum zone, but NO_2^- oxidation is detected within the oxygen minimum zone itself (Lipschultz *et al.*, 1990). This finding is consistent with the ability of NO_2^- oxidizers to persist and metabolize at very low or zero oxygen conditions, but their metabolism under these conditions would be expected to be dissimilatory rather than oxidative. The nitrous oxide that typically accumulates in the suboxic (low but not zero oxygen concentrations) regions of the oxygen minimum zones is thought to be due to nitrification (via NO_2^- reduction by AOB under low oxygen conditions), because nitrous oxide is depleted in the core of the oxygen minimum zone where denitrification rates are thought to be greatest. A direct tracer confirmation of this pathway in nature has not yet been accomplished. Stable isotope measurement of nitrous oxide from oxygen depleted waters in the Arabian Sea imply that both nitrification and denitrification may contribute to the signal (Naqvi *et al.*, 1998; Ward and Zafiriou, 1988). Ward and Zafiriou (1988) attributed the nitric oxide production they observed in the oxygen minimum zone of the eastern tropical North Pacific to nitrification and found that it was equivalent to 18% of the total NH_3 oxidation rate.

5. NITRIFICATION AND METHANE OXIDATION

As mentioned above in connection with the physiology of nitrifying bacteria, the NH_3 oxidizers and the methanotrophs have important biochemical similarities. These similarities extend to the nature of the primary enzyme in the NH_3 and methane oxidation pathways, the sensitivity of the enzymes to a wide range of metabolic inhibitors, the metabolic capabilities of the cell and to the ultrastructure of the cell (Bedard and Knowles, 1989). Methanotrophs, like NH_3 oxidizers, depend on two substrates with generally opposite sources. Methane, like NH_4^+, accumulates in anoxic habitats where it is produced by strictly anaerobic methanogens. Oxygen diffuses into surface waters or surface sediments from the overlying oxygenated habitats. The classical environment for significant contributions by methanotrophs to system–wide carbon cycling is stratified lakes, in which a large fraction of the annual carbon fixation is cycled through methanogenesis and methane oxidation. The methanotrophic activity is highest at the interface where oxygen and methane coincide. As described above for sediments, such an interface would also be an interface for NH_4^+ and oxygen and might be expected to harbor high nitrification activity as well.

Owing to the presence of high sulfate concentrations in seawater, methanogenesis is not as important in marine sediments and seawater as it is in freshwater systems. Nevertheless, there are a few marine environments where methane is found. The situation in Scan Bay or Cape Lookout Bight sediments, or in the water column of the Black Sea or the Cariaco Basin, is largely analogous to that in stratified lakes.

The observation from culture studies that methane oxidizers are capable of oxidizing NH_3 (Dalton, 1977) and that NH_3 oxidizers are capable of oxidizing

methane (Jones and Morita, 1983; Ward, 1987a), has led to uncertainty about which organisms are responsible for observed methane and NH_4^+ fluxes in nature. Although both groups of microorganisms show similar regulation by environmental variables and similar sensitivities to a variety of inhibitors (Bedard and Knowles, 1989), the possibility for differential regulation based on substrate affinity or competition suggested that ''cross oxidation'' might have important implications for the rate of NH_4^+ or methane oxidation in nature. Based on a combination of simulated *in situ* rate measurements, inhibitor studies and kinetic experiments with natural assemblages, it has been concluded largely that methanotrophs are mostly responsible for methane oxidation and nitrifiers for NH_4^+ oxidation in both freshwater and marine environments (Ward and Kilpatrick, 1990; Bedard and Knowles, 1997; Carini *et al.*, 2003). These conclusions do not rule out a role for cross oxidation or participation by both groups in some environments, and the two processes remain problematic to separate entirely in the environment.

6. Future Directions

Our understanding of the biogeochemistry of nitrification has advanced greatly in the past two decades. The basic patterns of distributions and rates have been discovered and are largely understood in terms of the characteristics of the organisms believed to be responsible for the process and their interactions with other components of the ocean's physical and biological systems. Unpredictable surprise discoveries that change our view of the N cycle are likely to appear, as they have done in recent years. Nevertheless, there are some avenues of future discovery that are more easily predictable on the basis of current research.

1. Two recent discoveries are presently causing major reevaluation of our understanding of the N cycle. The anammox process was first described in 1995 (Mulder *et al.*, 1995; van de Graaf *et al.*, 1995) and is reviewed elsewhere in this volume (chapter by Devol). In the time between the first draft of this review and its final revision, the NH_3 oxidizing archaea have been cultivated (Konnecke *et al.*, 2005), shown to be both ubiquitous (Francis *et al.*, 2005; Leininger *et al.*, 2006) and abundant (Wuchter *et al.*, 2006) in both aquatic and terrestrial environments. At this point, it is entirely unclear how extensively the discovery of the AOA will change our understanding of nitrification in the ocean. There are several obvious high priority questions that should be addressed immediately:
    i. What fraction of the ubiquitous Crenarchaeota in the ocean are AOA?
    ii. Are the marine AOA predominantly autotrophic or do they have a facultative or mixotrophic metabolism that allows them to utilize alternative energy generation modes?
    iii. What are the pathways of NH_3 oxidation in AOA? Are they homologous with those of AOB? Do AOA possess a microaerophilic or anaerobic metabolism similar to the nitrifier denitrification pathway of AOB?

iv. How do the physiological characteristics of the AOA compare with those of the AOB in terms of substrate affinity, light sensitivity, growth rates, oxygen requirements, N_2O production, etc.? I.e., are AOA and AOB regulated in the environment by similar factors?

v. What fraction of NH_3 oxidation in the ocean is due to AOA vs. AOB?

It would be very surprising if AOA turn out to have metabolisms and physiological characteristics quite different from those of the AOB, because the knowledge summarized above about nitrification in the environment obviously includes the influence of AOA. That is, conclusions about the distribution and environmental regulation of nitrification rates are derived from samples that contained the natural assemblage of AOA as well as AOB and NOB. What is still missing is the thorough characterization of organismal biochemistry from culture work, or from *in situ* characterizations using molecular methodology such as qPCR to evaluate gene expression and activities in response to environmental variables.

The discovery of AOA does not change the magnitude or distribution of nitrification rates. It may that AOA, rather than AOB, are predominantly responsible for the measured rates, but that does not alter the rates. Thus the *in situ* abundance and activities of AOA, when determined, must be compatible with the biogeochemical constraints discussed above.

Are AOA involved in N_2O production? Preliminary information from soil Archaeal metagenomic fragments reveal a *nirK* homolog similar to that found in *Nitrosomonas europaea* (Treusch *et al.*, 2005). The *nirK* of *N. europaea* is quite distinct from that found in conventional denitrifiers and from the closely related marine clades of AOB (Casciotti and Ward, 2001). The protein encoded by this type of *nirK* apparently is not involved in NO production leading to N_2O from NO_2^- (Beaumont *et al.*, 2002), but may play a role in NO_2^- detoxification or some more general electron transport function. The genome of *C. symbiosum* also contains elements of the NH_3 oxidation pathway, but it appears to be incomplete (Hallam *et al.*, 2006). There is no homolog for the second critical enzyme, hydroxylamine oxidoreductase, and again, the *nirK* homolog is not the type usually recognized as involved in NO_2^- reduction to NO. The pathways involved in NH_3 oxidation and the potential for nitrifier denitrification in AOA are likely to be resolved soon, and they will provide much insight in to the role of AOA in marine N cycling.

Why have AOA never been cultivated before? Although AOB were never thought to be abundant in seawater, extrapolation from *in situ* numbers and rates estimated from cultures usually resulted in a rough match such that no huge missing nitrification flux was identified. Such estimates and extrapolations are very poorly constrained, however, because even the most important/abundant AOB in seawater (the marine *Nitrosospira* type) have not been cultivated and therefore extrapolations from culture to environment could be wildly incorrect. Abundance estimates based on FISH indicate that AOA outnumber AOB in the open ocean by as much as 1000-fold (Wuchter *et al.*, 2006). Nevertheless, AOB have been cultivated from locations all over the world, even though they are notoriously difficult to cultivate and fastidious in culture. The evidence that AOA have only once been obtained in culture, and that from a marine aquarium (Konnecke *et al.*, 2005) suggests that AOA

have some fundamentally different metabolic requirements than AOB. The explanation could be the same that applied to *Pelagibacter ubique*, identified in 16S rRNA clone libraries as SAR11. Although ubiquitous in clone libraries, *P. ubique* was never cultivated until very low substrate clean culture techniques were applied (Rappe *et al.*, 2002). *P. ubique* and its close relatives are estimated to contribute up to 50% of the surface ocean microbial communities, yet it does not grow under standard rich media conditions. Perhaps AOB are the weeds of the marine nitrifiers and AOA are the ever present dominant assemblage that is important in normal—low substrate clean—environmental conditions, the *P. ubique* of NH_3 oxidizers. Alternatively, perhaps most AOA use NH_3 oxidation only as a background or support metabolism and therefore do not compete well in the obligately autotrophic conditions usually employed to enrich for nitrifiers. The only cultivated AOA appears to be obligately autotrophic, but the uncultivated marine Crenarchaeota assimilate organic compounds (Ouverney and Fuhrman, 2000), indicating that a more versatile metabolism may prevail in the AOA that dominate the marine environment.

Are NOA waiting to be discovered? Even less is known about the abundance and growth characteristics of important marine NOB than the AOB; it is entirely possible that NOA are also present. On the other hand, NO_2^- does not accumulate in most of the world's ocean, specifically in the deep ocean where the Crenarchaeota, suspected to be AOA, constitute up to 40% of the microbial cells. If AOA are abundant and active, the resident NO_2^- oxidizing assemblage is clearly capable of keeping up with them. On balance, this argues that most of that 40% of cells is not very active in NH_3 oxidation, or another 40% of the assemblage would be required to consume the NO_2^-.

2. Molecular ecological research has arisen as a powerful research tool and has led to major new ecological insights in recent years. As is the case for every process that has been investigated, this approach has led to the discovery of immense diversity at the genetic level among organisms involved in nitrification. The degree to which this genetic diversity in relation to distribution or regulation of biogeochemical reaction rates is the focus of much current research. Cultivated nitrifiers and rates measured in natural samples make it clear that different strains of nitrifiers have different physiological optima and are thus likely to inhabit different ecological niches. Although so far untested, this statement probably applies to AOA as well as AOB and NOB. Does the variety of physiological types ensure that ecosystem function is maintained in widely different environmental conditions by the activity of different assemblages under different conditions? What is the time scale for change in the microbial assemblage in response to environmental change? Which environmental changes are most likely to affect nitrification rates? A combination of biogeochemical and molecular methods can address these questions.

3. Our understanding of the biochemistry of nitrification is based largely on insights from cultivated strains. Molecular data obtained from clone libraries indicate that the most important AOB in the ocean are not represented in the culture collection. The same is probably true for NOB, but much less molecular information is available for these organisms. At this stage, the most

important AOA have not been identified even in clone libraries, but they certainly are not in culture. Because pure cultures are such a powerful way to investigate the biochemistry and regulation of microbial biogeochemistry, every effort should be made to cultivate both AOA and the so far uncultivated important AOB clades. It is clear that innovative cultivation techniques will be required, perhaps on the *P. ubique* model.

4. Rising CO_2 in the atmosphere or purposeful deep ocean CO_2 disposal may lead to decreases in oceanic pH. pH was not even considered as a factor that influences nitrification rate in the list of potential factors mentioned earlier, because ocean pH is reliably invariant over most of the ocean in today's world. But changes of 1–2 pH units in response to CO_2 increases could inhibit nitrification rates by up to 90% (Huesemann *et al.*, 2002). Long term inhibition of nitrification may lead to the accumulation of NH_4^+ in the oxic waters, decreases in NO_3^- available for denitrification, and changes in phytoplankton community composition as a result of nutrient preferences and competition.

5. Nitrifiers are responsible for the accumulations of N_2O that typically occur at the upper boundaries of OMZ regions. It has been suggested (Fuhrman and Capone, 1991; Jin and Gruber, 2003; Law and Ling, 2001) that increased productivity in the surface ocean, leading to increased organic flux to deeper waters, could increase the total volume of suboxic water, and thence to enhanced N_2O fluxes to the atmosphere. Global warming due to increased atmospheric inventories of greenhouse gases may cause increasingly stable thermal stratification, which could in turn lead to further enlargement of the major oxygen minimum zones of the world ocean. Increased stratification due to atmospheric warming might lead to increased N_2O fluxes to the atmosphere from this source, thus compounding the greenhouse warming effect of CO_2. These possibilities for changes in N_2O dynamics point to the necessity to understand factors that regulate trace gas metabolism by nitrifiers, as well as denitrifiers. The most likely avenues of progress in this area are through stable isotope analyses, including isotopomer approaches (Popp *et al.*, 2002; Sutka *et al.*, 2004), and through genomics. The former can elucidate the biochemical pathways responsible for the formation of compounds with multiple potential sources. Genomics can provide insights into the genetic capabilities, pathways and regulation of metabolic processes that result in trace gas production consumption. Genetic data provide the information necessary to develop specific probes and assays to identify which organisms are involved in particular transformations in particular times and places.

6. Better rate measurements: Although the currently available methods for direct assessment of nitrification rates and the geochemical constraints provide internally consistent estimates of the overall rates and distributions of nitrification, improvements in direct rate methods are sorely needed to investigate the effects of environmental variables on rates under realistic conditions. These improvements will likely come with the application of even greater sensitivity isotope tracer methods, which require less substrate perturbation for detection of signal in the product pool. In addition, stable isotope methods that do not require tracer addition may possess the sensitivity to detect small fractionation

changes in incubated samples. It is particularly important to apply these efforts to the measurement of NO_2^- oxidation rates. Nitrite oxidation rates have only rarely been measured and have suffered from methdological artifacts. Direct rate measurements would be useful in investigating the actual degree of coupling between the two steps in nitrification.

7. Nitrite oxidation and NO_2^- oxidizing bacteria have not received the attention accorded to the first half of the nitrification process. The discovery of AOA raises even more questions about the abundance and activity of NO_2^- oxidizers. With the publication of several NOB genomes (Starkenburg *et al.*, 2006; 2008), new tools are also at hand for the molecular ecological study of NOB to address the parallel questions of diversity and abundance raised by recent findings on the AOB and AOA. Finally, are the NOB responsible for the consumption of NO_2^- generated by AOB and AOA or are NOA awaiting discovery?

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Denitrification including Anammox

Allan H. Devol

Contents

1. Introduction

Denitrification is the process by which combined nitrogen is reduced to gaseous end products; combined nitrogen being nitrogen combined with any element other than itself, e.g., nitrate, ammonium or organic ammines. Originally "denitrification" referred to the phenomenon of loss of nitrogen from a microbial community, but the term was adopted by Kluyver and Donker (1926) when they defined denitrification as a metabolic pathway that allowed respiratory metabolism in the absence of oxygen. Canonical denitrification is a heterotrophic process in which nitrogen oxides serve as the terminal electron acceptor for organic carbon metabolism (Codispoti *et al.*, 2001). However, from a biogeochemical perspective any process that results in loss of combined nitrogen from the biosphere is a denitrifying process. Thus, metabolisms such as the newly discovered anammox reaction, an autotrophic metabolism (Strous *et al.*, 1999), as well as purely inorganic reactions between metal oxides and ammonium that may produce nitrogen

gas would also be denitrifying processes. This is the definition of denitrification adopted here.

In the oceans canonical denitrification takes place when dissolved oxygen concentrations are reduced to zero, or nearly so. At this point, a series of suboxic respiratory processes is initiated through which alternate electron acceptors are utilized during the oxidation of organic matter. These electron acceptors include NO_3^-, IO_3^-, Mn (III, IV) oxides, and Fe (III) oxides (Farrenkopf et al., 1997; Luther et al., 1997; Nealson and Saffarani, 1994). In the oceans the dominant of these electron acceptors is nitrate. Thermodynamic calculations suggest that oxidation of typical "Redfieldian" organic matter with NO_3^-, IO_3^-, or Mn (IV) all yield similar amounts of free energy, all of which are somewhat less than O_2, while Fe (III) oxidation yields are substantially less (Farrenkoph et al., 1997; Froelich et al., 1979; Hulth et al., 1999; Murray et al., 1995). Chemical distributions of oxidized and reduced species in the water column and sedimentary pore waters suggests that the sequence of electron acceptor utilization generally follows the thermodynamic energy yield, i.e., $O_2 > NO_3^- \cong IO_3^- \cong Mn(IV) > Fe(III) > SO_4^=$ (Farrenkoph et al., 1997; Froelich et al., 1979; Luther et al., 1998; Murray et al., 1995; King and Nedwell, 1985).

Nearly all heterotrophic denitrifiers are facultative anaerobes (Tiedje, 1988). Although various workers defined suboxia differently, the upper limit of oxygen concentration for the occurrence of denitrification is probably 5 µM, with quantitatively most denitrification in the marine environment taking place below about 2 µM (Cline and Richards, 1972; Codispoti et al., 2005; Devol, 1978; Murray et al., 1995). The ability to denitrify is not limited to the bacteria, archea and even some fungi are capable of denitrification (Knowles, 1996; Zumft and Körner, 1997).

Suboxic environments are found throughout the oceans. Three large water column suboxic zones are found in the modern ocean: one in the eastern tropical North Pacific off the west coasts of Mexico and Guatemala, one in the eastern tropical South Pacific off Peru and Chile, and one in the northern Arabian Sea (Codispoti et al., 2001). Suboxic zones are also found in marine sediments, primarily shelf and slope sediments, where dissolved oxygen is depleted relatively quickly below the sediment–water interface (Aller et al., 1985; Blackburn, 1987; Blackburn et al., 1996; Devol and Hartnett, 2001; Jørgensen and Sørensen, 1985; Lomstein et al., 1989; Reimers et al., 1992; Seitzinger and Giblin, 1996). Likewise, anoxic basins and fjords such as the Black Sea, Cariaco Trench, and Saanich Inlet contain a suboxic zone between the upper oxic layer and the lower sulfidic layer (Anderson and Devol, 1973; Murray and Yakushev, 2006; Richards, 1975; Thunell et al., 2004). Finally, the lower water column over productive shelf areas sometimes becomes suboxic. This situation is a persistent natural phenomenon off south west Africa (Kuypers et al., 2005; Tyrrell and Lucas, 2002), occurs ephemerally on others (Dugdale et al., 1977) and, due to anthropogenic stimulation of productivity, has become a problem on the shelf surrounding the Mississippi River delta (Rabalais et al., 1996) and on the continental shelf off the north west coast of India (Naqvi et al., 2000).

This review will cover various pathways of denitrification, important oceanic sites of denitrification, isotopic consequences of denitrification, and the role of

denitrification in the marine combined nitrogen budget. A detailed recent review of the metabolic pathways and cell biology of denitrification is given by Zumft (1997) and the ecology of denitrification was reviewed by Tiedje (1988).

2. Pathways and Controls of Nitrogen Oxide Reduction and Denitrification

2.1. Canonical or respiratory denitrification

Canonical denitrification is carried out by heterotrophic bacteria during which nitrate (or nitrite) serves as the terminal electron acceptor for organic matter oxidation and the nitrogen oxides are reduced mainly to nitrogen (some nitrous oxide may be formed). The characteristic feature of canonical denitrification is that the reduction of N-oxides is coupled to electron transport phosphorylation (Knowles, 1982, 1996; Koike and Hattori, 1975). The capacity for respiratory denitrification is widespread among bacteria and is distributed across various taxonomic subclasses, mainly within the Proteobacteria (Zumft, 1997).

   The N-oxide reduction pathway during canonical denitrification has been well worked out and involves the sequential reduction of nitrate to nitrite, followed by nitric oxide, nitrous oxide and finally to nitrogen gas (Fig. 6.1). This sequential reduction is facilitated by four well-studied enzyme systems, nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Zumft and Körner, 1997). The intermediate, NO_2^-, is known to escape the cell and is frequently found in denitrifying environments under certain conditions; likewise, N_2O also can accumulate externally (Codispoti et al., 2005; Tiedje, 1988). In denitrification favorable environments local maxima in both intermediates tend to occur near the boundaries of a suboxic zone. Interestingly, NO_2^- and N_2O are also intermediates that accumulate in the environment during nitrification (Betlach and Tiedje, 1981; Codispoti et al., 1992; Naqvi and Noronha, 1991), frequently complicating interpretation of oceanographic profile data (Yoshinari et al., 1997). As NO is actually a free radical and, thus, very reactive, and highly toxic to most bacteria including denitrifiers (Zumft, 1997), nitrite reductase and nitric oxide reductase are controlled interdependently at both the transcriptional and enzyme activity levels in order to minimize the accumulation of NO (Ferguson, 1994; Zumft, 1997). Although the distribution of NO in the environment has not been well-studied, it is generally undetectable in aerobic water columns but it has been detected at low levels in oxygen deficient waters (≤ 0.5 nM; Ward and Zafario, 1988).

$$NO_3^- \xrightarrow{\text{Nitrate reductase}} NO_2^- \xrightarrow{\text{Nitrite reductase}} NO \xrightarrow{\text{Nitric oxide reductase}} N_2O \xrightarrow{\text{Nitrous oxide reductase}} N_2$$

Figure 6.1 Denitrification pathway involved in canonical denitrification. The four enzymes involved in the sequential reduction of nitrate are show.

2.2. Chemodentirification and chemotrophic denitrification

In addition to canonical heterotrophic denitrification there are a number of reactions between inorganic and organic compounds and N-oxides that produce N_2 and yield significant free energy. Some of these reactions are known to be catalyzed by microbes, while others have been proposed to occur spontaneously. Under acidic conditions, pH ≤ 5, the acid catalyzed reduction of NO_2^- can become important (Broadbent and Clark, 1965; Van Cleemput et al., 1976). Another related reaction is a Van Slyke type reaction (Van Slyke, 1911), whereby organic ammines are oxidized by NO_2^- to form N_2. This reaction is also favored under mild acidic conditions and has been shown to be important in frozen soils where NO_2^- is concentrated by the salting out effect (King and Nedwell, 1985). However, due to the well-buffered nature of seawater these reactions are not thought to be important in the oceans (Cline and Richards, 1972; Richards, 1965). It is well documented that reduced sulfur compounds (*Beggiatoa*, *Thiobacilus*) and H_2 also (*Pseudomonas*) also support chemotrophic bacterial denitrification (Tiedje, 1988; Zumft, 1997).

Much attention has recently been focused on interactions between various manganese and nitrogen species because solute profiles through the suboxic zones of anoxic basins such as of the Black Sea and marine sediments suggest that reactions involving these two species may be occurring (Murray et al., 1995; Luther et al., 1997, 1998). Luther et al. (1997, 1998) have proposed two reactions with manganese that result in denitrification:

$$15MnO + 6HNO_3^- \rightarrow 15MnO_2 + 3N_2 + 3H_20 \qquad (6.1)$$

$$15MnO_2 + 10NH_3 \rightarrow 15MnO + 5N_2 + 15H_20 \qquad (6.2)$$
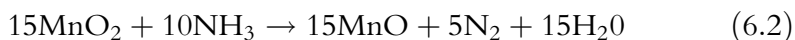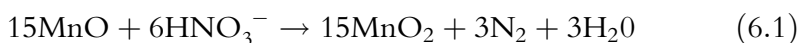
Interestingly, if the two reactions are coupled, the overall result is:

$$6HNO_3^- + 10NH_3 \rightarrow 8N_2 + 18H_2O. \qquad (6.3)$$
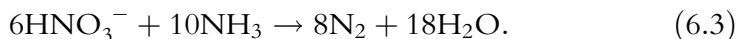
Similar reactions can be written for Fe and N, but the free energy yields are not as large and the Fe^{2+} oxidation coupled to NO_3^- reduction to N_2 is not favorable at typical seawater pH (Luther et al., 1997). Reaction 2 has been shown to proceed under laboratory conditions (Luther et al., 1997; Straub et al., 1996) and has been proposed to explain sediment pore-water distributions in the Laurentian Trough (Anschutz et al., 2000), but it would seem likely that any such reactions in the natural environment are microbially catalyzed.

2.3. Anammox

Canonical denitrifiers are mainly facultative anaerobes that can modify their electron transport system to accommodate N-oxides as the terminal electron acceptor for organic carbon oxidation. However, commitment with the oxidation of proteins to CO_2 and H_2O is the liberation of the amino group as NH_4^+. Thus, canonical denitrification should be accompanied by the build up of NH_4^+ concentrations, but

significant NH_4^+ accumulation is not observed in the water column of open-ocean denitrification zones (Codispoti, 1973). Similar observations led Richards (1965) and Cline and Richards (1972) to propose that a Van Slyke like reaction was responsible for the anaerobic oxidation of ammonium (similar to the reaction given in Eq. (6.3) above but with amino acids as the electron donor). Likewise, interpretation of solute profiles in sediments lead several groups to propose anaerobic ammonium oxidation there (Bender et al., 1989; Emerson et al., 1980), as well as in the water column of the Black Sea (Murray et al., 1995). The Black Sea data are perhaps the most compelling (Fig. 6.2). Here dissolved oxygen is depleted at a depth of about 60 m ($\sigma_\theta = 15.7$), whereas nitrate is depleted somewhat deeper at about 80 m ($\sigma_\theta = 15.95$). Ammonium is generated at depth via sulfate reduction and it is in high concentration there. As one moves upward these high ammonium concentrations decrease until they
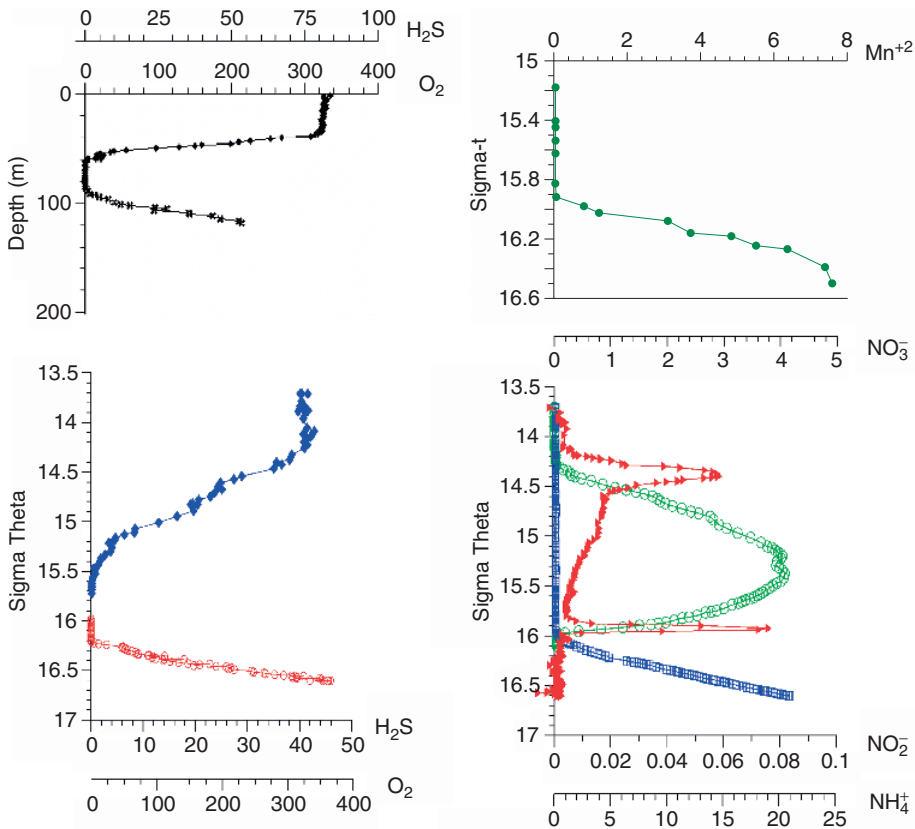
Figure 6.2 Water-column profiles from the western gyre of the Black Sea, 2003: Upper left— dissolved oxygen (diamonds) and hydrogen sulfide (x) versus depth; Upper right—dissolved Mn (II) versus sigma theta; Lower left—dissolved oxygen and hydrogen sulfide versus sigma theta; (lower right), Nitrate (open circles), nitrite (triangles), and ammonium (open squares) versus sigma theta. (Composite redrawn from Murray and Yakushev, 2006. Data are from the same station but different hydrocasts.).

reach zero very near the depth at which NO_3^- and NO_2^- reach zero. These gradients imply a flux of NO_3^- and NO_2^- downward and a flux of NH_4^+ upward into a zone where they are both consumed. However, as noted by Broda (1977) the organism that could carryout this reaction seemed to be "missing in Nature." In 1999, a Plancto-mycetes microbe capable of the "anammox" reaction, i.e., Eq. (6.3), except with NO_2^- substituted for NO_3^-, was isolated from a fluidized bed reactor (Strous *et al.*, 1999), and shortly thereafter other genetically similar organisms were identified in natural environments where the anammox reaction was shown to take place, includ-ing the Black Sea suboxic zone (Dalsgaard and Thamdrup, 2002; Jetten *et al.*, 2003; Kuypers *et al.*, 2003; Risgaard-Petersen *et al.*, 2004a). Although three genera of anammox organisms have been isolated so far—*Candidatus*, *Kuenenia*, and *Scalin-dua*—only *Scalindua* has been found in environmental samples to date.

Anammox bacteria are chemoautotrophic bacteria that fix CO_2 using NO_2^- as the electron donor (Güven *et al.*, 2005) and they are thought to be strict anaerobes. All anammox species found too date have evolved a membrane-bound intracyto-plasmic compartment called the anammoxasome. The anammoxasome membrane is made up of high-density lipids, called ladderanes because of their ladder-like struc-ture, that are thought to be specific to anammox bacteria. The proposed mechanism of anammox involves a hydrazine hydrolase, which catalyzes the combination of hydroxyl ammine and ammonium to form hydrazine. The hydrazine is subsequently oxidized to N_2 by a hydrazine-oxidizing enzyme (Fig. 6.3; Jetten *et al.*, 2003; van Niftrik *et al.*, 2004). The ladderane-lipid membrane is thought to act as a barrier to diffusion thus isolating the toxic intermediates of the anammox reaction within the anammoxasome. It is also believed that the anammoxasome helps create a proton motive force that is coupled with ATP production (van Niftrik *et al.*, 2004).

Anammox bacteria were originally thought to be specialists catalyzing only the oxidation anaerobic oxidation of NH_4^+. They were also considered slow growing organisms (Dalsgaard *et al.*, 2005). Recent evidence has changed this view some-what. It has been suggested that anammox bacteria are able to grow at much more rapid rates, doubling every 1.8 days versus the 11 day doubling time originally
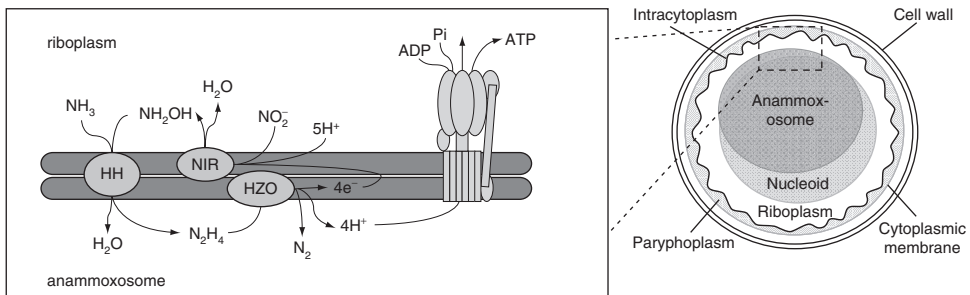
Figure 6.3 (Right) Schematic depiction of anammox cell showing the anammoxozome and nucleoid. (Left) Postulated pathway of anaerobic ammonium oxidation coupled to the ana-mmoxosome membrane resulting in a proton motive force and ATP synthesis via membrane-bound ATPases. HH, hydrazine hydrolase; HZO, hydrazine oxidizing enzyme; NIR, nitrite reductase. (Redrawn from van Niftrik *et al.*, 2004 and Kuypers *et al.*, 2006).

determined (Isaka *et al.*, 2005). More importantly, examination of the genome of *K. stuttgartiensis* revealed anammox bacteria to be much more metabolically versatile previously thought (Strous *et al.*, 2006). They are able to oxidize low molecular weight organic acids to CO_2 using NO_3^- or NO_2^- as the electron acceptor, while simultaneously carrying out the anammox reaction (Güven *et al.*, 2005). Kartal *et al.* (2007) went one step further. In incubations of *K. stuttgartiensis* that were given ^{13}C–formate as a energy source and $^{15}NO_3^-$ as an electron acceptor the main products of nitrate reduction were $^{13}CO_2$ and $^{15}N^{15}N$. In these experiments N_2 did not start to evolve until after a lag phase of several hours, during which $^{15}NO_2^-$ accumulated as an intermediate. They were able to show *K. stuttgartiensis* reduced NO_3^- to NH_4^+ with NO_2^- as an intermediate and that the NH_4^+ and NO_2^- produced were subsequently converted to N_2 via the anammox reaction.

2.4. Dissimilatory nitrate reduction

There are two types of dissimilatory denitrification that are considered here. The first is denitrification that takes place during the chemoautotrophic process of nitrification. Nitrification is the sequential oxidation of NH_4^+ to NO_2^-, which is followed by the oxidation of NO_2^- to NO_3^- (see Chapter 11 by Ward, this volume). These two steps are accomplished by two distinct microbes of the *Nitroso* and *Nitro* groups, respectively. Although it would seem that this obligately aerobic process would not be simultaneously a denitrifying process, a common byproduct of nitrification is N_2O (Tiedje, 1988). Originally, it was thought that this N_2O was produced during the oxidation of ammonium, but Poth (1986) and Poth and Focth (1985) used ^{15}N isotope tracers to show that N_2O was produced from NO_2^- by *Nitrosomonas europaea* and a *Nitrosomonas* sp.

A copper containing nitrite reductase was also isolated from *Nitrosomonas europaea* at about the same time (DiSpirito *et al.*, 1985). More recently, Casciotti and Ward isolated a copper containing nitrate reductase gene, *nirK* (Casciotti and Ward, 2001), as well as a nitric oxide reductase gene, *norB* (Casciotti and Ward, 2004), from several strains of nitrifying bacteria that were homologous to those of denitrifying organisms. Bock *et al.* (1995) showed that *N. europaea* and *N. eutropha* were able to nitrify and denitrify at the same time when grown under oxygen limitation (0.2 and 0.4 mg L^{-1}) and anaerobically. Under oxygen limitation, oxygen and nitrite served simultaneously as electron acceptors and both N_2 and N_2O were produced, whereas under anaerobic conditions N_2 was the predominant end product. (See also Schmidt and Bock, 1997 and Zart and Bock, 1998.) This type of nitrification–denitrification pathway may help explain why ammonium oxidizers remain totally active in nearly suboxic environments and the observation that the N_2O production is stimulated at reduced oxygen concentrations (Blackmer *et al.*, 1980; Goreau *et al.*, 1980). Furthermore, it may help explain the ^{15}N depleted N_2O found in some environmental samples (Dore *et al.*, 1998; Yoshida, 1988). The ability of nitrifiers to thrive at near suboxic oxygen concentrations and the common intermediates (NO_3^-, NO_2^-, NO, and N_2O) with canonical denitrification creates the potential for coupling between the two process at suboxic transition in the

water column and sediments and for a possible coupling of nitrifier-dentirification and anammox (Lam *et al.*, 2007; Schmidt *et al.*, 2002).

Although not strictly denitrification by the definition used here, the process of anaerobic dissimilatory nitrate reduction to ammonium, also known as nitrate fermentation, is worth mention. The reaction has been reported for anoxic sediments (Boon *et al.*, 1986; Kaspar, 1983; Sørensen, 1978) and sediments with substantial free sulfide, possibly due to sulfide inhibition of nitrification and denitrification (An and Gardner, 2002). Suboxic NO_3^- reduction to ammonium appears to occurr in environments where there is excess available carbon, such as estuarine sediments (Rysgaard *et al.*, 1996) and under fish culture net pens (Christensen *et al.*, 2000). Perhaps more important are the nitrate fermenting organisms such as *Thioploca* and *Thiomargarita*, that are found in sediments underlying the major suboxic-denitrifying water columns of the Arabian sea, eastern Tropical Pacific and Namibia (Farrias, 1998; Fossing *et al.*, 1995; Jørgensen and Gallardo, 1999; Schmaljohann *et al.*, 2001; Thamdrup and Canfield, 1996; Devol, unpublished data). These organisms are able to couple the reduction of NO_3^- to ammonium with the oxidation of reduced sulfur compounds. Both *Thioploca* and *Thiomargarita* are able to concentrate NO_3^- at up to 500 mM levels in large vacuoles within their cells for subsequent sulfide oxidation (Fossing *et al.*, 1995; Schulz *et al.*, 1999). *Thiomargarita*, the largest known bacteria, is actually visible with the naked eye (Schulz *et al.*, 1999). Although, the reduction of NO_3^- to NH_4^+ in these environments is not denitrification per se, because these environments underlie suboxic waters, when the ammonia produced by these organisms diffuses up to the denitrification zone it may well be oxidized to N_2 by anammox bacteria at the sediment–water interface or in the overlying suboxic-denitrifying water column (Codispoti *et al.*, 2001, 2005).

2.5. Environmental controls on denitrification

The two necessary requirements for denitrification in the environment appear to be low dissolved oxygen concentrations and the availability of N-oxides. Indeed, the inhibitory effects of oxygen were first reported by Gayon and Dupetit, 1882. The effect of oxygen on denitrification is usually described as an oxygen threshold below which denitrification is initiated, and the sharpness of this transition in the ocean's major oxygen deficient zones (ODZs) is clearly evident in high quality hydrographical data. When the concentration of the denitrification intermediate NO_2^- is plotted against dissolved oxygen, it is seen that NO_2^- does not appear in the water columns of the eastern tropical North Pacific or Arabian Sea until oxygen concentrations are reduced below about 2 μM (Fig. 6.4A and B). Interestingly, when a similar plot is made for data from the intermittently anoxic fjord Saanich Inlet (British Columbia, Canada) the oxygen threshold for NO_2^- accumulation is ~4 μM (Fig. 6.4C). This apparently elevated threshold for denitrification was interpreted as a response by the microbial population to the more transient suboxic environment in the coastal inlet (Devol, 1978). Ferguson (1974) suggested aerobic denitrification might be advantageous to populations growing in near suboxic conditions. Although it has long been known that oxygen represses synthesis and activity of denitrifying enzymes (Tiedje, 1988), denitrification in the presence of
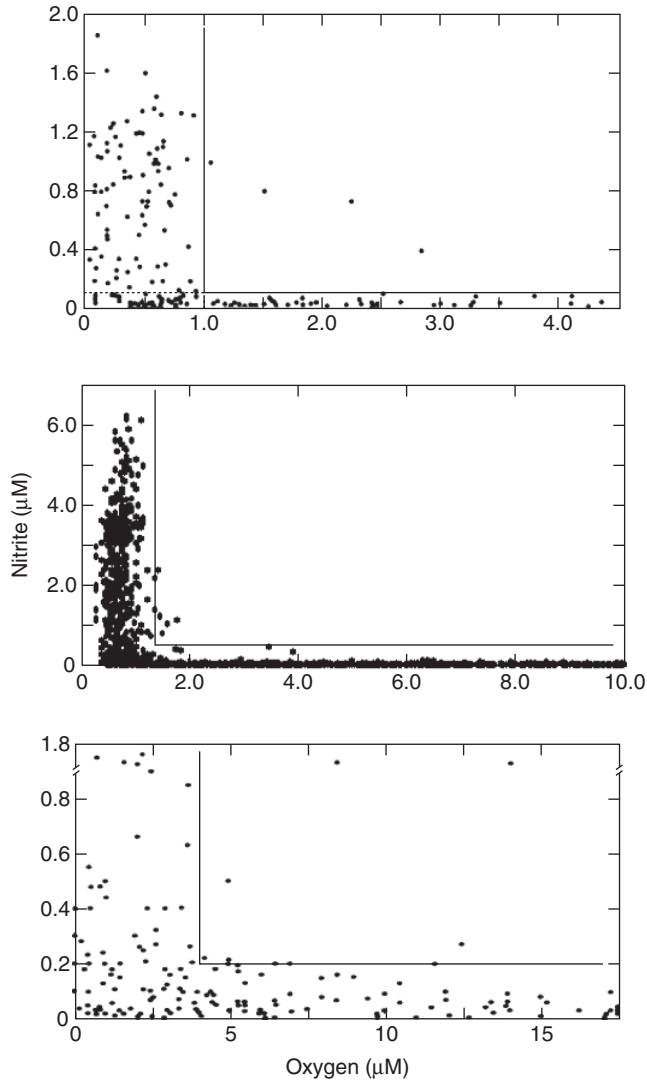
Figure 6.4 Dissolved oxygen versus nitrite concentration for the waters of the ODZ ($<20\,\mu M\,O_2$); the eastern tropical North Pacific (upper, redrawn from Cline and Richards, 1972), the Arabian Sea (center, redrawn after Morrison *et al.*, 1999), and Saanich Inlet (lower, redrawn from Devol, 1975). Note, axes are different on each plot. Vertical lines denote possible denitrification thresholds for each area.

oxygen has been observed in the laboratory (Bell and Ferguson, 1991; Lloyd, 1993; Yoshinari and Koike, 1994). Interestingly, although it is clear that low oxygen concentrations are a prerequisite for denitrification in both the water column (Fig. 6.4) and sediments (Rysgaard *et al.*, 1994), at least in the water column, the denitrification rate is likely controlled by the availability of oxidizable carbon (Lipschultz *et al.*, 1990).

3. Sites of Marine Denitrification

3.1. Water-column denitrification

3.1.1. Locations of pelagic water-column denitrification

Somewhat less than half the current marine denitrification is thought to occur in the three main pelagic ODZs that are located in the eastern tropical North Pacific (ETNP), the eastern tropical South Pacific (ETSP) and the northern Arabian Sea (Fig. 6.5). These zones occur in the intermediate waters (~150–1000 m) in locations where the ventilation rate is not great enough to meet the oxygen demand. In the Arabian Sea the ventilation of the intermediate waters is not particularly sluggish (Morrison *et al.*, 1999; Naqvi and Shailaja, 1993; Olson *et al.*, 1993). However, high primary productivity during both the Southeast Monsoon and the Northwest Monsoon (Barber *et al.*, 2001) results in a correspondingly elevated organic matter rain rate to the ODZ, which drives these waters to denitrification (see Chapter 14 by Naqvi, this volume). Off western Mexico the situation is reversed. Although primary productivity is only moderate in this area (Longhurst *et al.*, 1995), residence times of ODZ waters have been estimated at about 10 years based on geostrophic calculations (Codispoti, 1973) and CFC ages in the ODZ are the oldest on those
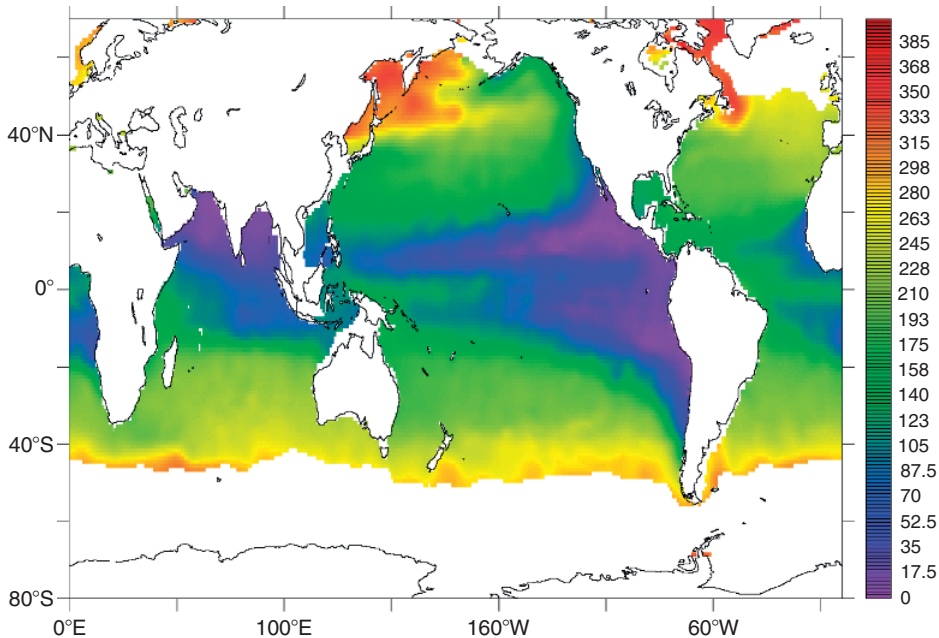
Figure 6.5 Dissolved oxygen on the 26.5 sigma theta surface. Note the three main pelagic water column ODZs, the eastern tropical North Pacific, the eastern tropical South Pacific and the Arabian Sea. Althouth oxygen levels are very low in the Bay of Bengal, this area does not appear to be a location of water column denitrification (Rao *et al.*, 1994).
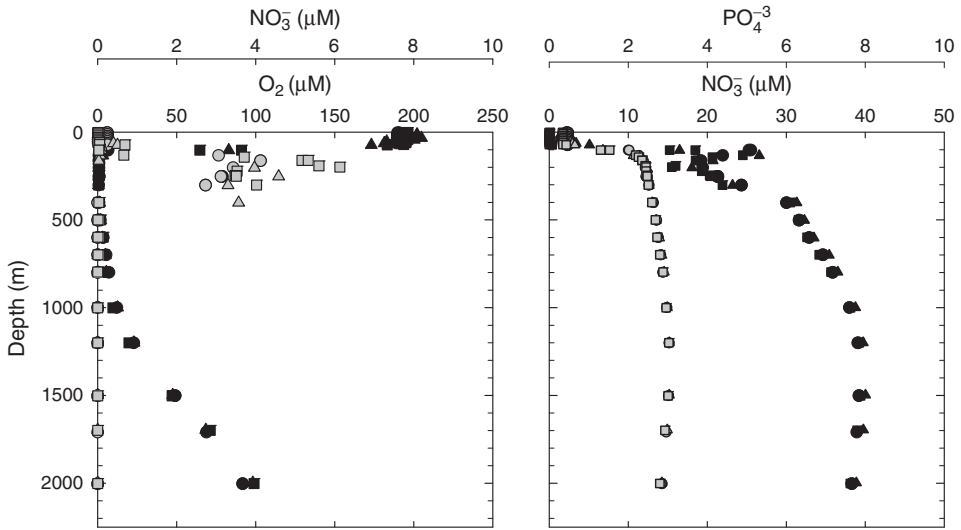
Figure 6.6 Dissolved oxygen nitrate, nitrite and phosphate in through the ODZ of the Arabian sea. Left—dissolved oxygen (solid black symbols) and nitrite (solid gray symbols). Right—Nitrate (solid black symbols) and phosphate (solid gray symbols). Data are from USJGOFS station M1 during cruises TGT O43 (circles), TGT 045 (triangles) and TGT 050 (squares).

density surfaces in the North Pacific (WOCE Electronic Atlas; www.awi. bremerhaven.de/eWOCE/gallery/index.html). Although a significant ODZ has been reported off south West Africa (Calvert and Price, 1971) little is known about denitrification in this area (except see Section 2.3).

In the ETNP and Arabian Sea dissolved oxygen is reduced to detection levels at about 150–180 m and remains so until about 800 m (Fig. 6.6), while in off Peru waters suboxic waters can be found as shallow as 50 m. Within the upper oxycline both nitrate and phosphate increase dramatically. In the depth zone of vanishingly low oxygen, phosphate concentrations continue to increase to maximal values more or less in the middle of the ODZ. The nitrate profile, however, exhibits a local minimum or ''divot'' in the ODZ where nitrate concentrations actually decline while phosphate increases. This ''divot'' has classically been interpreted as the consequence of canonical denitrification (Codispoti and Christensen, 1985; Codispoti and Richards, 1976; Codispoti and Packard, 1980; Fiadeiro and Strickland, 1968; Naqvi, 1987). Usually coincident with the divot in the nitrate profile is a local maximum in NO_2^- concentration, typically on the order of 2–4 μM. This local maximum is called the secondary nitrate maximum, SNM. Its association with low oxygen waters of the eastern tropical North Pacific was first noted by Gilson in 1937 and represents an accumulation of this intermediate in the denitrification process (Brandhorst, 1959; Codispoti and Richards, 1976; Naqvi, 1987; Thomas, 1966). (The primary NO_2^- maximum is located at the base of the euphotic zone in oxygenated water and is associated with the nitrification process.)
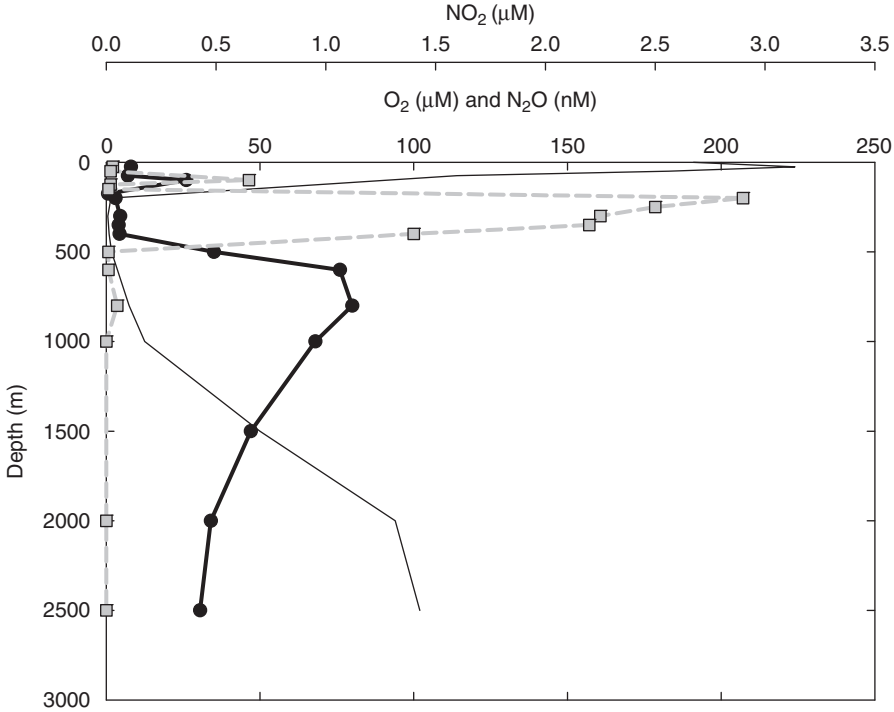
Figure 6.7 Dissolved oxygen (line), nitrite (gray squares) and nitrous oxide (profiles black circles) through the ODZ in the Arabian sea (redrawn from Devol *et al.*, 2007).

Nitrous oxide, the other accumulating intermediate during denitrification, typically displays a profile with two local maxima, one coincident with the base of the oxycline and the second, deeper local maxima near the lower transition from suboxic back to oxic conditions (Fig. 6.7). Concentrations of N_2O at these maxima are frequently an order of magnitude above saturation Below this deeper maximum, N_2O concentrations fall steadily with depth but remain well above saturation. Between the local maxima, N_2O concentrations decrease to extremely low values, presumably due to consumption during denitrification (see Chapter 2 by Bange, this volume; Bange *et al.*, 1996; Bange *et al.*, 2000; Codispoti and Christensen, 1985; Codispoti *et al.*, 1992; Cohen and Gordon, 1978; Naqvi and Noronha, 1991; Naqvi *et al.*, 1998a,b; Yoshinari *et al.*, 1997).

3.1.2. Rates of water-column denitrification
Various methods have been used to estimate water–column denitrification rates including stoichiometric relationships, measurements of the enzymatic activity of the electron transport system (ETS), and $^{15}NO_3^-$ incubation experiments. In combination with residence times or mass transport calculations these measurements yield areal denitrification rates.

Stoichiometric estimations involve calculation of the NO_3^- concentration in a parcel of ODZ water expected before denitrification takes place, i.e., the "expected NO_3^-" (*sensu* Codispoti and Richards, 1976). Given an expected NO_3^-, $^{exp}NO_3^-$, concentration and an analytical determination of the actual inorganic nitrogen concentration ($IN = NO_3^- + NO_2^- + NH_4^+$), the measured value can be subtracted from the expected to give a "NO_3^- deficit" or the amount of NO_3^- removed by denitrification, viz.,

$$^{def}NO_3^- = IN - {}^{exp}NO_3^-. \tag{6.4}$$

Three primary methods have been used to calculate expected NO_3^- and all are based on Redfieldian stoichiometry.

The first quantitative estimates of denitrification in the eastern tropical North Pacific were made by Codispoti and Richards (1976). Codispoti and Richards used apparent oxygen utilization, AOU, and phosphate data to stoichiometrically extrapolate back to the nitrate concentration present when a given water mass was previously at the surface. In this way they were able to develop a relationship between $^{exp}NO_3^-$ and sigma-*t* for waters of the ETNP-ODZ, from which they calculated nitrate deficit as outlined in Eq. (6.4).

A second method used to calculate nitrate deficit takes advantage of the "semi-conservative" water-mass tracer "*NO*" (Broecker, 1974). Stoichiometrically, every mole of O_2 respired will remineralize approximately 1/9 of a mole of NO_3^- so that *NO*, defined as

$$NO = [O_2] + 9.1[NO_3^-], \tag{6.5}$$

should be a conservative property in most water masses. Then, given *NO* in any particular water mass, the amount of NO_3^- expected can be calculated by rearranging the above equation to the following:

$$^{exp}NO_3^- = (NO - O_2)/9.1. \tag{6.6}$$

Again, one can then calculate the nitrate deficit from Eq. (6.4). This method has been used primarily to estimate denitrification rates in the Arabian Sea (Howell *et al.*, 1997; Mantoura *et al.*, 1993; Naqvi, 1987).

Yet another stoichiometric method involves the quantitiy N$\star$ (Deutsch *et al.*, 2001; Gruber and Sarmiento, 1997; Gruber and Sarmiento, 2002). Gruber and Sarmiento used high quality JGOFS or GEOSECS data to develop a general relationship between combined inorganic nitrogen, IN, and PO4 for the world's oceans. The relationship as given in Deutsch *et al.* (2001) is

$$N^* = IN - 16P + 2.90. \tag{6.7}$$

Here N$\star$ is the deviation from the amount of IN predicted stoichiometrically from phosphate and the world-ocean N:P regression relationship. Negative values of N$\star$ are interpreted to show net denitrification whereas positive values show net
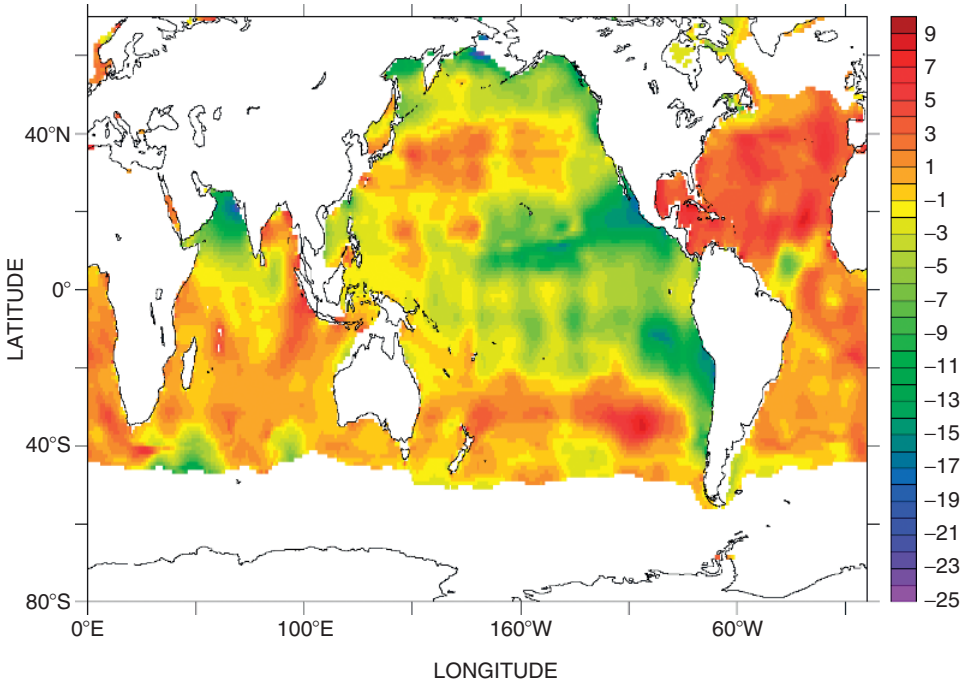
Figure 6.8 N⋆ as defined by Deutsch *et al.* (2001) on the 26.5 sigma theta surface. Note the large negative N⋆ values in the eastern tropical North Pacific, the eastern tropical South Pacific and the Arabian Sea. Also note the lack of negative N⋆ in the Bay of Bengal despite its low oxygen (c.f. Fig. 6.5).

nitrogen fixation. N⋆ contours on specific density surfaces clearly show the large pelagic ODZs along with several other possible denitrification features. For example, in Fig. 6.8, the large negative N⋆ values associated with the ETNP, ETSP, and Arabian Sea denitrification zones are clearly evident. Also evident are relatively highly negative N⋆ values in the deep Bering Sea. Indeed, Broecker and Peng (1982) noticed low N–P relationships in deep Bering Sea water and suggested denitrification as the source, and this has since been confirmed by Lehmann *et al.* (2005).

Although N⋆ is useful to demonstrate general patterns of oceanic N-cycling, as Gruber and Sarmiento (1997) point out, N⋆ only show net N-cycling relative to the general oceanic mean. To overcome this shortcoming and to quantitatively estimate nitrate deficits in the Arabian Sea, Codispoti *et al.* (2002) develop a N⋆-type relationship specific to the Arabian Sea from the high-quality JGOFS nutrient data. They utilized a Type II linear regressions of reactive phosphate (PO_4^{3-}) vs inorganic nitrogen ($IN = NO_3^- + NO_2^- + NH_4^+$) on samples from depths between 100 and 1500 db, with oxygen concentrations >65 μM/l to determine the N–P relationship for the intermediate water before the onset of denitrification. The resulting equation was:

$$N_{deficit} = [14.89PO_4^{3-} - 0.28) - IN] \mu M \times 0.86 (r^2 = 0.998), \quad (6.8)$$

where $N_{deficit}$ is the estimate of the inorganic nitrogen removed from a water parcel by denitrification, $14.89 = \Delta IN/\Delta PO_4^{3-}$ (by atoms), 0.28 is the PO_4^{3-} intercept at IN, and 0.86 accounts for the PO_4^{3-} released by the organic material re-mineralized by denitrification assuming that N/P in local organic matter is 14.89, and that consumption of 94.4 NO_3^- by denitrification releases one PO_4^{3-} (Froelich *et al.*, 1979; Richards, 1965). Partially because this equation is independent of dissolved oxygen, it does not result in large negative nitrate deficits in the surface layer (Fig. 6.9). The maximum predicted deficit is about 30% larger (12 vs 9 μM) and remains and extends about 1000 m deeper than the deficits calculated from NO–θ relationships. The observation that the denitrification signal penetrates well below the ODZ, to almost 2000 m is supported by isotopic data (see isotopic section).

Measurement of the enzyme activity of the respiratory electron transport system (ETS activity) has also been used to estimate denitrification rates in the Arabian Sea (Naqvi and Shailaja, 1993) and eastern tropical South Pacific (Codispoti and Packard, 1980) denitrification zones. To measure the ETS activity a crude enzyme extract is made by grinding filtered seawater samples in a buffered medium to liberate the enzymes, after which the ETS substrates NADH+ and NADPH+ along with tetrazolium salt are added as an artificial electron acceptor. Samples are then incubated for a
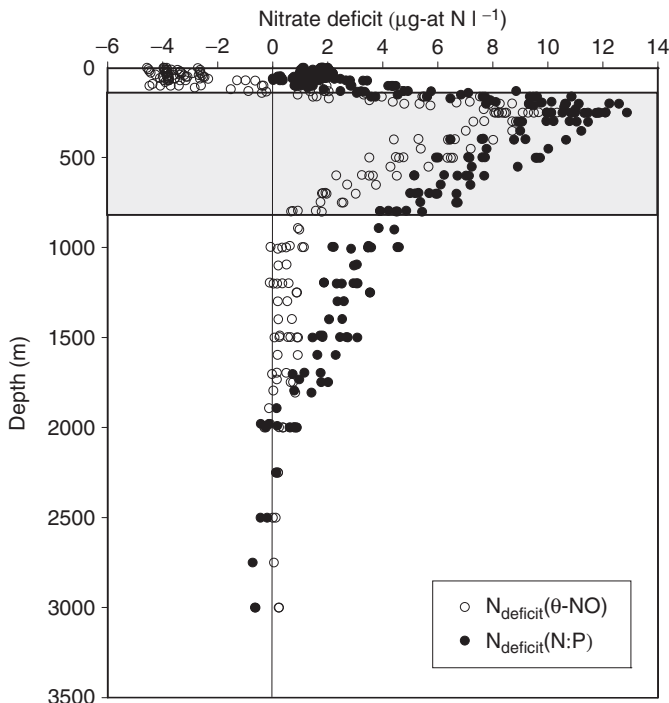
Figure 6.9 Nitrate deficit in the ODZ of the Arabian Sea. Solid cricles are Codispoti *et al.* (2001) modified N⋆ calculation, while the open circles are calculated from sigma theta relationships of Naqvi and Sen Gupta (1985). Shaded zone approximates the depth zone of the ODZ. (Redrawn from Devol *et al.*, (2007).

short time during which electrons are transferred from the substrates to the electron acceptor by the ETS system reducing the tetrazolium salt to a colored end product, which is quantified and from which the rate of electron transfer can be calculated and used to estimate denitrification rates (Devol, 1975; Codispoti and Packard, 1980; Packard, 1971). In both the Arabian Sea and eastern tropical South Pacific, rates estimated by ETS activity were in general agreement with those estimated by stoichiometric methods.

Recently, Devol et al. (2006) have used on-deck and in situ incubation of water samples with added $^{15}NO_3^-$ to determine the rate of denitrification by measuring the rate of $^{29}N_2$ production. Although these were not true tracer experiments, as the in vitro NO_3^- concentrations were increased 5 µM over the in situ concentration of 25 µM, it is likely that rates were not stimulated by the amendment. In the Arabian Sea ODZ the $\delta^{15}N$ of the N_2 typically increased by 0.4–0.8‰. The average denitrification rate for the ODZ waters they determined was 8.8 ± 3.8 (s.d.) nmol N l^{-1} d^{-1}. Although not directly comparable because of different time scales (days vs years), these rates are in general agreement with those estimated from nitrate deficits and residence times. Rates measured in suboxic shelf waters were moderately higher averaging 28.5 ± 48.9 (s.d).

Devol et al. (2006) were also able to determine the profile of excess N_2 gas throughout the Arabian Sea ODZ. They did this by measuring the N_2:Ar ratio using high precision isotope ratio. Mass spectrometry: The amount of excess N_2 was then computed from the increase in the N_2:Ar over that present in the source waters as measured south of the ODZ. Nitrogen excess gas measurements predicted a larger nitrogen anomaly than that estimated by nitrate deficit, maximum anomaly $=$ 23 µg-at N l^{-1} versus 13 µg-at N l^{-1}, respectively (Fig. 6.10). It was suggested that the mismatch may have resulted from incorrect assumptions of Redfield stoichiometry inherent in the nitrate deficit calculation (Van Mooy et al., 2002), inputs of new nitrogen through N-fixation, N_2 contributions from sedimentary denitrification along continental margins, the anammox reaction, or metal catalyzed denitrification reactions. Regardless, the data suggested that denitrification in the Arabian Sea may have previously been underestimated.

3.2. Sedimentary denitrification

3.2.1. Sites of sedimentary denitrification

Another place where oxygen is typically depleted and denitrification takes place is in marine sediments, especially continental margin and hemipelagic sediments. This is because the source of nitrate, and other respiratory electron acceptors, is the local overlying water, while the respiratory processes within the sediments act as sinks. The sedimentary pore-water profiles reflect the balance between sources and sinks, and the transport mechanism of electron acceptors into the sediment is either molecular diffusion or molecular diffusion plus macrobenthic irrigation. Thus, sedimentary pore-water profiles generally show the vertical zonation of respiratory modes according to the thermodynamic yield outlined previously. Going down core, oxygen is depleted first followed by nitrate, after which reduced manganese appears, followed by reduced iron and finally sulfate declines (Fig. 6.11). In most
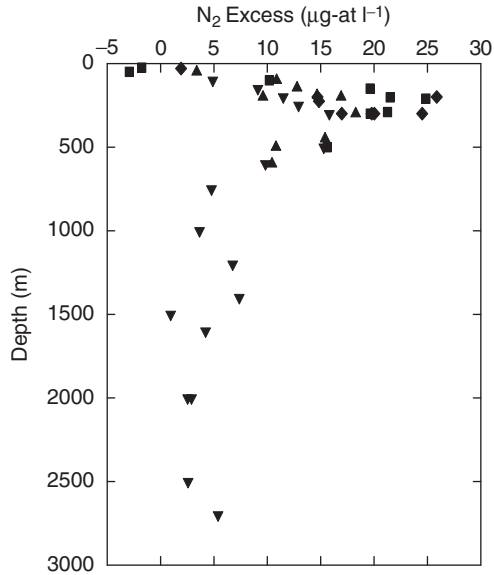
Figure 6.10 Excess nitrogen gas in the Arabian Sea. Excess N_2 gas values are presented in units of μg-at l^{-1} so there is a 1:1 stoichiometry with nitrate anomaly. Compare the maximum values presented here, $\sim$25 μg-at l^{-1} with the values of about 13 μg-at l^{-1} derived from nitrate deficit calculations shown in Fig. 6.9. (Redrawn from Devol *et al.*, 2007).
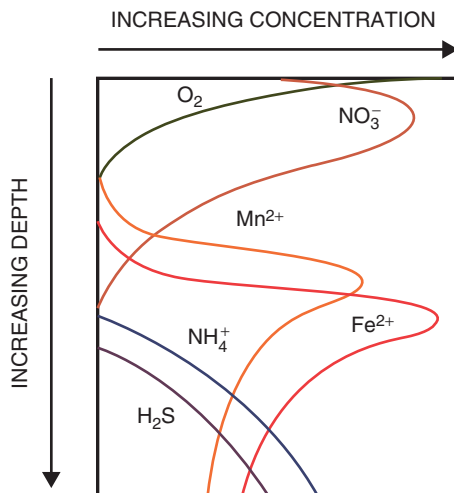
Figure 6.11 Schematic representation of the vertical zonation of electron acceptors in sediments. The highest energy yeilding acceptors are used first, thus oxygen is depleted before nitrate is depleted. Manganese oxide(s) reduction occurs next as shown by the appearance of reduced manganese followed by Iron oxide(s) reduction. Finally, Sulfate is reduced. Mehthanogenesis is not shown but it occurs deeper than sulfate reduction (after Froelich *et al.*, 1979).

continental shelf sediments oxygen penetrates to less than 1 cm below the sediment–water interface and even in most deep-sea sediment oxygen penetration is restricted to the upper 10 cm or so. Thus, there exist ample environments for denitrification in marine sediments.

Pore-water nitrate profiles in marine sediments typically show one of three profile shapes. In sediment with rapid rates of organic matter oxidation relative to rates of solute supply from the overlying water, both oxygen and nitrate concentrations decrease more or less exponentially from overlying water concentrations at the sediment–water interface to zero, with oxygen depletion preceding or simultaneous with nitrate depletion at shallow sediment depth (see 105 m and 440 m profiles in Fig. 6.12). These types of profiles are common in continental shelf and upper slope sediments, and are due to relatively large carbon rain to the sediments (relatively
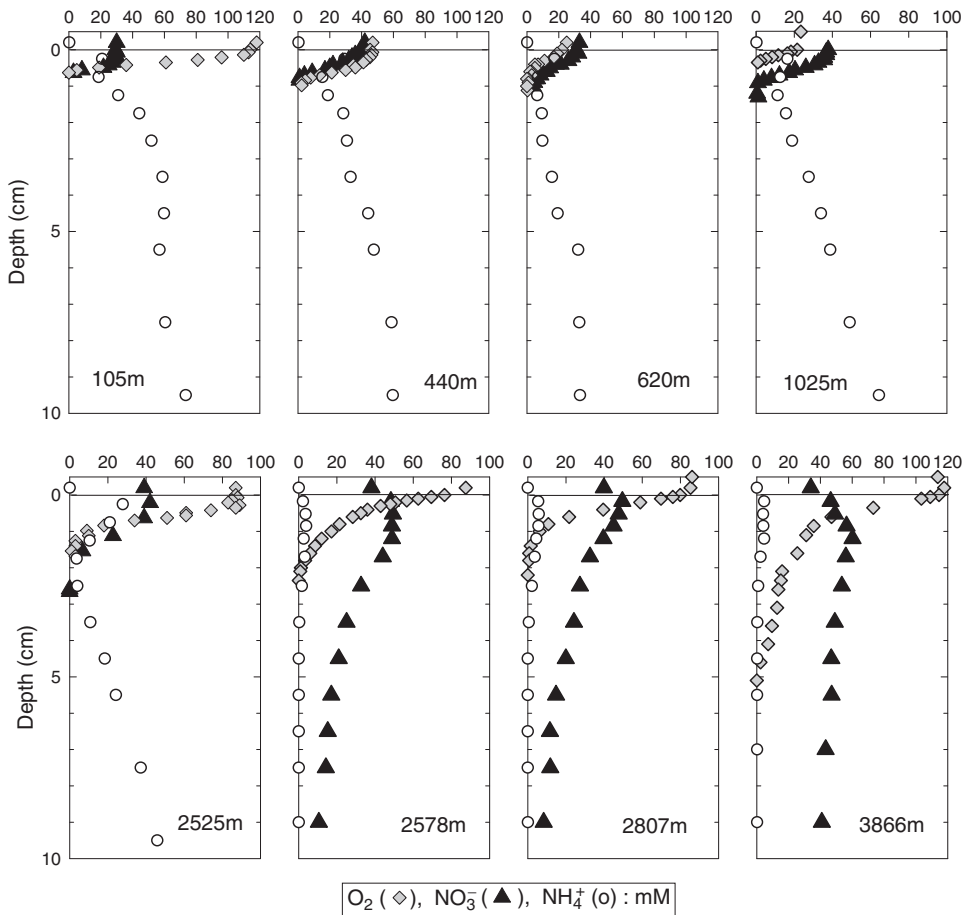
Figure 6.12 Pore-water profiles of oxygen, nitrate and ammonium along a zonal section off the Washington State continental margin at approximatly 47°N latitude extending 650 km offshore. Data from Hartnett and Devol (2003) and Emerson and Devol unpublished.

high shelf productivity and short travel time to sediments; e.g., Brandes and Devol, 1995; Glud *et al.*, 1998; Hartnett and Devol, 2003; Jørgensen and Sørensen, 1985; Kristensen *et al.* 1999; Reimers *et al.*, 1992). When organic matter degradation is less rapid relative to solute supply a subsurface nitrate maximum typically develops (2578 m and 2807 m profiles in Fig. 6.12). This is the result of nitrification in the oxygenated zone of the sediments. These profile shapes are commonly found in hemi–pelagic sediments (Hartnett and Devol, 2003; Reimers *et al.*, 1992). In sediments with very slow rates of organic matter respiration, nitrate profiles increase due to nitrification, but do not decrease because oxidizable organic matter is consumed before the oxygen supply is depleted (this situation is approached in the 3886 m profile in Fig. 6.12). Such profiles are found in deep-sea sediments and under oligotrophic conditions. Continental margins will typically show a progression from very shallow nitrate penetration depth to deeper penetration to a subsurface nitrate maximum that deepens going even further off-shore (Fig. 6.12).

3.2.2. Rates of sedimentary denitrification

Sedimentary denitrification rates have been estimated from measured pore-water solute profiles using diagenetic models, determined directly via sediment incubation both on deck and in situ, and determined from ^{15}N-incubation techniques. Sedimentary diagenetic process can be thought of as a simple reaction–diffusion–transport system (Berner, 1980; Boudreau, 1997). In a simple fine-grained sediment system, transport is via molecular diffusion and the diagenetic equation describing this system can be expressed as:

$$dN/dt = \rho D_s(\partial N/\partial z) - R_{NO_3} + P_{NO_3}, \qquad (6.8)$$

where *N* is nitrate concentration, ρ is sediment porosity, D_s is the sediment diffusion coefficient is depth, *R* is nitrate consumption rate (denitrification) and *P* is nitrate production rate (nitrification). Also note that the flux into the denitrification zone from the top must equal the integrated denitrification rate within the zone or on an areal basis:

$$\text{Flux in} = \int R_{NO_3} dz = \rho D_s(\partial N/\partial z_m), \qquad (6.9)$$

where ∂z_m is the maximum negative N gradient. Thus, given vertical stratification in the zones of oxygen consumption and denitrification, the areal denitrification rate can be calculated from the steepest part of the nitrate gradient.

Early examples of the use of the diagenetic equation are given in Jahnke *et al.* (1982) and Galloway and Bender (1982). Because nitrification is dependent on ammonium produced during oxic degradation of carbon there is a relationship between oxygen consumption and denitrification inherent in these models. Indeed, in a purely diffusive environment it is possible to predict the maximum amount of carbon that can be oxidized by denitrification, C_N, relative to that oxidized by oxygen, C_O. In the pelagic Pacific $C_N:C_O$ can theoretically reach about 0.33, while

in the pelagic Atlantic it can only be 0.13 at maximum (Jahnke *et al.*, 1982). This is because of the higher bottom water nitrate and lower bottom water oxygen in the Pacific than the Atlantic.

Although the simple advection diffusion model works well in open-ocean pelagic sediments, this is not where the majority of sedimentary denitrification occurs; rather it occurs in shallow, shelf and upper margin sediments. About 30% of shallow sediments are fine grained with the remander being sandy (Rao *et al.*, 2007). In these sediments there is rarely a subsurface nitrate maximum and solute transport due to the activities of benthic macrofauna and/or pore-water advection is important if not dominant (Aller *et al.*, 1998; Archer and Devol, 1992; Devol and Christensen, 1993; Glud *et al.*, 2003; Huettel and Rusch, 2000; Janssen *et al.*, 2005a and 2005b; Kristensen and Blackburn, 1987; Kristensen *et al.*, 1991). In shallow fine-grained sediments pore-water advection is not important and the diffusive supply of nitrate for denitrification can be calculated from the pore-water gradient, however the component due to macrobenthic activities, or "irrigation," must also be modeled. Typically this is done with a "biodiffusion" term or a non-local source function (Berner, 1980; Boudreau, 1997). Martin and Banta (1992) investigated the magnitude of macrobenthic irrigation by including a non-local source term of the form $\alpha(C_o-C)$ in the reaction-diffusion equation (here α is an exchange coefficient, cm s^{-1}, C is the concentration at a depth, z, and C_o is the concentration in the overlying water). They modeled both the penetration of added bromine in sediment core incubations and the natural deficit of ^{222}Rn for Long Island Sound sediments. Martin and Banta found that the flux of a given solute into the sediment due to irrigation was equal to that of molecular diffusion or up to several times greater than the molecular diffusive flux. In a more recent study they were able to show that irrigation increased the denitrification rate by about 50% at a upper continental slope site in the mid-Atlantic Bight (Martin and Sayles, 2004).

Devol and Christensen (1993) used a different approach to determine the importance of irrigation in fine-grained Washington State continental shelf sediments. They used in situ benthic flux chambers to measure the net flux of nitrate into the sediments and they also determined pore-water nitrate profiles. By comparing the total nitrate flux into the sediment with the purely diffusive flux calculated from the pore-water profiles, they were able to show that the irrigation flux could be as great as 3 times the molecular flux (this was also true for oxygen and silica fluxes). In addition to benthic nitrate fluxes into the sediment, Devol and Christensen (1993) also measured the flux of N_2 gas out of the sediments. For sediments at their study site the flux of N_2 out of the sediments was about twice the flux of NO_3^- into the sediments. The extra N_2 was attributed to coupled nitrification–denitrification, i.e., within sediment nitrification of NH_4^+ regenerated during organic matter oxidation coupled to subsequent denitrification. This conclusion was supported by a sedimentary nitrogen budget that showed that up to 75% of the regenerated NH_4^+ was nitrified (Devol and Christensen, 1993). The importance of irrigation and within-sediment nitrification as a supply of nitrate for denitrification has been shown by many workers (Christensen *et al.*, 1987; Devol *et al.*, 1997; Glud *et al.*, 1998; Howe *et al.*, 2004; Hulth *et al.*, 1996; Jenkins and Kemp, 1984; Lehmann *et al.*, 2004; Kristensen and Blackburn, 1987; Kristensen *et al.*, 1991).

In general it appears that the rate of sedimentary dentirificaiton is related to the rate of sediment oxygen consumption (Christensen, 1994; Laursen and Seitzinger, 2002; Seitzinger and Giblin, 1996).

Workers who use the nitrogen isotope pairing technique are able to quantitatively partition denitrification into that supplied by nitrification and that supplied by the flux from the overlying water (Christensen *et al.*, 2000; Jensen *et al.*, 1996; Loshe *et al.*, 1996; Nielsen, 1992; Risgaard-Petersen *et al.*, 1998; Wang *et al.*, 2003). However, depending on the degree to which anammox is present, the isotope pairing technique would overestimate canonical denitrification, although this would not be a large problem in sediments where anammox is a minor component of the N-cycling (Risgaard-Petersen *et al.*, 2004b; see following section).

Interestingly, in fine-grained sediments off the west coast of Mexico where the ODZ impinges upon the continental margin denitrification rates are a little less than half those of the Washington margin even though pore-water profiles are about the same (Hartnett and Devol, 2003). Apparently, the lack of dissolved oxygen in the overlying waters precludes macrobenthic organisms and thus pore water irrigation. Lack of oxygen and irrigation prevents both enhanced exchange of overlying-water nitrate into the sediments via macrobentihc irrigation and the possibility of within sediment nitrification and coupled denitrification. This results in the rather counter intuitive observation that denitrification rates are actually decreased by a lack of oxygen in the overlying waters (Hartnett and Devol, 2003).

Though sediments are generally treated as vertically zoned, this may not be the case for all situations. Brandes and Devol (1995) presented pore-water profiles from shallow, rapidly respiring sediments of Washington State and the Chukchi Sea that show oxygen depletion and nitrate depletion at the same depth. Additionally, when both solutes were normalized to their overlying water values, the profiles were nearly identical as were their first derivatives. Brandes and Devol proposed the existence highly respiring microsites within the sediments and they were able to successfully model their data with a microsite model. One of the consequences of this model is that concentrations of both nitrate and oxygen at the microsite are very small and, thus the isotopic fractionation of both solutes should be very much smaller than the observed biological fractionation (Brandes and Devol, 1995). Indeed, subsequent studies have found near zero isotopic fractionation for dissolved oxygen, nitrate and nitrogen gas in sediment incubations Brandes and Devol, 1997; Lehmann *et al.*, 2005; Sigman *et al.*, 2003).

In sandy sediments pressure gradients caused by waves and flow over irregular topography (sand waves or mounds) can force an advective flow of pore water through sediments (Huettel *et al.*, 1998; Precht and Huettel, 2003). Although it is well documented that pore-water advective flow through coarse-grained sediments increases sedimentary oxygen consumption (Huettel and Rusch 2000; Reimers *et al.*, 2004), little is known about its effect on sedimentary denitrification. However, Rao *et al.* (2007) investigated denitrification rates in sandy sediments from the South Atlantic Bight. Their experiments were done by pumping water through natural sediment cores at environmentally determined rates and measuring the nitrate and

nitrogen gas differences between the inflow and outflow. Measured rates were similar to those typically found in fined grained sediments.

3.3. Sites and rates of anammox

Like canonical denitrification anammox favorable conditions are those where oxygen has been depleted, and, like canonical denitrification, anammox has been found both in marine sediments and within the water column. Nitrogen stable isotope incubations are commonly used to distinguish between anammox and canonical denitrification. Typically, additions of $^{15}NH_4^+$, $^{15}NH_4^+ + {}^{14}NO_3^-$, and $^{15}NO_3^-$ are made to anaerobic samples that are then incubated for hours to several days, after which the samples are sacrificed and the isotopic composition of N_2 is determined. If $^{29}N_2$ is formed during the incubations with added $^{15}NH_4^+$ this is taken as evidence of anammox, whereas formation of $^{30}N_2$ in the $^{15}NO_3^-$ treatment is indicative of canonical denitrification. The treatment with both $^{15}NH_4^+ + {}^{14}NO_3^-$ is used to detect anammox in samples with little or no ambient NO_3 or samples that have been preincubated to remove traces of O_2 and NO_3^-. That NO_2^- rather than NO_3^- is the electron acceptor during anammox has been confirmed by the isotopic composition of N_2 at the end of the experiment. With $^{15}NO_3^-$ as the electron acceptor, the reaction stoichiometry would be

$$5{}^{15}NO_3^- + 3{}^{14}NH_4^+ = 4N_2 + H^+ + H_2O, \qquad (6.10)$$
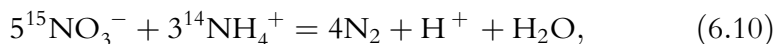
whereas, with 15 NH_4^+ and $^{14}NO_2^-$ as the electron acceptor the stoichiometry would be

$$^{14}NO_2^- + {}^{15}NH_4^+ = N_2 + H_2O. \qquad (6.11)$$
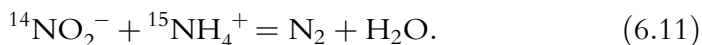
The former stoichiometry would yield 75% $^{29}N_2$ and 25% $^{30}N_2$ while the latter would yield 100% $^{29}N_2$. As 100% $^{29}N_2$ is typically found experimentally in relatively pure anammox cultures, NO_2^- appears to be the oxidant for anammox (Dalsgaard et al., 2005).

In the oceans anammox has been found to occur in the sediments and in the water column, as well as in Arctic Sea ice (Kuypers et al., 2003; Rysgaard and Glud, 2004; Thamdrup and Dalsgaard, 2002). Anammox was first observed in sediments from the Skagerrak and Arahus Bay by Thamdrup and Dalsgaard (2002). It was discovered through ^{15}N-isotope paring experiments such as those described above, and from the relative yields of $^{29}N_2$ and $^{30}N_2$ in the different incubations it was concluded that 24% and 67% of the total N_2 produced at two continental margin sites (Skagerrak) was attributable to anammox, but anammox was insignificant in Arahus bay. Subsequently, anammox has been reported from sediments in the Thames estuary, United Kingdom (Trimmer et al., 2003), Arctic sediments around Svalbard (Rysgaard et al., 2004), several Swedish fjords and Long Island Sound, New York (Engström et al., 2005) and Boston Harbor, Massachusetts (Tal et al., 2005). The range of rates reported by Egström et al. of between 0.14 and
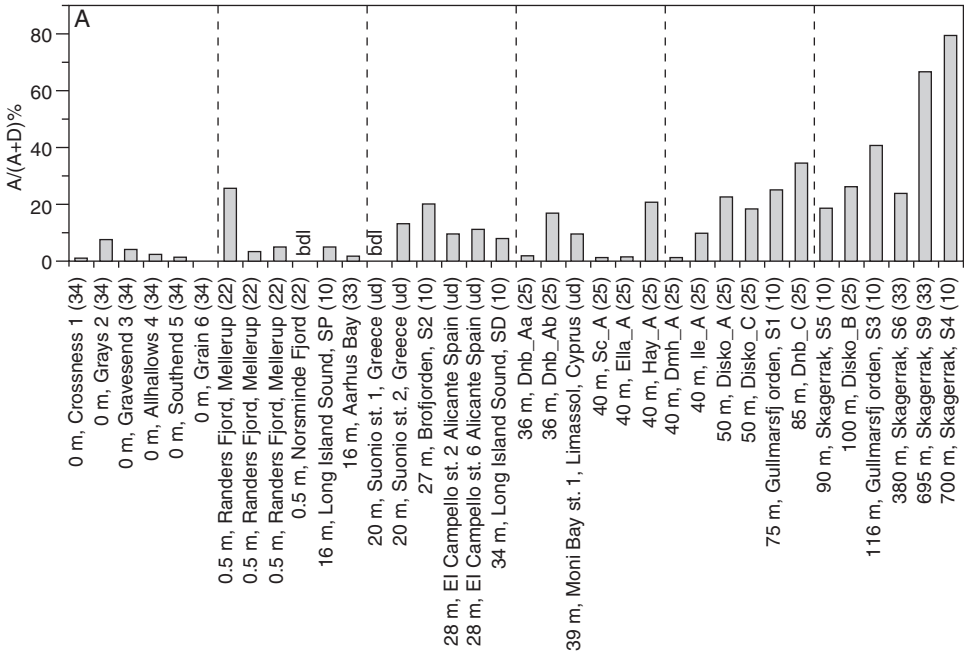
Figure 6.13 Relative rate of N_2 production from anammox as a percentage of the total N_2 production rate as determined from ^{15}N incubation experiments described in text. Although the relative rate of anammox increases with water depth, the ablsolute rate of N_2 production generally decreases with increasing water depth. (Redrawn from Dalsgaard *et al.*, 2005).

16 μM N_2 h^{-1}, more or less brackets the entire reported range sedimentary anammox, as does their range of importance of anammox relative to total N_2 production of 4%–79%. Although, the study of anammox in marine sediments is in its infancy, it appears that the relative importance of anammox increases as overlying water depth increases (Fig. 6.13).

Anammox has also been reported to occur in the water columns of the Gulfo Dulce, Costa Rica (Dalsgaard *et al.*, 2003), the Black Sea (Kuypers *et al.*, 2003), and in the bottom waters over the Namibian shelf (Kuypers *et al.*, 2005). To see if the anammox organism in the Black Sea was similar to well–characterized Planctomycetes isolated from the fluidized bed reactors, Kuypers *et al.* looked for the characteristic ladderane lipid signatures of anammox bacteria and found that ladderane lipid distributions in the water column closely mirrored the anammox reaction as indicated by the isotope labeling experiments. Additionally, molecular DNA analysis showed that organisms closely related to known anammox bacteria were also present at this depth (Fig. 6.14). Taken together, this was striking confirmation of the anammox process in the Black Sea. Subsequently Kuypers *et al.* have also demonstrated anammox in the suboxic bottom waters of the Nambian shelf, this time occurring at very low but measurable oxygen concentration (Kuypers *et al.*, 2005). In fact, at the time of their observations, anammox was the only process responsible for
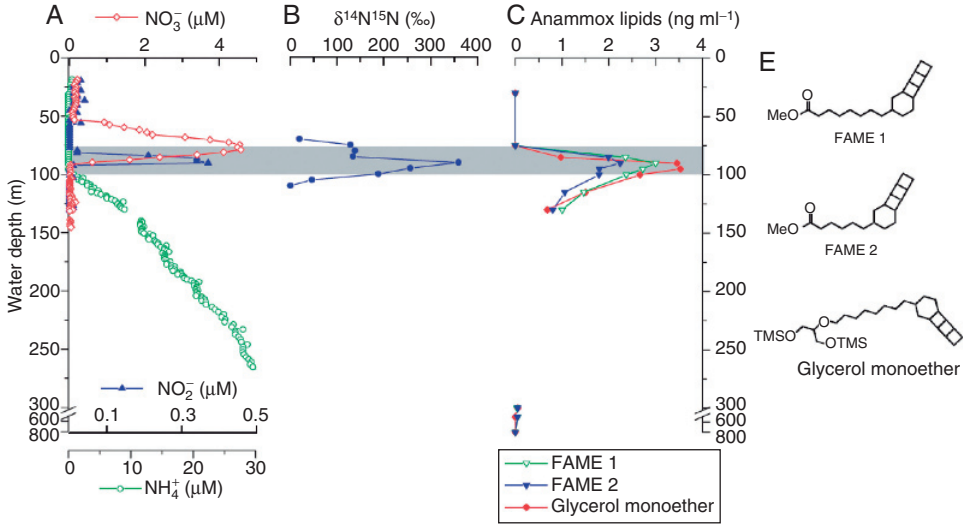
Figure 6.14 Anammox indicators in the suboxic transition of the Black Sea. (A) Nitrate (red) nitrite (blue) and ammonia (green) concentrations, (B) the dirtribution of $^{15}N^{14}N$ produced in incubation experiments similar to those described in the text, and (C) distributions of the three ladderane lipids Fame 1, Fame 2 and glycerol monoether. The suboxic transition zone is shown as the gray shaded area. The molecular structures of the three ladderane lipids are also shown on the right. (Redrawn from Kuypers *et al.*, 2003).

N_2 production in the oxygen depleted waters over the shelf, canonical denitrification was not detectable.

Thamdrup *et al.* (2006) investigated denitrification and anammox in the oxygen deficient waters of the ETSP using the ^{15}N labeling technique and found that anammox was by far the dominant the N_2 production mechanism with canonical denitrification playing only a minor role. They postulated that during the time they were there organic matter decomposition was proceeding mainly through nitrate reduction to nitrite and that anammox converted the remineralized NH_4^+ to N_2. However, given a Redfield stoichiometry for organic matter decomposition this could only be a transient situation because with the estimated nitrate deficit the nitrite concentration would need to be much greater than observed. In contrast to the ETSP, Nicholls *et al.* (2007) found little evidence for anammox in the Arabian Sea ODZ based on conversion of $^{15}NH_4^+$ to $^{29}N_2$. They also concluded that denitrification could not be the only source of N_2 production because if that were the case the percentage of ^{15}N label found as $^{29}N_2$ in the N_2 pool should equal the percentage of label found as $^{15}N_2O$ in the N_2O pool and that was not what they observed in their incubations.

Up until a few years ago the general understanding was that in the world's oxygen deficient zones, heterotrophic denitrification was the sole mechanism of N_2 production and NO_3^- was the sole electron acceptor. Given the results of

the recent incubation work with ^{15}N tracers it appears that our understanding of the processes producing N_2 in the world's ODZ is not really all that well understood.

4. Isotopic Consequences of Denitrification

Two stable isotopes of nitrogen exist, ^{14}N (99.634%) and ^{15}N (0.366%). Because of this mass difference the heavier isotope reacts at a slightly slower rate, producing a kinetic isotope fractionation in many biological reactions. The increase in the $^{15}N/^{14}N$ in the reaction products is typically expressed as the parts per thousand change in the ratio relative to that of a standard (air), or the del change, $\delta^{15}N‰$. With respect to denitrification, the change in isotopic composition or ''fractionation'' that occurs during canonical denitrification is relatively well-studied, both in the water column and sediments, while those of other denitrifying processes, anammox, chemodenitirification and nitrifier denitrification, are virtually unknown.

Early studies with cultures of heterotrophic denitrifiers suggested fraction factors, ε, of 17–30‰ (Delwiche and Steyn, 1970; Mariotti *et al.*, 1981: $\varepsilon = 1000[\alpha-1]$, where $\alpha =$ ratio of the rate constants for the light and heavy isotopes, respectively). These have been confirmed by a recent study in which fractionation by steady-state cultures of *Paracoccus dentirificans* was investigated at oxygen concentrations between 0 and 1.2 μM (Barford *et al.*, 1999). Barford *et al.* found that the overall ε for denitrification was 28.6‰ and that it was independent of oxygen concentration. They were also able to determine the fractionation associated with the $N_2O \rightarrow N_2$ step of denitrification and it was 12.9‰. This results in the N_2O formed during denitrification being about 15‰ lighter than the nitrate from which it was produced and about 13‰ heavier than the N_2 that is produced.

The isotopic fractionation is easily seen in $\delta^{15}NO_3^-$ and $\delta^{15}N_2$ distributions in the major open-ocean denitrification zones (Altabet *et al.*, 1999; Brandes *et al.*, 1998; Cline and Kaplan, 1975). Typical open ocean values of sub-euphotic zone nitrate are about 5‰ (Lehmann *et al.*, 2005; Sigman *et al.*, 2000; Wu *et al.*, 1997) but within the ODZ they climb to upwards of 15‰. Concomitant with this increase is a decrease in the $\delta^{15}N_2$ from about 0.6‰ to 0.2‰ (Fig. 6.15). The large enrichment of $^{15}N-NO_3^-$ and the mirror image decrease in $^{15}N-N_2$ is undoubtedly due to fractionation during denitrification. It is also possible to derive a fraction factor, ε, from the isotope distributions in the ODZ if one makes some assumption about the amount of nitrate that has been removed by denitrification, i.e., the nitrate deficit. For the eastern tropical North Pacific Brandes *et al.* (1998) assumed a Raleigh fraction mechanism and both open (advection-reaction) and closed (diffusion-reaction) systems to derive fractionation factors from the data. in Fig. 6.15. (Raleigh fractionation; $\delta^{15}NO_3^- = 10^{3(\alpha-1)}f_{NO3}$, where $\delta^{15}NO_3^-$ is the isotopic composition of the fraction, f, of nitrate remaining after denitrification.) Brandes *et al.*, found that ε was between 22‰ (Arabian Sea closed system model) and 30‰ (eastern tropical North Pacific open-system model). Altabet *et al.* (1999), Cline and Kaplan (1975),
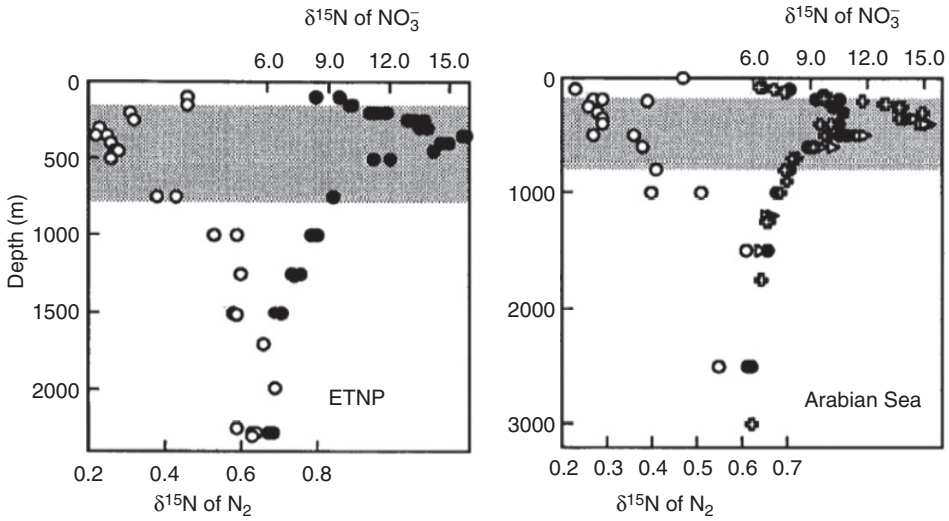
Figure 6.15 Profiles of del ^{15}N of N_2 and NO_3^- through the oxygen deficient water of the eastern tropical North Pacific (left) and Arabian Sea (right). The shaded area represents the oxygen deficient zone. Redrawn from Brandes *et al.*, 1998.

Sigman *et al.* (2003), and Voss *et al.* (2001) have similarly used observed isotopic values and nitrate deficits to estimate ε from ODZs (Fig. 6.16) and all of these estimates cluster around an ε of 25‰.

In sediments the situation is much different. Brandes and Devol (1997) measured the isotopic change in NO_3^-, N_2 and O_2 in the overlying water of benthic flux chambers. While NO_3^- and O_2 decreased and N_2 increased within the flux chamber during the incubation the isotopic concentrations of all three remained virtually constant. This implied that there was near zero net isotopic fractionation during sedimentary denitrification and oxygen consumption. This was a surprising result because given the large biological isotope fractionation associated with both denitrification and oxygen consumption (ε for O_2 uptake ∼20‰; Kiddon *et al.*, 1993) and a reaction diffusion system such as that given by Eq. (6.8), the observed fractionation in the overlying water theoretically should be half that of the true biological fractionation or 12.5‰ (Bender, 1990). This apparent lack of fractionation has since been confirmed by observations in the Santa Barbara Basin (Sigman *et al.*, 2003), the deep Bering Sea (Lehman *et al.*, in press), as well as the freshwater lake sediment (Lehmann *et al.*, 2003).

The small isotopic effect expressed during sedimentary denitrification could be due to diffusion limitation as proposed by Brandes and Devol (1997), but it could also result from a balance between the isotopic effects of denitrification being offset by an input of low $^{15}N/^{14}N$ NO_3^- from sedimentary nitrification. However in an elegant study of the isotopic effects of denitrification during benthic flux chamber deployments, Lehmann *et al.* (2004) were able to show that not only was there little N-fractionation, there was also little O-fractionation in the residual NO_3^-. Because
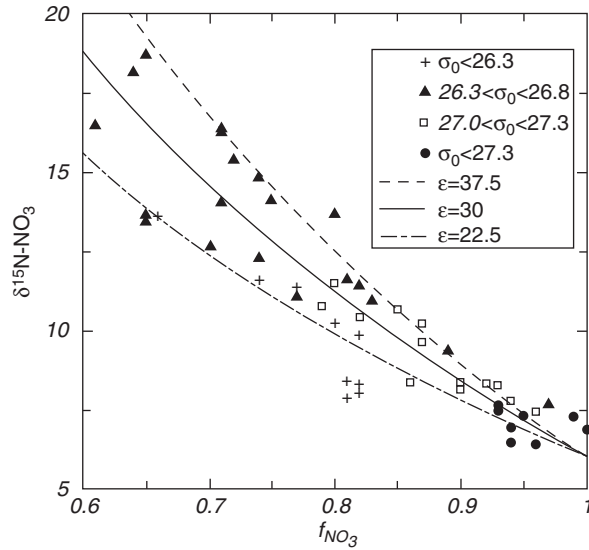
Figure 6.16 Solutions to a horizontal ventilation model of denitrification for waters of the eastern tropical North Pacific for $\delta^{15}NO_3$ vs f, the fraction of nitrate remaining, i.e., the measured nitrate minus the nitrate deficit. Nitrate deficit was calculated as $NO_{3(deficit)} = 14.8 \times PO_4 - NO_3$, 'where NO_3 and PO_4 are the measured concentrations. The two equations describing the steady-state nitrate isotope distribution are: $\partial[^{14}NO_3]/\partial t - J[^{14}NO_3] + A\partial^2[^{14}NO_3]/\partial x^2$ and $\partial[^{15}NO_3]/\partial t - \alpha QJ[^{14}NO_3] + A\partial^2[^{15}NO_3]/\partial x^2$, where A is the eddy diffusion coefficient in the x direction, J is the denitrification rate, α is the fraction factor, and Q is a $^{15}N{:}^{14}N$ ratio that makes the system non-linear. (See Voss *et al.*, 2001 for solution details). Solutions for three different values of ε are given ($\varepsilon = [1-\alpha] \times 1000$).

the ratio of N and O isotopic fractionation seems to be close to unity for denitrification in seawater (Casciotti *et al.*, 2002; Sigman *et al.*, 2003) and because sedimentary nitrification should actually enrich ^{18}O in the nitrate produced (Lehmann *et al.*, 2004), the near lack of fractionation in both N and O isotopes strongly suggested that the isotopic fractionation specifically associated with sedimentary denitrification was, indeed, small. That water-column and sedimentary denitrification have different apparent fractionation factors provides a valuable constraint on the relative rates of the two processes globally.

5. DENITRIFICATION AND THE MARINE COMBINED NITROGEN BUDGET

Nearly all quantitative estimates of open-ocean water column denitrification are based on some type of nitrate deficit calculation. In the Arabian Sea, Naqvi (1987) have used nutrient-potential temperature relationships to estimate a denitrification rate of ~30 Tg/y. In a later study Naqvi and Shailaja (1993) arrived at a

similar value, $\sim$33 Tg/y, based on denitrification rates estimated enzymatically from electron transport system activity. Howell *et al.*, 1997, likewise, used nutrient-temperature relationships in their estimate of $\sim$22 Tg/y for the Arabian Sea. However, as as noted above Codispoti *et al.*'s (2002) N$\star$ relationship predicts nitrate deficit values about 30% larger than those predicted from nutrient-potential temperature relationships, so it is likely that the denitrification rate in the Arabian Sea is similarly greater. Because they are based on nitrate deficit, these rates are strictly for canonical denitrification (N$_2$ produced from NO$_3^-$) but new information suggests that such estimates may be too low due to inappropriate stoichiometries (Van Mooy *et al.*, 2002) and under-appreciated pathways, such as anammox (Codispoti *et al.*, 2002) or chemodenitrification (Farrenkopf *et al.*, 1997; Luther *et al.*, 1997). Indeed, the measurements of dissolved nitrogen gas in the Arabian Sea (Codispoti *et al.*, 2002; Devol *et al.*, 2006) suggested that the N$_2$ supersaturation is almost twice as great as can be accounted for by amount of nitrate removed (nitrate deficit). Consequently, denitrification in the Arabian Sea ODZ can be conservatively estimated to be at least 50 Tg/y.

Denitrification rates in the other two main ODZs, the ETNP and ETSP; appear to be about 25 Tg a^{-1} each Codispoti *et al.* (2002). In the ETNP denitrification was estimated by the geostrophic flux of nitrate deficit out of the ODZ area, with nitrate deficit determined from nutrient-density relationships (Codispoti, 1973; Codispoti and Richards, 1976). Volumetric estimates of denitrification rate based on ETS activity are in concert with this rate (Codispoti and Richards, 1973; Devol, 1975). The ETSP the denitrification rate is based on measurements of ETS activity (Codispoti and Packard, 1980). As with the Arabian Sea these estimates are again for canonical denitrification. It is likely that processes identified in the Arabian Sea are also occurring in the ETNP and ETSP. Thus, the rate of 25 Tg a^{-1} might be raised to 50 Tg a^{-1}. Given a denitrification rate between 30 and 50 Tg a^{-1} for each of the major ODZ's, global water-column denitrification would appear to between 90 and 150 Tg a^{-1}.

There is also water column denitrification in Black Sea, Cariaco Trench and smaller anoxic basins and fjords, as well as within the more transient hypoxic zones over the continental shelves. The recent work by Kuypers *et al.* (2005) indicated that the denitrification rate off south west Africa is about 1.5 Tg a^{-1}. Codispoti *et al.*, 2002 have estimated the total denitrification in these smaller/more transient sites to be $\sim$10 Tg a^{-1}. Finally, denitrification has been reported to take place in microenvironments within the oxygenated water column such as organic aggregates Aldredge and Cohen, 1987; Wolgast *et al.*, 1998). However no quantitative estimates of the global importance of microsite denitrification are available.

The amount of sedimentary denitrification occurring in the ocean today is one of the most poorly quantified terms in the marine combined nitrogen budget. Most modern measurements of denitrification rate have utilized pore-water NO$_3^-$ profiles (estimated from diffusion calculations–reaction models); whole-sediment incubations, either on deck or in situ, or by the isotope paring technique. It appears that shelf and upper slope sediments are quantitatively the most important sites of sedimentary denitrification (Christensen, *et al.*, 1996; Christensen *et al.*, 1987; Devol, 1991; Devol and Christensen, 1993; Gruber and Sarmiento, 1997; Kristensen, *et al.*, 1999; Middelburg *et al.*, 1996). Typical denitrification rates in these areas

are from 0.1 to 4 mMoles m^{-2} d^{-1} (Dalsgaard *et al.*, 2005; Devol and Christensen, 1993; Devol *et al.*, 1997; Hartnett and Devol, 2003; Middelburg *et al.*, 1996; Seitzinger, 1988; Seitzinger and Giblin, 1996). Given rates of this magnitude, global sedimentary denitrification has been estimated to be somewhere between 100 Tg a^{-1} (Gruber and Sarmeinto, 1997) and 400 Tg a^{-1} (Codispoti, 1997; Codispoti *et al.*, 2001; Middelburg *et al.*, 1997).

Assuming that pelagic water-column and sedimentary denitrification represent the bulk of the combined nitrogen loss from the oceans, adding the two would lead to a total denitrification rate in the range of 190–550 Tg a^{-1}, with the majority of it taking place in the sediments. The total amount of combined nitrogen in the ocean is on order of 550×10^3 Tg, which would put the residence time of combined nitrogen at 1000–3000 years.

Although there is a large range in the estimate of the absolute denitrification, it is possible to constrain the relative amounts of sedimentary and water-column denitrification with stable isotope data (Brandes and Devol, 2002). There is some sedimentary evidence that away from the ODZs the stable isotopic composition of combined nitrogen in the ocean has been relatively constant over the last 10,000 years (Kienast, 2000, also see Deutsch *et al.*, 2004). If this is the case, then the stable isotope composition of combined nitrogen (almost entirely nitrate) is determined by the mass balance of the sources, primarily nitrogen fixation, and the sinks, primarily denitrification. For the $\delta^{15}N$ of combined nitrogen in the ocean to remain constant, the $\delta^{15}N$ of denitrification sink must equal the $\delta^{15}N$ of the nitrogen fixation source, which is ~0‰ (Carpenter *et al.*, 1997). Given an average $\delta^{15}N$ of 5‰ for oceanic nitrate, the net fractionation factor for the sum of water-column denitrification, ~25‰, plus sedimentary denitrification, ~0‰, must be −5‰ to produce a total denitrification sink at 0‰. To achieve this sedimentary denitrification must be about 4 times greater than water column denitrification. Including a loss term for combined nitrogen burial in margin sediments only reduces this ratio to about 3 times water column denitrification (assuming a burial rate one third that of water column denitrification and an isotopic value of the buried material of 6‰).

The current literature would thus suggest a denitrification rate of 200–500 Tg a^{-1}, with 2/3 to 3/4 occurring in the sediments and the remainder in the water column (Codispoti *et al.*, 2001; Gruber and Sarmiento, 1997; Middelburg *et al.*, 1997). Given a combined nitrogen residence time of several thousand years this implies either an equally rapid nitrogen fixation rate (to achieve steady-state) or that the ocean is ''losing nitrate'' (Codispoti, 1995). There are relatively large uncertainties in the estimates of both the nitrogen-fixation rate and the denitrification rate, especially the sedimentary component. Recent work by Vance-Harris and Ingall (2005) suggest that denitrification rates in sandy continental shelf sediments, which comprise 70% of continental shelves, is two orders of magnitude less than in fine grained sediments where the vast majority denitrification work has been done to data. This raises the possibility that sedimentary denitrification has been over estimated. Nevertheless, even if we take a low estimate of the water-column denitrification, the isotopic constraints discussed above still put the total denitrification rate well over 200 Tg a^{-1}, far larger than can be explained by current nitrogen fixation rates, ~100 Tg a^{-1} (Capone *et al.*, 1997; Gruber and

Sarmiento, 1997). However, it is likely that the nitrogen fixation rate has been underestimated. Most nitrogen fixation rates are extrapolated from measurements on a single species, *Trichodesmium*, but it now appears that unicellular diazotrophs in the oligothropic Pacific (and presumably the Atlantic and Indian) Ocean are capable of high nitrogen fixation rates (Montoya *et al.*, 2004, Chapter 4 by Carpenter and Capone, this volume) and that rates of fixation by *Trichodesmium* may also be low (Capone *et al.*, 2005). Also, it may be that nitrogen fixation is more significant at higher latitudes than previously recognized (Sigman *et al.*, 2005) or that it occurs closely associated with denitrification zones, which are areas where N_2 fixation studies are scarce (Deutsch *et al.*, 2007; Capone and Knapp, 2007; see also Chapter 14 by Naqvi, this volume). Nevertheless, based on a global analysis of $N^\star$ in surface waters, Deutsch *et al.* (2007) argue that the total rate of nitrogen fixation can not be pushed beyond about 150 Tg a^{-1}.

This again brings us back to the issue of an unbalanced marine combined–nitrogen budget. Codispoti *et al.* (2001) suggest that perhaps the budget is out of balance because our measurements are being made during a period of rapid change as we enter the "anthropocene" (*sensu* Falkowski *et al.*, 2000). On the other hand, the current estimates of the major terms, denitrification and N_2 fixation, are continuing to evolve so the possibility of a balanced budget can not be ruled out. Possibly we will have the budget resolved by the time the third edition of *Nitrogen in the Marine Environment* is published.

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CHAPTER 7

Nitrogen Uptake and Assimilation

Margaret R. Mulholland *and* Michael W. Lomas

Contents

1. Introduction

Since the original volume of *Nitrogen in the Marine Environment* was published in 1983, our views of the marine nitrogen (N) cycle and the utilization of dissolved N by microorganisms have expanded substantially. In the earlier volume, N uptake was reviewed mainly in terms of the kinetics of ammonium (NH_4^+) and nitrate (NO_3^-) uptake and the linkages to internally versus externally controlled cellular growth rate by phytoplankton (Goldman and Glibert, 1983). At that time, these two compounds were thought to be the major nitrogenous nutrients support-ing oceanic primary production and so measuring their uptake, the relative affinity of phytoplankton for NH_4^+ and NO_3^-, and the applicability of the different models of marine phytoplankton growth were of primary concern. These efforts were limited by the inability to accurately determine low concentrations of these compounds in many oligotrophic oceanic environments and the inability to correctly predict growth rates from experiments that measured only instantaneous uptake rates. Since 1983, our ability to measure nanomolar concentrations of nitrogen has greatly

improved (e.g., NO_3^- – Garside, 1985; Raimbault *et al.*, 1990; Steimle *et al.*, 2002; Yao *et al.*, 1998; Zhang, 2000; NH_4^+ – Brzezinski, 1987). However, few researchers have employed these new methods to revisit old questions such as "what are the kinetic constants of open ocean phytoplankton" and "what are *in situ* nutrient uptake rates in the oligotrophic ocean". In general, those studies that have employed these new analytical techniques suggest their use has not significantly altered our perception of kinetic constants in the oligotrophic ocean (*cf.* Goldman and Glibert, 1983 and references therein; versus Garside and Glover, 1991; Harrison *et al.*, 1996; Lipschultz, 2001). In addition to NO_3^- and NH_4^+, we now recognize that organic compounds contribute substantially to primary productivity and methods for measuring dissolved organic nitrogen (DON) and specific components of the DON pool have improved dramatically since 1983 (see Chapter 3 by Aluwihare and Meador, this volume; Chapter 28 by Bronk and McCarthy, this volume).

It has also become increasingly clear that kinetic parameters are themselves physiological "variables." Kinetic parameters vary with physiological state, nutrient prehistory, and phytoplankton species, thus complicating the interpretation of field results, where the physiological state of collected organisms is generally unknown. In coastal areas, we have the additional limitation of being unable to evaluate *in situ* nutrient uptake kinetics because ambient nutrient concentrations are often greater than half-saturation constants, thereby making it impossible to measure non-saturated uptake rates and formulate kinetic parameters. Because of these limitations, measurements in the environment characterize transient responses/capacities in natural assemblages, where there are competitive interactions among multiple nutrient substrates and mixed microalgal populations (species and size). In any case, this may be more informative and relevant than understanding steady state Michaelis–Menten type kinetics in natural or culture systems.

In 1983, studies of N uptake and assimilation in marine systems were scarcer than today and were dominated by investigations of the relative contributions of NO_3^- and NH_4^+ (Table 7.1; and sometimes urea) to total N uptake. On the basis of the models of new and regenerated production at the time (Dugdale and Goering, 1967; Eppley and Peterson, 1979; Ryther and Dunstan, 1971), NO_3^- and NH_4^+ were thought to be the dominant N species influencing total phytoplankton production in the world ocean; NO_3^- was considered the primary source of nitrogen fueling "new" production and NH_4^+ was the dominant regenerated N source. New inputs of N through dinitrogen (N_2) fixation were thought to be trivial and DON was thought to be relatively unavailable to phytoplankton. Since this time, we have many more uptake measurements, not only for NO_3^- and NH_4^+ but also for a variety of other N substrates. In addition, our view of the N cycle has expanded; global estimates of new production from marine N_2 fixation have been revised upward (see Chapter 4 by Carpenter and Capone, this volume), and uptake of DON regenerated through a variety of processes by phytoplankton in the upper water column is now widely recognized (see also Chapter 8 by Bronk and Steinberg, this volume). Unfortunately, never have all things been measured everywhere at one time (see Table 7.1).

Further complications in interpreting uptake data in the context of "new production" arise when one considers that water column regeneration of NO_3^- (i.e.,

Table 7.1 Summary of selected nitrogen (NO_3^-, NO_2^-, NH_4^+, urea, dissolved free amino acids [DFAA], DON) uptake rates from the major ocean basins, and upwelling, coastal and estuarine systems using ^{15}N tracer techniques. Rates were converted to common units (nmol N L^{-1} h^{-1}) using data provided in the original publication or using conversion factors specified in the footnotes

Location	Season	Depth	Fraction	NO_3^- (nmol N$L^{-1}h^{-1}$)	NO_2^- (nmol N$L^{-1}h^{-1}$)	Uptake NH_4^+ (nmol N$L^{-1}h^{-1}$)	Urea (nmol N$L^{-1}h^{-1}$)	DFAA (nmol N$L^{-1}h^{-1}$)	DON (nmol N$L^{-1}h^{-1}$)	Reference
Oceanic Atlantic Ocean										
Subpolar NE nitrogen Atlantic	Summer	0–25 m; warm core eddy	WW	<2.1–29.1		0.42–10				Boyd et al., 1997
Subarctic nitrogen Atlantic	Summer	5 m	WW	5.8–16.9						Sambrotto et al., 1993
North Atlantic	Fall	Euphotic zone	WW	<0.1–2.5		0.2–4.0	0.1–2.5			Varela et al., 2005
NE N. Atlantic[a]	Summer	Euphotic zone	WW	0.2–9.6		1.2–12.0				Donald et al., 2001
NE N. Atlantic[b]	Spring	Euphotic zone	WW	3.0–16.2		2.2–5.2				Bury et al., 2001
NE N. Atlantic	Annual	0–20 m	WW	0.4–75.2		1.2–19.8				Elskens et al., 1997
NE N. Atlantic	Summer	Upper 30 m	WW	0.40–3.75		4.2–8.5				Rees et al., 1999
NW N. Atlantic (BATS)	Summer– oligotrophic	Euphotic zone	WW	<2		1–10				Lipschultz, 2001
NW N. Atlantic (BATS)	Winter–bloom	Euphotic zone	WW	15–170		<12				Lipschultz, 2001
NW N. Atlantic (BATS)	Spring and Summer	Euphotic zone	WW	0.2–5.3		0.4–5.3				Mulholland, unpublished data
NW N. Atlantic Bermuda	Fall/Winter	15 m	WW	0.4		5.7				Dugdale and Goering, 1967
NW N. Atlantic	Summer	Euphotic zone	WW	0.2–3.9		2.1–20.5				Harrison and Wood, 1988
NW N. Atlantic	Summer	Euphotic zone	<1µm	0–1.1		0–7.8				Harrison and Wood, 1988
Sargasso Sea	Summer–June/July	5% and 60% LD	WW	1.5–7.6		1.5–20.4				Glibert and McCarthy, 1984
Sargasso Sea	Spring–May/June	3, 22, and 60% LD	<153µm	0.2–0.4		1.2–3.5	0.4–7.8			Glibert et al., 1988
N Atlantic Subtropical Gyre	Spring	Euphotic zone	WW	0.1–2.5						Eppley and Koeve, 1990
N Atlantic Subtropical Gyre	Annual	Euphotic zone	WW	0–0.12		0.02–0.05				Rees et al., 2006
N Atlantic Subtropical Gyre	Mar–Apr	Euphotic zone	WW	0.09–1.57		0.15–2.9				Planas et al., 1999

Table 7.1 Summary of selected nitrogen (NO_3^-, NO_2^-, NH_4^+, urea, dissolved free amino acids [DFAA], DON) uptake rates from the major ocean basins, and upwelling, coastal and estuarine systems using ^{15}N tracer techniques. Rates were converted to common units (nmol N L^{-1} h^{-1}) using data provided in the original publication or using conversion factors specified in the footnotes (*continued*)

Location	Season	Depth	Fraction	NO_3^- (nmol N L^{-1} h^{-1})	NO_2^- (nmol N L^{-1} h^{-1})	Uptake NH_4^+ (nmol N L^{-1} h^{-1})	Urea (nmol N L^{-1} h^{-1})	DFAA (nmol N L^{-1} h^{-1})	DON (nmol N L^{-1} h^{-1})	Reference
N Atlantic Subtropical Gyre	Summer	Upper 30 m	WW	0.08–3.8		4–9				Rees *et al.*, 1999a
N Atlantic Subtropical Gyre	June	Euphotic zone	WW	0.45–1.0		1.5–2.6				Lewis *et al.*, 1986
N. Atlantic Subtropical Gyre[c]	Spring	Euphotic zone	WW	0–5.42						Eppley and Koeve, 1990
N Atlantic Subtropical Gyre	Summer	Euphotic zone	WW	1.2–2.8		3.4–5.9				Mulholland, unpublished data
NE Atlantic Subtropical Gyre–African Coast	Spring	Euphotic zone	WW	0.5–6.0						Eppley and Koeve, 1990
NE subtropical Atlantic	June	Euphotic zone	WW	0.5–1.4		1.5–2.6				Lewis *et al.*, 1986
NW Atlantic–Gulf Stream	Spring–May/June	3, 22, and 60% LD	<153 μm	<0.1–0.2		0.4–2.8	0.2–0.5			Glibert *et al.*, 1988
Tropical Western Atlantic	Summer–Aug	Euphotic zone	WW	0.1–51.1		0.4–8.1				Mulholland, unpublished data
Tropical and equatorial Atlantic[d]	Aug–Oct	Euphotic zone	<200 μm	3.8	2					Collos and Slawyk, 1983
Equatorial Atlantic	Boreal Fall	Euphotic zone	WW	3.5–7.0		0.5–1.2	0–12.5			Varela *et al.*, 2005
Equatorial Atlantic	Mar–Apr	Euphotic zone	WW	0.05–2.98		0.27–2.91				Planas *et al.*, 1999
Equatorial Atlantic	Annual	Euphotic zone	WW	1–21		2.5–27.5				Le Bouteiller, 1986
South Atlantic	Winter–March	50% LD	WW	0.1–140		0.5–28.1	0.2–5.2			Metzler *et al.* 1997
South Atlantic	Mar–Apr	Euphotic zone	WW	0.005–0.95		0.13–2.08				Planas *et al.*, 1999
South Atlantic	Spring	Euphotic zone	WW	<0.5–5.5		0.2–1.0	<0.5–12.0			Varela *et al.*, 2005
South Atlantic – Scotic Sea	Summer	60% LD	WW			2.7–20.4				Glibert *et al.*, 1982a

Pacific Ocean

Subarctic Pacific	May–Sept	Euphotic zone	WW	0.6–3.8			Wheeler, 1993
NE Pacific (includes Ocean Station P)[c]	Winter–Feb/Mar	Euphotic zone	WW	0.32–1.38	0.91–2.39	0.17–1.04	Varela and Harrison, 1999b
NE Pacific (includes Ocean Station P)[c]	Spring–May	Euphotic zone	WW	1.20–2.95	2.57–12.3	0.56–8.49	Varela and Harrison, 1999b
NE Pacific (includes Ocean Station P)[c]	Summer–Sept	Euphotic zone	WW	0.14–2.70	1.28–4.46	0.22–2.02	Varela and Harrison, 1999b
Subarctic Pacific (near Station P)	May–Oct	Surface	WW	1.2–6.7	1.3–14.6	0.5–9.2	Wheeler and Kokkinakis, 1990
Subarctic Pacific (near Station P)[d]	May & Aug	Euphotic zone	WW	70–452	77–322		Cochlan et al., 1991a
NW Subarctic Pacific	Summer	1 m	WW	6.3–23.2	10.6–37.5		Shiomoto and Maita, 1990
NW Pacific[e]	Spring–May	Surface	WW	3.94–13.9	11.0–20.5		Shiomoto et al., 1994
NW Pacific[e]	Fall–Sept	Surface	WW	8.7–16.3	19.3–38.3		Shiomoto et al., 1994
SubArctic Pacific	Summer	10 m	WW	2.7–21.7	5.6–16.6		Wheeler et al., 1989
Oligotrophic NE Pacific–VERTEX	Annual	Euphotic zone	WW	0.06–0.22	0.74–3.64		Harrison et al., 1992
Central North Pacific	Winter–Summer	Euphotic zone	WW	0.01–0.18	0.05–0.79	0.04–0.92	Eppley et al., 1977
N Pacific[e]	Winter	Surface	WW	0.59–1.44	1.84–5.82	0.42–1.05	Kanda et al., 1985
Eastern Tropical North Pacific–eutrophic[f]	Winter	Euphotic zone	WW	5.19–51.3	231–313		MacIsaac and Dugdale, 1972
Tropical nitrogen Pacific[e]	Winter	Surface	WW	0.32–1.08	1.62–6.47	0.88–4.00	Kanda et al., 1985
Subtropical nitrogen Pacific[e]	Winter	Surface	WW	0.23–1.24	1.44–3.67	0.89–1.80	Kanda et al., 1985
N Central Pacific gyre	Summer	15–25 m	WW	0.7–1.9	3.3–6.5	1.6–3.3	Sahlsten, 1987
NW Pacific gyre	Winter–Summer	Euphotic zone	WW	0.01–0.2	0.04–0.8	0.04–0.9	Eppley et al., 1977

(Continued)

Table 7.1 Summary of selected nitrogen (NO_3^-, NO_2^-, NH_4^+, urea, dissolved free amino acids [DFAA], DON) uptake rates from the major ocean basins, and upwelling, coastal and estuarine systems using ^{15}N tracer techniques. Rates were converted to common units (nmol N L^{-1} h^{-1}) using data provided in the original publication or using conversion factors specified in the footnotes (*continued*)

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N equatorial Pacific[c]	April	Euphotic zone	WW	0.32–0.72		0.79–23.1				Peña *et al.*, 1992
Equatorial Pacific[c]	April	Euphotic zone	WW	0.59–2.10		0.21–13.78				Peña *et al.*, 1992
S equatorial Pacific[c]	April	Euphotic zone	WW	0.15–0.26		0.65–7.93				Peña *et al.*, 1992
Tropical Pacific[g]	Aug–Sept	Euphotic zone	WW	0.21–0.94	0.09–0.42	0.6–13.2				McCarthy *et al.*, 1996
Tropical Pacific[g]	Feb–Mar	Euphotic zone	WW	0.42–0.78	0.04–0.45	1.9–4.2				McCarthy *et al.*, 1996
Equatorial Pacific (3°N–3°S)[g]	Aug–Sept	Euphotic zone	WW	1.25–5.0	0.16–0.84	10.8–14.8				McCarthy *et al.*, 1996
Equatorial Pacific (3°N–3°S)[g]	Feb–Mar	Euphotic zone	WW	0.47–1.25	0.06–0.24	3.5–5.2				McCarthy *et al.*, 1996
Equatorial Pacific – Equator[h]	Summer–Nov	Euphotic zone	WW	1.5–2.7		5.3–10.4				Raimbault *et al.*, 1999
Equatorial S Pacific – Mesotrophic[h]	Summer–Nov	Euphotic zone	WW	0.9–1.6		4.9–6.1				Raimbault *et al.*, 1999
N Tropical Pacific	Winter	Euphotic zone	WW	0.04–0.26		0.6–3.4				Wilkerson and Dugdale, 1992
Equatorial Pacific	Winter	Euphotic zone	WW	0.16–2.4		0.6–6.9				Wilkerson and Dugdale, 1992
S Tropical Pacific	Winter	Euphotic zone	WW	0.18–0.33		2.0–7.6				Wilkerson and Dugdale, 1992
Eastern Tropical Pacific–Costa Rica	Summer	5–15 m	WW	17.5 (2.1)						Franck *et al.*, 2005
Eastern Tropical Pacific–Humboldt	Summer	5–15 m	WW	7.5 (0)						Franck *et al.*, 2005
Eastern Tropical Pacific – Peru	Summer	5–15 m	WW	12.5 (1.3)						Franck *et al.*, 2005
Eastern Tropical North Pacific– eutrophic[f]	Winter	Euphotic zone	WW	117–3332		133–1481				MacIsaac and Dugdale, 1972
	Winter	Euphotic zone	WW	0–33.6		9.9–34.4				MacIsaac and Dugdale, 1972

Eastern Tropical North Pacific–oligotrophic[f]								
Equatorial S Pacific – Oligotrophic[h]	Summer–Nov	Euphotic zone	WW	0.4–1.0		2.9–5.3		Raimbault et al., 1999
Tropical S Pacific	Summer–Nov	Euphotic zone	WW	0–6.25				Raimbault et al., 2000
Eastern Equatorial Pacific	June	Euphotic zone	WW	0.2–6.5		0.2–9.6		Murray et al., 1989
Eastern Subtropical Pacific[i]	Spring–Apr	Euphotic zone	WW	0–0.006		0.001–0.010		Goering et al., 1970
Indian Ocean								
Arabian Sea	SW monsoon	Euphotic zone	WW	0–90		0–25	0–13	Sambrotto, 2001
Arabian Sea	Spring intermonsoon	Euphotic zone	WW	0–10		0–100	0–20	Sambrotto, 2001
Arabian Sea	Not stated	15 or 25 m	WW			6–167		Dugdale and Goering, 1967
Western Indian Ocean	Spring intermonsoon	10 m	WW	12.8–90.0	10.0–65.0	42.3–373.3		Mengesha et al., 1999
Western Indian Ocean	SW monsoon	10 m	WW	0.1–4.9		56.3–1083		Mengesha et al., 1999
Western Indian Ocean–oligotrophic[b]	Fall intermonsoon	Euphotic zone	WW	1.7–4.5		12.1–33.3		Owens et al., 1993
Western Indian Ocean–upwelling[b]	Fall intermonsoon	Euphotic zone	WW	12.6–185.3		16.1–102.2		Owens et al., 1993
Western Indian Ocean[b]	NE monsoon–early	Euphotic zone	WW	0.2–4.3		0.1–9.8	0–3.0	Watts and Owens, 1999
Arabian Sea[j]	NE monsoon–late	Euphotic zone	WW	0.7–8.3	0.25–4.4	4.8–33.2		McCarthy et al., 1999
Arabian Sea[j]	NE monsoon–early	Euphotic zone	WW	0.4–4.8	0.06–2.6	2.8–17.9		McCarthy et al., 1999
Arctic Ocean								
Barents Sea	Summer	0.25–15 m	WW	0.2–2.0		8.0–13.6		Kristiansen et al., 1994
Barrow Strait	Spring	Ice algae	WW	870–59,450		2,640–36,150	2,000–5,370	Harrison et al., 1990a,b
Canada Basin[k]	Summer	1 m	WW	0.072 (0.072)		0.138 (0.138)		Lee and Whitledge, 2005
Canada Basin[k]	Summer		WW	1.03 (1.1)		4.9 (3.6)		Lee and Whitledge, 2005

(Continued)

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Location	Season	Depth	Fraction	NO$_3^-$ (nmol N L^{-1}h^{-1})	NO$_2^-$ (nmol N L^{-1}h^{-1})	Uptake NH$_4^+$ (nmol N L^{-1}h^{-1})	Urea (nmol N L^{-1}h^{-1})	DFAA (nmol N L^{-1}h^{-1})	DON (nmol N L^{-1}h^{-1})	Reference
		Deep chlorophyll maximum								
Canada Basin[k]	Summer	Ice algae	WW	0.021 (0.015)		0.055 (0.020)				Lee and Whitledge, 2005
Northeast Water Polynya[k]	Summer	Euphotic zone	WW	1.6–13		0.8–2.5				Smith, 1995
Northeast Water Polynya[k]	Spring and Summer	Surface	WW	2.6 (2.5)– 5.6 (12)		1.8 (2.1)– 2.7 (2.4)	0.7 (2.1)– 2.1 (3.9)			Smith et al., 1997
North Water Polyna	Summer/Fall–Sept	Surface	WW	0.6–13.6		0.6–17.8	0.2–3.7			Fouilland et al., in press
Barents Sea	Winter	0.25–15 m	WW	0.7 (0.5)		0.2 ± 0.1	<0.1			Kristiansen et al., 1994
Barents Sea	Spring	0.25–15 m	WW	0.3–10.2		3.8–15.3	0.7–3.8			Kristiansen et al., 1994
Greenland Sea[l]	Spring	Euphotic zone	WW	2.5–152.2		3.2–42.9				Smith, 1993
Southern Ocean										
Antarctic Polar Front[m]	Summer–Dec avg	Euphotic zone	WW	2.5–9		18.3–29.1	0–6.9			Sambrotto and Mace, 2000
Antarctic Polar Front[m]	Summer–Feb avg	Euphotic zone	WW	1.5–2		5.2–18.3	0.9–2.2			Sambrotto and Mace, 2000
Scotia Sea	Summer/Fall	Euphotic zone	WW	0.2–5.7		0.1–26.6				Glibert et al., 1982
Scotia/Weddell Sea	Summer	Euphotic zone	WW	0.8–20.1		1.2–6.1				Goeyens et al., 1991
Scotia/Weddell Sea[a]	Winter	Euphotic zone	WW	0.69–10.1		0.3–2.6				Cota et al., 1992
N of Antarctic Peninsula	Spring	2 m	WW	3.2–3.9		0.1–5.5	0.4–59.0			Bury et al., 1995
N of Antarctic Peninsula	Spring	Under ice	WW	0.1		1.2	1.3			Bury et al. 1995
N of Antarctic Peninsula	Spring	Ice	Ice	5.1–47.2		0.4–31.2	0.5–1.0			Bury et al., 1995
Atlantic Sector	Fall	1 m	<200 μm	88–365		64–192	22–277			Probyn and Painting, 1985
Atlantic Sector	Fall	1 m	<15 μm	56–194		46–133	16–153			Probyn and Painting, 1985
Atlantic Sector	Fall	1 m	<1 μm	0–124		21–85	12–129			Probyn and Painting, 1985
Atlantic Sector	Summer	Surface	WW	0.95–38.3		0.38–5.0				Semeneh et al., 1998
Pacific Sector–Ross Sea	Summer	25 m	WW	5–30		3–5				Cochlan et al., 2002

Indian Sector	Spring	Surface	WW	1.3–4.3	0.4–1.5			Mengesha et al., 1998
Indian Sector[g]	Fall–Mar	Euphotic zone	WW	0.8–6.3				Collos and Slawyk, 1986
Indian Sector	Summer	Surface	WW	0.21–0.92	0.17–1.4			Mengesha et al., 1998
Indian Sector	Summer	Surface	WW	0.95–2.6	0.47–3.6			Semeneh et al., 1998
Pacific Sector[k]	Spring	Euphotic zone	WW	0.9–7.6	1.3–2.7	0.2–1.8		Savoye et al., 2004
Bellingshausen Sea	Spring	Euphotic zone	<200 μm	0.3–60.3	1.2–17.0	0.2–9.9		Waldron et al., 1995
Bellingshausen Sea	Spring	1 m	20–200 μm	1.3–58.7	0.2–4.8	0–4.3		Waldron et al., 1995
Bellingshausen Sea	Spring	1 m	2–20 μm	0–2.5	0.3–2.0	0.2–1.9		Waldron et al., 1995
Bellingshausen Sea	Spring	1 m	<2 μm	0–1.6	0.1–0.4	0.04–0.5		Waldron et al., 1995
Other basins								
Gulf of Mexico	Summer/Fall	Euphotic zone	WW	1.3–246	6.0–190	3.9–1950	1.1–246	Mulholland et al., 2006
South China Sea	March	Euphotic zone	WW	0.43–21.5				Chen et al., 2004
East China Sea–Coastal[b]	Spring	Euphotic zone	WW	22.1				Chen and Chen, 2003
East China Sea[b]	Spring	Euphotic zone	WW	0.2–4.0				Chen and Chen, 2003
East China Sea[b]	Winter	Euphotic zone	WW	0.4–4.9				Chen et al., 2001
East China Sea[b]	Summer	Euphotic zone	WW	1.2–122.9				Chen et al., 2001
East China Sea	Spring	Euphotic zone	WW	0–43				Chen et al., 1999
East China Sea	Summer	Euphotic zone	WW	0–67				Chen et al., 1999
East China Sea	Fall	Euphotic zone	WW	0–21				Chen et al., 1999
East China Sea–Coastal/frontal	May	Euphotic zone	WW	3.3–36.5				Hung et al., 2000
East China Sea–Upwelling	May	Euphotic zone	WW	13.5				Hung et al., 2000
East China Sea–Kuroshio	May	Euphotic zone	WW	1.4				Hung et al., 2000
E Mediterranean Sea–eutrophic[f]	Spring–Mar	Euphotic zone	WW	0–5.95	44.4–83.3			MacIsaac and Dugdale, 1972
E Mediterranean Sea–oligotrophic[f]	Spring–Mar	Euphotic zone	WW	1.2–8.5	2.6–21.6			MacIsaac and Dugdale, 1972
NW Mediterranean Sea–coastal	Winter/Spring	Euphotic zone	WW	0.8–25.8				Diaz et al., 2001
NW Mediterranean Sea–coastal	Spring–Mar to May	Euphotic zone	WW	0–3.3	0.2–9.2			Diaz et al., 2000

(*Continued*)

Table 7.1 Summary of selected nitrogen (NO_3^-, NO_2^-, NH_4^+, urea, dissolved free amino acids [DFAA], DON) uptake rates from the major ocean basins, and upwelling, coastal and estuarine systems using ^{15}N tracer techniques. Rates were converted to common units (nmol N L^{-1} h^{-1}) using data provided in the original publication or using conversion factors specified in the footnotes (*continued*)

Location	Season	Depth	Fraction	NO_3^- (nmol N $L^{-1}h^{-1}$)	NO_2^- (nmol N $L^{-1}h^{-1}$)	Uptake NH_4^+ (nmol N $L^{-1}h^{-1}$)	Urea (nmol N $L^{-1}h^{-1}$)	DFAA (nmol N $L^{-1}h^{-1}$)	DON (nmol N $L^{-1}h^{-1}$)	Reference
NW Mediterranean Sea	Fall	5 m	WW	0.8		1.5–1.7				Selmer *et al.*, 1993
NW Mediterranean Sea	Spring	5 m	WW	0.02–0.3		1.2–9.5				Selmer *et al.*, 1993
SW Mediterranean Sea–integrated	Spring	Euphotic zone	WW	0.8–2.4		4.6–7.5				L'Helguen *et al.*, 2002
SW Mediterranean Sea–profiles	Spring	Euphotic zone	WW	0–6.5		0–18				L'Helguen *et al.*, 2002
Upwelling										
NW Iberian upwelling	Summer	Euphotic zone	WW	0.3–0.8		0.4–1.1	4.0–7.9			Joint *et al.*, 2001
NW Africa upwelling	Fall	Euphotic zone	WW	<0.2–30		<0.2–1.0	2.0–11.0			Varela *et al.*, 2005
NW Africa upwelling–Cape Blanc[n]	not specified	50% and 100% LD	WW	18.8–31.2						Dugdale *et al.*, 1990
Namibian upwelling	Spring	Euphotic zone	WW	1.3–29.8		3.7–39.4	1.4–9.3			Probyn, 1988
Benguela upwelling	Annual	Euphotic zone	WW	0–4000		50–1000				Rees *et al.*, 2006
Benguela upwelling	Summer	Euphotic zone	WW	4–273		36–168				Probyn *et al.*, 1996
Benguela upwelling	Summer	Sub-photic	WW	1–95		5–151				Probyn *et al.*, 1996
Benguela upwelling	Summer	1% LD	20–200 μm	0–12.2		0–55.8	0–19.3			Probyn *et al.*, 1990
Benguela upwelling	Summer	1% LD	2–20 μm	0–4.2		0–39.4	0.5–11.5			Probyn *et al.*, 1990
Benguela upwelling	Summer	1% LD	<2 μm	0–3.5		2.1–25.6	0–6.7			Probyn *et al.*, 1990
Benguela upwelling	Summer–Dec	Surface	<212 μm	0–436		18–114	0–79			Probyn, 1985
Benguela upwelling	Summer–Dec	Surface	<10 μm	0–153		8.3–67	0–28			Probyn, 1985
Benguela upwelling	Summer–Dec	Surface	<1μm	0–16.2		3.8–22	0–11			Probyn, 1985
Peru upwelling[f]	Fall–Mar to May	50% LD	WW	15–1045		0–50				MacIsaac *et al.*, 1985
Peru upwelling[n]	Not specified	50% and 100% LD	WW	22.4–197.2						Dugdale *et al.*, 1990
North Sea	Spring	10 m	WW	0.8–20.4		0.14–2.6	<0.07–1.1			Riegman *et al.*, 1998
North Sea	Summer	10 and 40 m	WW	0.5–6.4		1.2–39.9	0.2–22.1			

							Riegman and Noordeloos, 1998
North Sea	Mar–June	10 and 40 m	WW	0–200	2–70	0–19	Tungaraza et al., 2003b
Westland, New Zealand	Winter–July	upper 10 m	<200 μm	0.3–12.8	0.1–3.4	0.1–1.0	Chang et al., 1992
Westland, New Zealand	Winter–July	upper 10 m	<20 μm	0.02–6.4	0.05–2.7	0.1–0.5	Chang et al., 1992
Westland, New Zealand	Winter–July	upper 10 m	<2 μm	0.1–4.2	0.04–1.3	0.01–0.25	Chang et al., 1992
Westland, New Zealand	Summer–Feb	10 m	<200 μm	0.9–13.3	1.9–4.4	0.6–13.2	Chang et al., 1995
Westland, New Zealand	Summer–Feb	10 m	<20 μm	0.8–10.3	0.9–4.1	0.6–9.9	Chang et al., 1995
Westland, New Zealand	Summer–Feb	10 m	<2 μm	0.5–3.8	0.3–4.0	0.4–2.6	Chang et al., 1995
Oregon Coast	Summer–low NO_3^-	15 m	WW	4–151	13–95	2–69	Kokkinakis and Wheeler, 1987
Oregon Coast	Summer–high NO_4^-	15 m	WW	245–1250	26–232	9–59	Kokkinakis and Wheeler, 1987
Oregon Coast	Summer	10–15 m	10–200 μm		0–125	0–42	Kokkinakis and Wheeler, 1988
Oregon Coast	Summer	10–15 m	<10 μm		11–47	2–30	Kokkinakis and Wheeler, 1988
Oregon Coast	Upwelling	Euphotic zone	WW	24–96			Dickson and Wheeler, 1995
Oregon Coast	Bloom	Euphotic zone	WW	253–1292			Dickson and Wheeler, 1995
Oregon Coast	Post-bloom and winter	Euphotic Zone	WW	10–83			Dickson and Wheeler, 1995
California Coast[n]	Not specified	50% and 100% LD	WW	7.5–19.9			Dugdale et al., 1990
Cape Sines upwelling	Fall	Euphotic zone	WW	0–19.0	0–9.0		Slawyk et al., 1997
Westland, New Zealand	Winter	Euphotic zone	<200 μm	0.57–1.1	0.4–10.3	0.25–0.62	Chang et al., 1992
Westland, New Zealand	Winter	Euphotic zone	<20 μm	0.26–1.7	0.43–3.6	0.17–0.35	Chang et al., 1992

(Continued)

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Location	Season	Depth	Fraction	NO_3^- (nmol N L^{-1} h^{-1})	NO_2^- (nmol N L^{-1} h^{-1})	Uptake NH_4^+ (nmol N L^{-1} h^{-1})	Urea (nmol N L^{-1} h^{-1})	DFAA (nmol N L^{-1} h^{-1})	DON (nmol N L^{-1} h^{-1})	Reference
Westland, New Zealand	Winter	Euphotic zone	<2 μm	0.08–0.5		0.13–1.6	0.07–0.13			Chang *et al.*, 1992
Coastal										
Coastal NE Atlantic– Galacia, Spain	Annual[o]	Euphotic zone[e]	WW			0–>650				Bode *et al.*, 2004
Coastal S Atlantic– Brazil	Spring	Euphotic zone	WW	1.5–2.5		<0.5–1.5	2.5–12.0			Varela *et al.*, 2005
Coastal S Atlantic– Brazil	Winter–March	50% LD	WW	0.8–156		18.1–806	4.5–123			Metzler *et al.*, 1997
Oslofjord, Norway	Annual	1 m	WW	0–3710		20–5600				Paasche and Kristiansen, 1982
Oslofjord, Norway	Annual	1 m	WW				0–3250			Kristiansen, 1983
Norwegian Fjords	Spring	upper 15 m	WW	0.7–40.7		6.4–75	2.1–35			Fernandez *et al.*, 1996
Skagerrak, Sweden	Annual	50% LD	WW	0.8–99.2		1.7–54.2	0.6–16.7			Pettersson, 1991
Skagerrak, Sweden	Spring	Above, in and below pycnocline	WW	0.7–11.7	0.25	2.5–26.7	0.9–17.5			Pettersson and Sahlsten, 1990
Skagerrak, Sweden– coastal	Spring	Above, in and below pycnocline	WW	3.6–21.4		1.2–9.5	3.0–10.7			Rosenberg *et al.*, 1990
Skagerrak, Sweden– open	Spring	Above, in and below pycnocline	WW	4.9–10.4		2.1–3.1	4.4–9.0			Rosenberg *et al.*, 1990
Western English Channel	Annual	50% LD	<200 μm	1–48	0–14	2–40	2–15			L'Helguen *et al.*, 1996
Humber Estuary Plume, England	Spring/Summer	5 m	WW	0–216		16–59	5–40			Shaw *et al.*, 1998
Humber Estuary Plume, England	Spring/Summer	5 m	WW	0–63.4		14.6–46.3	6.1–22			Shaw *et al.*, 1998
Menai Strait, Wales	Spring	1 m	WW	25–800		0–450				

Location	Season	Depth	Fraction							Reference
										Rodriques and Williams, 2002
Carmarthen Bay, Wales[b]	Spring	Euphotic zone	WW	13.0–233		3.9–76.7				Owens et al., 1986
Carmarthen Bay, Wales[b]	Summer	Euphotic zone	WW	3.0–11.2		9.0–27.4				Owens et al., 1986
Carmarthen Bay, Wales[b]	Fall	Euphotic zone	WW	12.8		0.3				Owens et al., 1986
Carmarthen Bay, Wales[b]	Winter	Euphotic zone	WW	4.4–9.2		1.5–3.4				Owens et al., 1986
Celtic Sea Shelf Edge[b]	Spring	Euphotic zone	WW	5.7–33.7		3.6–11.6				Rees et al., 1999b
Amorican Shelf, Belgium	Summer	Euphotic zone	<200 μm	2.1–26.3		3.2–38.1				Maguer et al., 2000
Belgian Coastal waters	Feb–June	Surface	WW	<2–127		3.2–19.2	0.2–17			Tungaraza et al., 2003a
Baltic Sea–Gulf of Riga	Spring–May	Upper mixed layer	WW	100–160		63–300	27–42	50–90		Berg et al., 2001
Baltic Sea–Gulf of Riga	Summer–July	Upper mixed layer	WW	25–60		300–350	40–220	25–60		Berg et al., 2001
Baltic Sea–Gulf of Riga[p]	Spring–May	Surface	<200 μm	2.9–110.5		4.3–97.2	1.7–22.1	1.4–33.1	0.7–3.8[q]	Berg et al., 2003a
Baltic Sea–Gulf of Riga[p]	Spring–June	Surface	<200 μm	0.2–24.2		0.8–11.4	0.15–4.2	0.2–2.3	0.2–1.7[q]	Berg et al., 2003a
Baltic Sea–Gulf of Riga[p]	Spring–June	Surface	<200 μm	0.2–24.2		0.8–11.4	0.15–4.2	0.2–2.3	0.2–1.7[q]	Berg et al., 2003a
Baltic Sea–Gulf of Riga[p]	Summer–July	Surface	<200 μm	1.9–17.0		13.3–88.1	4.7–51.6	1.3–13.8	0.9–8.3[q]	Berg et al., 2003a
SW Finland[n]	Summer	3–4 m	WW				37–94			Tamminen and Irmisch, 1996
Gulf of St. Lawrence[m]	Annual[o]	Euphotic zone	WW	~0.8–34.3		1.3–22.3	~0.8–5.7			Tremblay et al., 2000
Gulf of Maine, USA	Summer	Euphotic zone	WW	1.1–295.2		0.5–64.1				Harrison and Wood, 1988
Gulf of Maine, USA	Summer	Euphotic zone	<1 μm	0.4–76.7		0.2–21.1				Harrison and Wood, 1988
Gulf of Maine, USA	Spring	25 m	WW	150–350		200–440				Dugdale and Goering, 1967
Chesapeake Bay plume, USA	Winter	Surface	WW	2.5–26	1.5–11.5	5–84	8–72	1–4		Glibert et al., 1991; Glibert and Garside, 1992

(Continued)

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Location	Season	Depth	Fraction	NO_3^- (nmol N $L^{-1}h^{-1}$)	NO_2^- (nmol N $L^{-1}h^{-1}$)	Uptake NH_4^+ (nmol N $L^{-1}h^{-1}$)	Urea (nmol N $L^{-1}h^{-1}$)	DFAA (nmol N $L^{-1}h^{-1}$)	DON (nmol N $L^{-1}h^{-1}$)	Reference
Chesapeake Bay plume, USA	Spring	Surface	WW	100–480	30–140	50–850	5–140	3–23		Glibert *et al.*, 1991; Glibert and Garside, 1992
Chesapeake Bay plume, USA	Summer	Surface	WW	7–115	12–30	25–510	35–650	5–90		Glibert *et al.*, 1991; Glibert and Garside, 1992
Chesapeake Bay plume, USA	Spring	Surface	WW	0–120	0–71	3.2–341	0–267	0–111		Filippino *et al.*, in revision
Chesapeake Bay plume, USA	Summer	Surface	WW	4.9–190	3.2–224	8.5–748	2.3–179	3.2–126		Filippino *et al.*, in revision
Chesapeake Bay plume, USA	Fall	Surface	WW	68–156	118–149	133–211	24.1–54.5	9.7–13.0		Filippino *et al.*, in revision
Mississippi River plume, USA	Annual	Surface	<200 μm	0–3917		8–4444				Bode and Dortch, 1996
Mid-Atlantic shelf, USA	Spring	Surface	WW	0.01–29.1	0.04–19.5	3.22–125	0.04–25.5	0.04–30.6		Filippino *et al.*, submitted
Mid-Atlantic shelf, USA	Fall	Surface	WW	4.2–36.2	6.8–14.0	10.5–134.3	6.0–21.7	1.6–5.9		Filippino *et al.*, submitted
Mid-Atlantic shelf, USA	Summer	Surface	WW	4.9–79.1	3.2–24.6	8.53–317	2.3–248	3.1–60		Filippino *et al.*, submitted
Norfolk Canyon, USA	Spring	Euphotic zone	WW	1.6–16.2		0.4–5.3				Mulholland, unpublished data
Norfolk Canyon, USA	Summer	Euphotic zone	WW	5.6–42.5		0.21–8.3				Mulholland, unpublished data
Strait of Georgia, Canada	Summer	Upper 15 m	WW				7.4–25.6			Cochlan *et al.*, 1991
Strait of Georgia, Canada	Summer	50% LD–stratified	WW	35–45		60–105	40–60			Price *et al.*, 1985
Strait of Georgia, Canada	Summer	50% LD–frontal	WW	300–410		200–230	100–150			Price *et al.*, 1985
Monterey Bay, USA	Spring–Apr	5–15 m	WW	47.9–141.7						Franck *et al.*, 2005

Location	Season	Depth	Filter						Reference
Monterey Bay, USA	Fall	Euphotic zone	WW	3–40		17–46			Bronk and Ward, 1999
Monterey Bay, USA	Spring	Euphotic zone	WW	<5–75		<25–480			Bronk and Ward, 1999
Monterey Bay, USA	Spring	Surface–5 m	WW	1150–3400					Franck et al., 2005
Monterey Bay	Spring	Euphotic zone	WW	0–10		0–200			Bronk and Ward, 1999
S California Bight, USA–offshore	Spring–Apr	Euphotic zone	WW	2–42		4–34			Bronk and Ward, 2005
S California Bight, USA–nearshore	Fall–Oct	Euphotic zone	WW	1–5		25–160			Bronk and Ward, 2005
S California Bight, USA–offshore	Fall–Oct	Euphotic zone	WW	3–30		10–54			Bronk and Ward, 2005
Southern California Bight[g]	Summer	Euphotic zone	<183 µm	0.2–43.8		0.1–12.8	0–18.8		McCarthy, 1972
Kahe Point, Hawaii	Summer	15 to 25 m	WW			4.65			Sahlsten, 1987
Menai Strait	Summer	1 m	WW	<20		25–700			Rodriques and Williams, 2002
Menai Strait	Summer	1 m	WW	<20		25–700			Rodriques and Williams, 2002
Eastern Agulhas Bank	Summer–Jan	Euphotic zone	WW	0.3–16.4		2.9–30.8	3.2–11.1		Probyn et al., 1995
Westland, New Zealand	Winter	10 m	<200 µm	4.9–13.5		1.0–4.2	1.8–6.5		Chang et al., 1989
Westland, New Zealand	Winter	10 m	<20 µm	2.1–8.0		0.6–3.1	1.1–5.6		Chang et al., 1989
Westland, New Zealand	Winter	10 m	< 2 µm	0.9–6.3		0.2–2.1	0.7–3.1		Chang et al., 1989
Gulf of Carpentaria	Annual	Euphotic zone	WW	1.3–2.8					Burford and Rothlisberg, 1999
Moreton Bay, Australia	Summer	1 m	WW	50–200		600–800			O'Donohue et al., 2000
Estuarine									
Vineyard Sound, MA, USA	Annual	1 m	<202 µm	0–100		<10–180			Glibert et al. 1982
Bellport Bay (Great South Bay), NY, USA	Annual	Surface	WW	~20–750		~100–1800	~20–270		Carpenter and Dunham, 1985
Long Island Sound, NY, USA	Spring	Surface	WW	5.3–19.8	0–0.7	32.7–339	0.3–15.7	0.4–5.7	Mulholland et al., unpublished data

(*Continued*)

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Location	Season	Depth	Fraction	NO_3^- (nmol N$L^{-1}h^{-1}$)	NO_2^- (nmol N$L^{-1}h^{-1}$)	Uptake NH_4^+ (nmol N$L^{-1}h^{-1}$)	Urea (nmol N$L^{-1}h^{-1}$)	DFAA (nmol N$L^{-1}h^{-1}$)	DON (nmol N$L^{-1}h^{-1}$)	Reference
Great South Bay, Long Island Sound, USA	Annual	Surface	WW	0–160		<50–1400	<50–900			Kaufman *et al.*, 1983
Great South Bay, Long Island Sound, USA	Annual	Surface	WW	20–750		100–1800	20–270			Carpenter and Dunham, 1985
Delaware River estuary, USA	Summer	Surface	WW	0—127	0–10	100–1600				Lipshultz *et al.*, 1986
Chincoteague Bay, VA, USA	Apr–Sept	Surface	WW	0–45		81–780	70–4690	34–290		Boneillo and Mulholland, unpublished data
Hog Island Bay, VA, USA	Spring	Surface	WW			17.4–168	2.0–36.9	0–156		Mulholland, unpublished data
Neuse River Estuary, USA	Annual	Surface	WW	0–4500		200–5800	<100–3750			Twomey *et al.*, 2005
North Carolina estuaries	Annual	Surface	WW			0.2–530				Fisher *et al.*, 1982
Choptank River (Ches. Bay), USA	Summer	1 m	<202 μm			600–1000				Bronk and Glibert, 1993
Choptank River (Ches. Bay), USA	Summer	1 m	<1.2 μm			100–300				Bronk and Glibert, 1993
Lafayette River (Ches. Bay), USA	Summer	Surface	WW	0–162	0–930	20–590	0–200			Morse *et al.*, unpublished data
Lafayette River (Ches. Bay), USA	Spring	Surface	WW	90–140	40–100	440–1000	90–120			Morse *et al.*, unpublished data
York River (Ches. Bay), USA	Spring	Surface	WW	91–424		75–1063	221–7662	36–303		Mulholland and Watson, unpublished data
York River (Ches. Bay), USA	Summer	Surface	WW	169–1221		119–3257	202–3652	3.9–343		Mulholland and Watson, unpublished data
Chesapeake Bay, mesohaline, USA	Spring	Surface	WW	90–260		40–440	130–360			Bronk *et al.*, 1998

Location	Season	Depth								Reference
Chesapeake Bay, mesohaline, USA	Summer	Surface	WW	0–220		90–1290	90–580			Bronk et al., 1998
Chesapeake Bay, mesohaline, USA	Fall	Surface	WW	9.3–34	3–12.5	33–82	3–17.2			Bronk et al., 1998
Chesapeake Bay, mesohaline, USA[r]	May	Surface	WW	90–360		90–340			310–540	Bronk and Glibert, 1993
Chesapeake Bay, mesohaline, USA[r]	August	Surface	WW	12–70	20–260	90–510	60–260		140–510	Bronk and Glibert, 1993
Chesapeake Bay, USA	Winter	Surface	WW	3–26	1.5–11.5	5–85	7–70			Glibert et al., 1992
Chesapeake Bay, USA	Spring	Surface	WW	5–480	12–140	40–850	8–550			Glibert et al., 1992
Chesapeake Bay, USA	Summer	Surface	WW	25–700	12–18	25–500	35–660			Glibert et al., 1992
Chesapeake Bay, USA	Spring	1 m	WW	81–525	21–128	90–400	63–105			Glibert et al., 1995
Chesapeake Bay, USA	Spring	Surface	WW			153–1165				Glibert et al., 1982
Chesapeake Bay, USA	Summer	Surface	WW	8–166	46–816	8–743				Horrigan et al., 1990
San Francisco Bay System	Summer/Fall	1 m	WW	4.7–13.1		24.6–70.1				Wilkerson et al., 2006
San Francisco Bay System	Summer/Fall	1 m	>5 µm	2.2–9.6		16.4–44.0				Wilkerson et al., 2006
San Francisco Bay System, USA	Winter/Spring	1 m	WW	2.0–59.3		11.1–75.6				Wilkerson et al., 2006
San Francisco Bay System, USA	Winter/Spring	1 m	>5 µm	0.5–57.9		4.7–61.1				Wilkerson et al., 2006
Australian Rivers–Moreton Bay, Australia	Summer	1 m	WW	50–600		400–1000				O'Donohue et al., 2000
Randers Fjord, Denmark	April	Surface	WW	1–43		10–150	4–43	1–12	<5–60	Veuger et al., 2004
Randers Fjord, Denmark	August	Surface	WW	0–175		15–275	2–130	4–180	130–430	Veuger et al., 2004
Scheldt estuary, Netherlands[s]	Annual[o]	2 m	WW	6–188	1.0–113	1.3–1283	0—148	0–110		Andersson et al., 2006
Thames estuary, England	Winter–Feb	Surface	WW	0–1450		1–420	0–7	15–75		Middelburg and Nieuwenhuize, 2000

(Continued)

Table 7.1 Summary of selected nitrogen (NO$_3^-$, NO$_2^-$, NH$_4^+$, urea, dissolved free amino acids [DFAA], DON) uptake rates from the major ocean basins, and upwelling, coastal and estuarine systems using ^{15}N tracer techniques. Rates were converted to common units (nmol N L^{-1} h^{-1}) using data provided in the original publication or using conversion factors specified in the footnotes (*continued*)

Location	Season	Depth	Fraction	NO$_3^-$ (nmol N L^{-1} h^{-1})	NO$_2^-$ (nmol N L^{-1} h^{-1})	Uptake NH$_4^+$ (nmol N L^{-1} h^{-1})	Urea (nmol N L^{-1} h^{-1})	DFAA (nmol N L^{-1} h^{-1})	DON (nmol N L^{-1} h^{-1})	Reference
Ria de Ferrol, Spain	Annual[o]	Euphotic zone	WW	0.2–5.4		0.2–10.8				Bode *et al.*, 2005
Humber Estuary, England	Spring/Summer	5 m	WW	0–0.22		0–70	0–30			Shaw *et al.*, 1998

[a] Converted from areal rates using the average euphotic depth (120m) and an average photoperiod (8 h) indicated by the authors.

[b] Originally reported as an areal rate so obtained volumetric rate using the depth over which the authors integrated. Volumetric rates are averages for the entire water column integrated.

[c] Originally reported as mmol m^{-2} (12 h)$^{-1}$.

[d] Divided value reported by 24 to get hourly rate.

[e] ρ max values.

[f] Calculated or measured *in situ* specific uptake rates from depth profiles were converted to absolute rates using particulate nitrogen values in tables and figures.

[g] Areal rates reported were averaged over 1–100% light level; this depth varied from 60–100 m so volumetric rates were calculated using an average euphotic depth of 80 m; daily rates reported were based on observed daily variation in uptake, the authors state these were similar to those observed by Dugdale *et al.* (1992) and so hourly rates were calculated using a 12 h uptake period for NO$_3^-$ and NO$_2^-$ and 18 hour uptake period for NO$_4^+$. An 18 h uptake period was used for urea.

[h] Areal rates were integrated over the 1–100% light level; volumetric rates were calculated using 1% light level depth and hourly rates were calculated based on 12 h accounting for the fact that some ammonium was taken up at night (the authors multiplied their 12 h rate by 1.3).

[i] Units are h^{-1}; there was insufficient information to convert units to nmol nitrogen 1^{-1} h^{-1}.

[j] Areal rates were integrated over the 1–100% light level or the euphotic depth so volumetric rates were calculated from mean 1% light depth; daily rates were converted to hourly rates based on the percent daily nitrogen uptake occurring at night reported by the author.

[k] Areal rates were converted based on an average day length of 16 h during the southern transit and an average 70 m euphotic depth.

[k] Mean values.

[l] Areal rates were converted based on an average euphotic depth (to 0.1% light level of 72 m) and converted to hourly rates based on 24 h incubations

[m] Insufficient information to convert areal daily rates (mmol m^{-2} d^{-1}) to volumetric hourly rates.

[n] Rates were obtained using ^{14}C tracer.

[o] Annual = annual range of values.

[p] Absolute rates were obtained by multiplying specific rates by total nitrogen biomass. Total nitrogen biomass was calculated from C biomass using a C:N of 6.6.

[q] Rates are for adenine uptake.

[r] Daily ranges.

[s] Excluded freshwater station.

[+] Only daytime rates are reported here.

Incubations were conducted under simulated *in situ* conditions. WW is whole water. Values in parentheses are standard deviations. If not specified otherwise, daily rates were divided by 24 to obtain hourly rates.

nitrification), can be an important source of regenerated NO_3^- and NO_2^- at least regionally (see Chapter 5 by Ward, this volume), and that bacteria and phytoplankton seem to compete for the same inorganic and organic N sources (see following). While conceptually, the new production paradigm can accommodate these modifications, we must reevaluate the application of traditional ''f-ratios'' (*sensu* Eppley and Peterson, 1979) when estimating new and export production in marine systems.

A major development in the last two decades is the recognition that marine cyanobacteria, such as *Prochlorococcus* and *Synechococcus*, are the numerically dominant primary producers in many open ocean oligotrophic environments. These marine cyanobacteria can account for over half of the observed primary production (e.g., Partensky *et al.*, 1999) and nutrient uptake (e.g., Cavender–Bares *et al.*, 2001) where they occur; however, numerically less abundant picoeukaryote populations are the larger fraction of autotrophic carbon biomass (e.g., DuRand *et al.*, 2001). More recently, it has been hypothesized that picoplanktonic cyanobacteria may be distributed based not only on their ability to adapt to various light environments (Moore *et al.*, 1995) but also on their ability to use different forms of N (e.g., Moore *et al.*, 2002b), including N_2 (Mulholland *et al.*, submitted for publication), and trace metals (e.g., Mann and Chisholm, 2000; Mann *et al.*, 2002; Saito *et al.*, 2002). These observations are only the tip of the iceberg as the growing literature on genomics, proteomics and cellular regulatory mechanisms (see Chapter 30 by Jenkins and Zehr, this volume) highlight further species-specific differences with respect to the marine N cycle.

High nutrient low chlorophyll (HNLC) regions in the eastern equatorial Pacific, open sub-arctic North Pacific, and the Southern Ocean represent about 20% of the world's ocean, and have been the target of intensive studies over the last two decades since the recognition that iron availability may limit productivity in these areas of the ocean (see Chapter 38 by Hutchins and Fu, this volume; Martin and Fitzwater, 1988). In HNLC regions, NO_3^- uptake remains low despite high NO_3^- concentrations (Dugdale and Wilkerson, 1991). These have been sites of large-scale iron fertilization experiments aimed at stimulating primary productivity and CO_2 and nutrient drawdown (DeBaar *et al.*, 2005; Martin *et al.*, 1993).

Our improved, but still incomplete, understanding of water column N regeneration, species and taxa-specific capabilities for N utilization, the discovery of new quantitatively significant taxa, and the exploration of genomes have altered our view of the marine N cycle. We will begin this chapter with a discussion of N limitation in the sea and then discuss the bioavailability of particular forms of N, pathways and regulation of N uptake and assimilation. This chapter will focus on general patterns of N uptake and assimilation, primarily in the open ocean because N cycling in coastal and estuarine systems are considered elsewhere in this volume (see Chapter 11 by Paerl and Piehler, this volume and Chapter 18 by Boynton and Kemp, this volume). While we try to provide a general description of N uptake in the world's oceans, we also refer the reader to the chapters focusing on N cycling in specific marine systems for details. We conclude by outlining some future research directions.

2. Re-Evaluation of Nitrogen Limitation and New Production in the Sea

The idea that marine primary productivity is N limited arose from efforts to understand factors controlling phytoplankton productivity in the sea and the observation that N was often the nutrient factor in shortest supply relative to other macronutrients in marine environments (e.g., McCarthy and Carpenter, 1983; Ryther and Dunstan, 1971). While N is still thought to be limiting in many marine environments, the view of a single most-limiting nutrient has been revised into a more dynamic view of nutrient limitation in marine ecosystems, with multiple and even co-limiting nutrients (e.g., N vs. phosphorus [P], or P vs. iron [Fe]), seasonal shifts between limiting nutrients (Arrigo, 2005; Fisher *et al.*, 1992; Holmboe *et al.*, 1999; Chapter 38 by Huthins and Fu, this volume; Chapter 11 by Paerl and Piehler, this volume), and multi-decadal oscillations between limiting nutrients (e.g., Karl *et al.*, 2001) regulating phytoplankton primary production. For example, NO_3^- uptake can be limited by Si requirements (for diatoms; Dortch and Whitledge, 1992; Dugdale and Wilkerson, 1998; Chapter 37 by Kudela, this volume) or trace metals (Franck *et al.*, 2000; Martin *et al.*, 1993; Milligan and Harrison, 2000), and utilization of N_2 can be limited by cellular Fe or P requirements (Rueter *et al.*, 1992). Organisms that can use N_2 are not N limited by definition but may become P or Fe limited (Chapter 38 by Hutchins and Fu, this volume; Karl *et al.*, 1997; Mills *et al.*, 2004; Sanudo-Wilhelmy *et al.*, 2001).

Nitrogen limitation in the ocean is difficult to assess experimentally because both autotrophic and heterotrophic populations and processes change during nutrient enrichment bioassays and addition of N substrates frequently causes other elements to rapidly become limiting, unless they are also added to incubation bottles. Physiological or biochemical targets such as probes for *ntcA*; (Lindell *et al.*, 1998) and cell surface proteins (Palenik and Koke, 1995) have been used as a means of identifying potential N limitation. However, use of genetic markers as indicators of N limitation, while possible, must be done cautiously because the presence of a gene does not always equate with its expression or activity. Nitrogen limitation in the ocean is also difficult to assess using stoichiometric arguments because many elements are at or near the analytical detection limits in marine environments, organisms have flexible elemental stoichiometry, and many elements have complex cycles in which there are multiple chemical forms that are more or less available. In addition, the relative turnover times of even the major elements are poorly understood with respect to individual organisms and populations. Although the Redfield Ratio, an average of major nutrient ratios in marine particles, might be a useful integrative indicator, and has been used to qualitatively identify shifts from nitrogen to P limitation, experimental evidence suggests that the critical N:P ratio may be substantially greater than 16 (Bertilsson *et al.*, 2003; Leonardos and Geider, 2004; Terry *et al.*, 1985) and highly variable, calling to question the 'numerical value' at which we decide a system is N or P limited.

Dugdale and Goering (1967) distinguished new and regenerated primary productivity based on the ultimate source of N taken up by phytoplankton; new production supported net growth (which could be exported; Eppley and Peterson, 1979) while regenerated production maintained populations. Since it was first articulated, this paradigm has shaped our view and perhaps the way we study N uptake and assimilation in the sea. New production is supported by N sources imported into the euphotic zone from external sources and includes: NO_3^- diffusion and convection from deep water, N_2 fixation, atmospheric deposition, and riverine inputs. At the time this theory was articulated, the primary source of 'new' N to the euphotic zone in the open ocean was thought to be upward diffusion and convection of NO_3^-, while in coastal systems upwelled NO_3^- could be augmented by runoff and riverine inputs. N_2 fixation, another new N source and the ultimate process by which new N is introduced into the ocean, was thought to be of limited importance in most marine systems, although this view has changed in the past several decades (Chapter 4 by Carpenter and Capone, this volume).

Regenerated primary production, on the other hand, described productivity supported by N internally recycled within the system. This component of primary productivity was assumed to be supported mainly by NH_4^+ (nitrification was not detected and assumed to be negligible, and DON was not considered) remineralized through microbial processes (Dugdale and Goering, 1967). In this context, Harrison (1990) reviewed new production and the f-ratio (the ratio of NO_3^- uptake to total NO_3^- plus NH_4^+ uptake) in 152 vertical profiles from temperate coastal and ocean waters. He found that the primary productivity maximum was shallower than the Chlorophyll a (Chl a) maximum and that the f-ratio was lower at the primary productivity maximum than at the Chl a maximum, which was associated with the nitracline. From this, he inferred that the source of N fueling the primary productivity maximum was derived from regenerative processes in the euphotic zone, and productivity associated with the Chl a maximum was fueled by NO_3^- upwelled from below. However, over the last two decades, a number of examples have been put forth to call into question the 'assumptions' of the new and regenerated production paradigms using the simple relationships between NH_4^+ and NO_3^- uptake.

Failing to consider other sources of new or regenerated N can bias estimates of new production and the f-ratio in nature. For example, it has long been recognized that urea uptake can support a significant amount of regenerated production in a wide variety of environments (Bronk, 2002; McCarthy, 1972). Exclusion of urea uptake from estimates of regenerated production has been shown to result in overestimates of the f-ratio, the extent of which depends on the ecosystem type. For example, the f-ratio was overestimated by 6, 17, 24, 42 and 55%, respectively, in upwelling, coastal, polar, oceanic and estuarine waters when urea uptake was not considered (Wafar et al., 1995). Further, we now recognize that the large DON pool may be more labile and available to phytoplankton than was originally thought, which if utilized, would further bias the regenerated production term.

On the other hand annual rates of new production and the f-ratio can be seriously underestimated by failing to consider alternative sources or pathways delivering new N inputs. For example, the upward transport of NO_3^- (Villareal *et al.*, 1999) and cellular release of NH_4^+ and NO_2^- (Singler and Villareal, 2005) by vertically migrating diatom mats has been shown to represent ~20% of the upward diffusive NO_3^- flux in subtropical ocean gyres. In addition, the passage of cold fronts during the period of winter convective mixing can amplify NO_3^- intrusion maintaining high phytoplankton growth rates and leading to enhanced rates of new production (Lomas *et al.*, 2004). In subtropical gyres where conditions are generally downwelling-favorable this is a particular problem as these areas are thought to account for half the ocean export production. Recent studies have focused on new N inputs into the subtropical North Atlantic gyre from N_2 fixation or lateral supply of DON (Mahaffey *et al.*, 2004; Williams and Follows, 1998).

We now recognize that NO_3^- is not always the dominant source of new N in the open ocean (Table 7.1). N_2 fixation is more widespread than previously thought. Filamentous diazotrophs (e.g., *Trichodesmium* sp.; Capone *et al.*, 1997), diazotrophic endosymbionts (Carpenter *et al.*, 1999), and unicellular cyanobacteria all fix N_2 at high rates in nature (Carpenter and Capone, this volume; Montoya *et al.*, 2004; Zehr *et al.*, 2001). In tropical and subtropical waters, it has been calculated that N_2 fixation by *Trichodesmium* sp. alone fuels 50% of the new production (Capone *et al.*, 2005; Karl *et al.*, 1997). Montoya *et al.* (2004) estimated that diazotrophic unicells may contribute up to 10% of total oceanic new production globally.

In most cases, the picture is far more complicated than simply classifying specific N compounds as supporting either new or regenerated production, even within a single aquatic environment. For example, nitrite (NO_2^-) in the water column is not often considered at all but can arise as a result of both assimilatory and dissimilatory metabolisms (e.g., Capone, 2000; Collos, 1998; Collos and Slawyk, 1984; see also Section 3.1.3 following and Chapter 5 by Ward, this volume). Therefore the mechanisms by which NO_2^- is produced blurs the distinction between new and regenerated production derived from its uptake and can lead to either underestimation or overestimation of new production rates from bulk NO_3^- uptake measurements (e.g., Collos and Slawyk, 1983). In the North and South Atlantic, nutrification rates were so intense that the entire NO_3^- pool could be turned over in less than 8 hours (Clark *et al.*, 2008). Lipschultz (2001) and Collos and Slawyk (1984) found high rates of NO_3^- turnover (i.e., nitrification) in the subtropical and tropical Atlantic during periods of summer stratification, while Raimbault *et al.* (1999a) and Ward *et al.* (1989) observed equally high rates of nitrification under similar conditions in the tropical and subtropical Pacific. In the Mediterranean and North Seas, rates of nitrification are so intense throughout the entire euphotic zone that they can account for 90% to >100% of the measured phytoplankton NO_3^- demand (Diaz and Raimbault, 2000; Rees *et al.*, 2002). Moreover, modeling studies for the subtropical Atlantic (Bermuda Atlantic Time-series Study, BATS) suggest that nitrification produces from 55% to 72% of the NO_3^- taken up per day (Martin and Pondaven, 2006; Mongin *et al.*, 2003).

Both reductive and oxidative pathways can produce NO_2^- in the ocean, and until recently it was believed that oxidative pathways dominated. Specifically, it was

thought that the differential inhibition of microbially mediated NH_4^+ and NO_2^- oxidation (Chapter 5 by Ward, this volume) at the base of the euphotic zone resulted in the accumulation of NO_2^- (Olson, 1981). Although it was also speculated that some of the primary NO_2^- maximum might result from the incomplete reduction of NO_3^- by phytoplankton (Kiefer and Kremer, 1981), this conjecture was overlooked for a long time. Increasingly, evidence suggests that both processes are important. Specifically, large shallower NO_2^- peaks have been attributed to incomplete NO_3^- reduction while the broad deeper NO_2^- maximum has been ascribed to uncoupling of the two-step nitrification process (Al-Qutob *et al.*, 2002; Dore and Karl, 1996a,b; Lomas and Lipschultz, 2006; Zafiriou *et al.*, 1992).

Inputs of new N into coastal systems are more diverse than inputs to the open ocean and include compounds considered regenerated N sources in the open ocean. New N can be delivered by rivers, runoff events, and atmospheric deposition and can include a whole spectrum of N compounds including NH_4^+, urea, and DON (e.g., Anderson *et al.*, 2002; Glibert *et al.*, 2001, 2005c; Mulholland *et al.*, 2007). Similarly NH_4^+ derived from natural processes and anthropogenic activities can support 'new' production in estuarine and coastal systems (see Chapter 9 by Seitzinger and Harrison and Chapter 11 by Paerl and Piehler, this volume).

Another complication that arises when estimating new and regenerated production from N uptake is the release of recently assimilated N by cells. We now recognize that a significant fraction of the N taken up during even short-term (hours) incubations can be released as a downstream metabolite (see Chapter 8 by Bronk and Steinberg, this volume). In general, N is taken up by cells, reduced intracellularly to NH_4^+ and then assimilated into amino acids (see following). However, release of $^{15}NH_4^+$ and $DO^{15}N$ has been observed during incubations with $^{15}NO_3^-$ (Bronk and Ward, 1999, 2000; Diaz and Raimbault, 2000; Raimbault *et al.*, 1999b) and $^{15}N_2$ (Glibert and Bronk, 1994; Mulholland and Bernhardt, 2005; Mulholland *et al.*, 2004c). It is unclear why cells would release metabolites either prior to or after their assimilation into organic matter (Bronk, 2002), but N release is highly variable among systems and complicates estimates of f-ratios because NO_3^- and N_2 uptake can be seriously underestimated. For example, natural assemblages in Monterey Bay released up to 80% of the recently assimilated NO_3^- as DON on timescales of hours (Bronk and Ward, 1999, 2000). In contrast, in the equatorial Pacific Ocean <20% of the NO_3^- taken up was released as DON (Raimbault *et al.*, 1999b). Marine N_2 fixers can release 80% or more of recently fixed N_2 as DON and NH_4^+ on similar timescales (Glibert and Bronk, 1994; Mulholland *et al.*, 2004b,c, 2006), but this release is also highly variable both temporally and spatially (Mulholland and Bernhardt, 2005; Mulholland *et al.*, 2004c, 2006).

Another consideration when calculating f-ratios is the diurnal variability of N versus carbon (C) metabolism. When relating new production to C export (*sensu* Eppley and Peterson, 1979), stoichiometric calculations of C export from N uptake can be in error when C and N uptake are not evaluated over appropriate timescales. Diel variability in uptake rates of various N compounds has been demonstrated (e.g., Cochlan and Harrison, 1991b; Cochlan *et al.*, 1991a; Tremblay *et al.*, 2000; Wilkerson and Grunseich, 1990) and failure to integrate daily uptake rates over an entire diel cycle may seriously bias estimates of f-ratios.

Bury *et al.* (2000), on the other hand, found uncoupling between N and C uptake on longer timescales. Similarly, C and N_2 fixation rates appear to be uncoupled or stoichiometrically different from particulate C:N ratios (Mulholland *et al.*, 2007; Mulholland and Capone, 2000; Mulholland *et al.*, 2006) making it difficult or impossible to estimate C export from N_2 fixation.

In addition to complicating our view of the marine N cycle, high rates of inorganic N uptake by bacteria and organic N uptake by phytoplankton may compromise stoichiometric calculation of C assimilation from N uptake (or vice versa) using the Redfield C:N ratio in the euphotic zone (Wheeler and Kirchman, 1986). Further, bacterial uptake of NH_4^+ and NO_3^- can confound computation of the f-ratio leading to over- or underestimates of new production. Consequently, estimating C export from N-based estimates of new production needs to be re-evaluated for a variety of reasons.

In summary, to accurately depict new production in the ocean, even without inputs from rivers, runoff, and atmospheric deposition, one needs to carefully evaluate new N inputs from NO_3^- upwelling and diffusion, and N_2 fixation, and regenerated production from NH_4^+ as well as NO_2^- (and NO_3^- if nitrification is complete), urea, and DON. In addition, one must consider the relative contribution of autotrophic versus heterotrophic processes to N cycling and temporal and spatial scales of variability in N uptake when calculating f-ratios and extrapolating these to C export in the ocean. In short, f-ratios, as currently used, may be misleading or just wrong in depicting new and export production.

3. BIOAVAILABILITY OF NITROGEN COMPOUNDS

The dominant N sources and their relative concentrations are important in determining microbial community structure in aquatic environments. Nitrogen is stable in a variety of oxidative states and so has a complex cycle in the ocean. Different N compounds vary in their abundances, distributions and residence times as a result of environmental constraints and interactions that affect their utilization and their relative availability to a diverse group of microorganisms with different genetic capabilities (Chapter 1 by Gruber, this volume; Sharp, 1983). For example, although N_2 gas is found throughout most oceanic and coastal waters at concentrations near saturation, it is considered to be more biologically inert than other inorganic N forms. On the other hand NO_3^- is abundant and biologically reactive; however, the highest oceanic concentrations are in the reservoirs of deep water where it cannot be accessed by photosynthetic microbes.

Frequently, compounds that are rapidly turning over are highly bioavailable (e.g., NH_4^+, amino acids). NH_4^+ concentrations, at least in the open ocean, are generally at the limits of analytical detection yet NH_4^+ accounts for the bulk of the measured N uptake in many systems (see below). The DON pool, formerly thought to be largely unavailable to primary producers, is the largest dissolved N pool (excluding N_2) in the surface ocean but poorly characterized (Bronk, 2002). Recent studies have demonstrated that portions of this pool are more biologically reactive

and may turn over on shorter timescales than previously thought (see Chapter 3 by Aluwihare and Meador, this volume). Because microorganisms are both consumers and producers of dissolved N, their activity leads to complex temporal and spatial patterns of nutrient distributions and utilization. In this section, we will discuss the various chemical forms of N and their bioavailability to marine microbes, including interactions between bacteria and phytoplankton, followed by a discussion of nutrient interactions and examples of taxon–specific N uptake. We have also compiled a table of representative uptake rates for various N compounds across environments (Table 7.1) demonstrating the wide temporal and spatial variability in uptake rates and the relative contribution of different forms of N across environments. The latter is more difficult to assess across environments because the oceanic database is skewed towards measurements of NO_3^- and NH_4^+ uptake.

3.1. Uptake of dissolved inorganic nitrogen

Two decades ago, our view of N cycling was simpler; relative uptake of NH_4^+ and NO_3^- provided an index of new production (Table 7.1), consequently, rate measurements of NH_4^+ and NO_3^- dominate our database of N uptake across systems; and phytoplankton were primary consumers of dissolved inorganic N (DIN) (NH_4^+, NO_3^-, NO_2^-) and urea in the euphotic zone while bacterial heterotrophs and grazers were primary consumers of organic compounds and regenerators of reduced inorganic compounds (Fig. 7.1A). We now recognize that bacteria and phytoplankton can be both consumers and producers of most forms of N and that their role as producers or consumers can change on a variety of temporal and spatial scales as well as with taxonomic diversity (Fig. 7.1B). Because their size spectra overlap, it is often difficult to distinguish which group or which cells are taking up which compounds in bulk nutrient uptake bioassays. Further, in the age of genomics, genetic capacity determines whether cells can produce transportors and enzymes necessary to take up, reduce, and assimilate various N compounds. Even if genes are present, it doesn't mean that they are expressed, and even if genes are expressed, it doesn't mean enzymes are active (see Chapter 32 by Berges and Mulholland, this volume).

3.1.1. NH_4^+

NH_4^+ is often the dominant form of dissolved N taken up in a variety of marine and estuarine systems (Table 7.1). Some notable exceptions are upwelling regimes (see Chapter 17 by Wilkerson and Dugdale, this volume) and eutrophic coastal systems (see Chapter 11 by Paerl and Piehler, this volume). This form of N is energetically efficient for cells to use because it is already reduced and is a common cellular transient in N metabolism, requiring little additional energy for assimilation (Fig. 7.2). Despite the low NH_4^+ concentrations in oceanic systems, uptake and regeneration of NH_4^+ are tightly coupled (Glibert, 1993) resulting in rapid turnover times of the relatively small standing stock. Consequently, low ambient NH_4^+ concentrations still support high rates of NH_4^+ uptake (and therefore growth rates, e.g., Furnas and Crosbie, 1999). Because many NH_4^+ uptake measurements
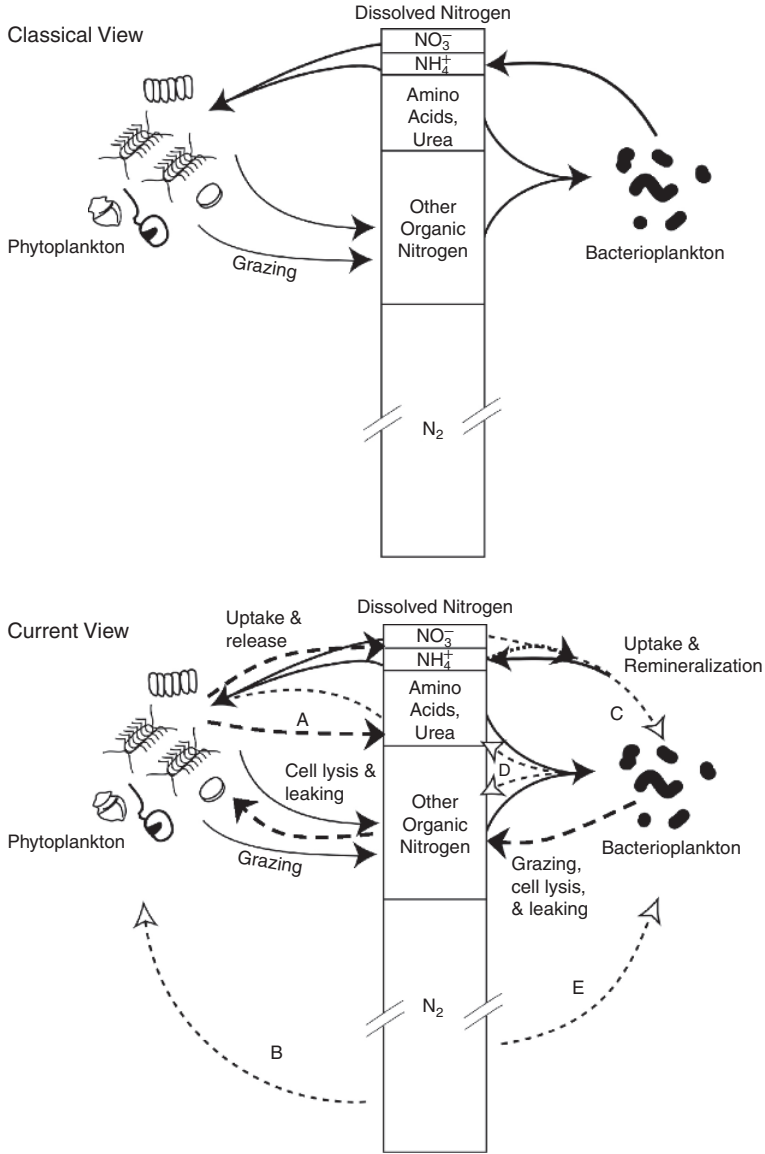
Figure 7.1 Classical and current views of the N cycle in the surface waters of oligotrophic oceans. The composition of the dissolved N pool is shown with approximate relative concentrations of inorganic and organic constituents indicated by the sizes of the boxes. Dashed lines indicate transformations and processes included in the newer view of N cycling. (A) Some phytoplankton use simple and more complex organic compounds as a source of N and phytoplankton can be sources of inorganic and organic N as well. (B) There are multiple species of phytoplankton (cyanobacteria) in the open ocean that fix N_2. (C) Bacteria compete for NO_3^- and NH_4^+. (D) Bacteria can take up and excrete urea and also be a source of DON. (E) Some oceanic bacterioplankton appear to fix N_2 (modified from Zehr and Ward, 2002).
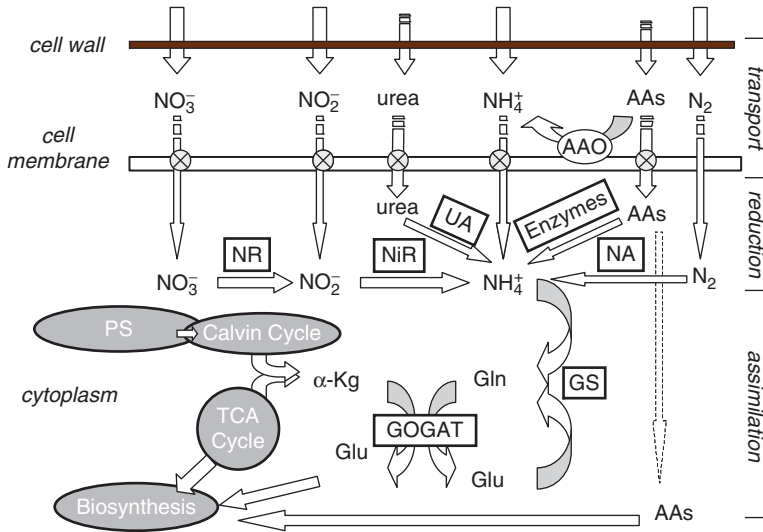
Figure 7.2 Intracellular pathways and enzymes involved with N transport, reduction and assimilation (modified from Capone, 2000).

made using stable isotopes do not account for NH_4^+ isotope dilution caused by regeneration of NH_4^+ in incubation bottles (Glibert *et al.*, 1982) it is likely that the importance of NH_4^+ uptake to total N nutrition of cells is underestimated, at least in some systems and during long incubations (see Chapter 31 by Lipschultz, this volume for a further discussion of isotope dilution).

In most studies examining the relative uptake of different N sources, NH_4^+ is commonly found to be preferred over urea, NO_3^- or NO_2^- for all taxa and this is attributed largely to the low energetic demand for its uptake and assimilation (Syrett, 1981). However, the relative preference for uptake of N compounds is difficult to assess in natural communities where nutrient pre-history is largely unkown and communities include a mixture of algae and bacteria. McCarthy *et al.* (1977) developed a Relative Preference Index (RPI) to evaluate nutrient preferences for phytoplankton N uptake, but it has since been argued that the RPI does not reflect real physiological preference under all conditions (Stolte and Riegman, 1996). Moreover, the use of the RPI is often biased by the dominant nutrient present as well as by substrates present at low concentrations for which uptake and regeneration are tightly coupled (e.g., NH_4^+ or urea). Nanomolar N uptake kinetic studies in the North Atlantic have shown that picoplankton have an enhanced capacity for NH_4^+ uptake and this has been argued as evidence of physiological preference (Harrison *et al.*, 1996). However, it is important to keep in mind that low rates of NH_4^+ uptake by autotrophs, or uptake of any N substrate for that matter, need not reflect low NH_4^+ bioavailability. Genetic capability and the availability of trace metals and other micronutrients (e.g., N, P, Si) can potentially reduce uptake rates of particular N compounds thereby complicating assessments of physiological preference (see Chapter 38 by Hutchins and Fu, this volume). In addition, estuarine and coastal populations appear to use a variety of N compounds simultaneously and the relative

uptake of various N compounds changes on short time scales (e.g., days) (Morse *et al.*, in preparation; Mulholland *et al.*, in preparation).

Without question, NH_4^+ is efficiently used by aquatic autotrophs; however, bacteria also consume significant amounts of NH_4^+ in natural systems. Uptake of NH_4^+ by bacteria is relatively high in oligotrophic gyres and in the coastal ocean. In Atlantic coastal waters, about 78% of total NH_4^+ uptake was by prokaryotes and a significant portion of that by heterotrophic bacteria (Wheeler and Kirchman, 1986), comparable to findings in the Pacific Ocean near the Hawaiian Islands (Laws *et al.*, 1985). A smaller fraction of the bacterial N demand was met by NH_4^+ in some eutrophic systems, where concentrations of free and combined amino acids were high (Fuhrman *et al.*, 1988; Hoch and Kirchman, 1993). However, in an extreme case, the very turbid and eutrophic Thames River, heterotrophic uptake of NH_4^+ exceeded 80% of the total NH_4^+ uptake (Middelburg and Nieuwenhuize, 2000). High rates of heterotrophic uptake of NH_4^+ in turbid environments could be due light limitation of autotrophic C fixation (e.g., Vincent, 1992). Uptake of NH_4^+ by bacteria also appears to vary seasonally, with higher uptake rates in summer (Fuhrman *et al.*, 1988; Hoch and Kirchman, 1995) when thermal stratification increases in most ocean systems and NH_4^+ concentrations reach annual minima (e.g., Lipschultz, 2001), and NH_4^+ turnover is rapid.

Because they are primarily heterotrophic, bacterial uptake of NH_4^+ may also depend on the availability of a labile C source (e.g., Gardner *et al.*, 1996). Goldman and Dennett (2001) found that NH_4^+ uptake by bacteria was dependent upon the availability of labile organic C sources to stoichiometrically balance N uptake. NH_4^+ uptake by bacteria was previously thought to be inhibited by amino acids; however, this does not appear to be the case (Jorgensen *et al.*, 1999b; Kirchman, 1994; Kirchman *et al.*, 1989). In fact, bacteria can simultaneously metabolize amino acids and NH_4^+ (Goldman and Dennett, 1991, 2000, 2001; Tupas and Koike, 1990, 1991). In N and C-limited bacterial cultures, dissolved combined amino acids (DCAA) were more important during stationary phase growth while dissolved free amino acids (DFAA) and NH_4^+ were more important N sources during exponential growth (Middelboe *et al.*, 1995b). These observations suggest that bacteria have flexible metabolisms and can satisfy their N demand using a variety of compounds depending both upon their relative availability and the physiological state of cells in the environment.

3.1.2. NO_3^-

Nitrate was thought to be the predominant source of ''new N'' to the euphotic zone and so its uptake has been more widely measured that any other N compound (Table 7.1). That said, NO_3^- upwelled from the deep ocean is not ''new N'' in the context of oceanic N inventories in general (see discussion of N_2 fixation below and Chapter 4 by Carpenter and Capone, this volume).

Assimilation of NO_3^- is more energetically demanding than NH_4^+, because assimilation of NO_3^- requires the synthesis of NO_3^- and NO_2^- reductases, associ–ciated active transport systems, and the turnover of cellular ATP and NADPH (Chapter 32 by Berges and Mulholland, this volume; McCarthy, 1981; Syrett, 1981). Further, supply of NO_3^- is limited by rates of nitrification and vertical

mixing. Diatoms have traditionally been thought to be the primary consumers of NO_3^- in the environment because they are often the dominant taxa in upwelling systems where NO_3^- concentrations are often high (Kokkinakis and Wheeler, 1987, see Chapter 17 by Wilkerson and Dugdale, this volume). However, because diatoms also require silicate, this element can limit their growth in high NO_3^- environments (Dugdale and Wilkerson, 1998). Alternatively, Fe can limit NO_3^- and silicate uptake in vast expanses of the ocean that are characterized by high nutrient and low chlorophyll concentrations (Martin and Fitzwater, 1988; Martin *et al.*, 1989; Timmermans *et al.*, 1998, 2004). NH_4^+ recycling and high concentrations of NH_4^+ are also thought to limit NO_3^- uptake in some oceanic regions (Wheeler and Kokkinakis, 1990, also see section on nutrient interactions below).

In order to assimilate NO_3^- for growth, phytoplankton must first possess the genetic capacity to synthesize the necessary enzymes and transport systems; not all phytoplankton do. For example, most of the strains of *Prochlorococcus* that have been studied in culture to date have been found to be unable to grow on either NO_3^- or NO_2^- as a sole N source (El Alaoui *et al.*, 2001; Moore *et al.*, 2002b). Notable exceptions are some recent strains isolated from the Red Sea (Fuller *et al.*, 2003). In contrast, most of the *Synechococcus* strains studied so far can use both NO_3^- and NO_2^- as sole N sources (e.g., Bird and Wyman, 2003; Moore *et al.*, 2002b). These observations have particular ecological significance because these two genera of marine cyanobacteria are numerically dominant in the Atlantic and Pacific oligotrophic ocean gyres (e.g., Campbell *et al.*, 1994; DuRand *et al.*, 2001) and contribute significantly to ocean primary productivity, overall autotrophic biomass, and export production (Goericke *et al.*, 2000).

Prochlorococcus and *Synechococcus* often display seasonal and geographic patterns that are consistent with their genetic capabilities (e.g., Johnson *et al.*, 2006). In the Sargasso Sea, *Synechococcus* has an annual biomass maximum in the late spring/early summer immediately following the period of winter convection and entrainment of deep NO_3^- concentrations in surface waters, whereas *Prochlorococcus* has an annual maximum late in the summer during the period of maximum thermal stratification when NO_3^- concentrations and upward mixing are low (e.g., Lipschultz, 2001; Steinberg *et al.*, 2001). Geographically, both *Synechococcus* and *Prochlorococcu,* can be found in shallow coastal environments where a variety of N substrates are available (see references in Partensky *et al.*, 1999; Scanlan, 2003). Based on the observation that *Synechococcus* but not *Prochlorococcus* can utilize NO_3^-, Bird and Wyman (2003) proposed that *Synechococcus* is more important in oceanic new production. However, although growth of a *Synechococcus* bloom in the Sargasso Sea was supported by NO_3^-, this 'new production' was not linked to export production (Glover *et al.*, 1988; *sensu* Eppley and Peterson, 1979). Current dogma holds that *Prochlorococcus* does *not* assimilate NO_3^- (nor fix N_2), and therefore, by definition, *Prochlorococcus* does *not* contribute to oceanic new production (*sensu* Dugdale and Goering, 1967). Indeed, it is paradoxical that although cultured *Prochlorococcus* strains cannot utilize NO_3^-, *Prochlorococcus* is the most abundant picoplankton population near the nitracline at the base of the euphotic zone, while *Synechococcus* is dominant in the surface ocean during periods of convective mixing; DuRand *et al.*, 2001).

Recent flow cytometry-stable isotope data suggests that natural populations of *Prochlorococcus* inhabiting the Sargasso Sea deep chlorophyll maximum contribute to new production (Casey *et al.*, 2007). Furthermore Sedwick *et al.* (2005) observed minima in dissolved Fe and in total dissolvable Fe in the Sargasso Sea deep chlorophyll maximum and suggested that these minima arise because of significant export production from this depth horizon. Mass sedimentation of picoplankton embedded in aggregates has been observed (Waite *et al.*, 2000) and cyanobacteria have been found in salp fecal pellets or aggregates (Goericke *et al.*, 2000; Pfannkuche and Lochte, 1993; Turley and Mackie, 1995) and fecal pellets of the copepod *Calanus finmarchicus* and the tunicate *Oikopleura* (Urban *et al.*, 1993a,b). These observations differ significantly from the view that diatoms and other species more prone to gravitational settling and less tightly controlled by grazers dominate new and export production from the euphotic zone (e.g., Barber and Hiscock, 2006; Buesseler, 2001; Gaines and Elbrachter, 1987; Goldman, 1993; Goldman and Mcgillicuddy, 2003; Goldman *et al.*, 1992). Indeed, mounting evidence suggests that the relative contribution of picoplankton and nanoplankton to oceanic export production needs to be reevaluated (Richardson *et al.*, 2004, 2006).

Heterotrophic bacteria can also take up NO_3^- and their capacity to do so varies according to their genetic make-up, physiological status, and nutrient environment. As for some clones of *Prochlorococcus*, the genome of *Silicibacter pomeroyi*, a bacterium whose relatives comprise 10–20% of coastal and oceanic mixed layer bacteria, contained genes for NH_4^+ and urea transport and assimilation, yet genes for NO_3^- and NO_2^- assimilation were not found (Moran *et al.*, 2004). In contrast, other bacterial groups appear to have the genetic capacity to take up and assimilate NO_3^- and NO_2^- (Allen *et al.*, 2001), and this has been confirmed in field incubations (Kirchman, 2000; Middelburg and Nieuwenhuize, 2000).

3.1.3. NO_2^-

Because nitrite reductase (NiR) is required for NO_3^- assimilation, organisms that assimilate NO_3^- can readily assimilate NO_2^-. However, some organisms that cannot use NO_3^- can use NO_2^- (e.g., some clones of *Prochlorococcus*; Moore *et al.*, 2002b). It has been suggested that this rather unique N physiology has arisen from the evolutionary loss of genes necessary for assimilation of NO_3^- (Garcia-Fernandez and Diez, 2004; Garcia-Fernandez *et al.*, 2004) and the resultant evolution of many ecotypes specifically adapted for unique environments where NO_2^- production can be high (see Chapter 5 by Ward, this volume). This could offer another explanation for the deep maximum in *Prochlorococcus* abundance; this maximum co-occurs with both the nitracline (Olson *et al.*, 1990; Partensky *et al.*, 1999) and the deep NO_2^- maximum.

Generally speaking, when NO_2^- is abundant in the euphotic zone, NO_2^- uptake can be significant (e.g., Glibert *et al.*, 1991), however, NO_2^- uptake is measured far less routinely than NO_3^- and NH_4^+ uptake. NO_2^- concentrations are generally low, but can at times accumulate throughout the entire euphotic zone (Al-Qutob *et al.*, 2002; Lomas and Lipschultz, 2006) as well as at the base of the euphotic zone in what has been termed the primary NO_2^- maximum (e.g., Rakestraw, 1936). Further, high NO_2^- concentrations have been found in estuaries and coastal systems at least seasonally (Filippino *et al.*, in revision; Morse *et al.*, in preparation; Bronk, personal

communication). There remains an active debate as to the proximate source of this NO_2^-, phytoplankton release or nitrification (Lomas and Lipschultz, 2006), and could likely be different between oceanic and coastal regions. The determination of the "source" of the observed NO_2^- in the ocean is not merely a pragmatic issue; it has large implications for the definition of new production (*sensu* Dugdale and Goering, 1967). One of the major assumptions of the new production paradigm is that euphotic zone nitrification is negligible (i.e., NO_3^- is entrained with deep water, not regenerated within the euphotic zone). We now know this assumption is untrue in stratified surface waters (Lipschultz, 2001; Raimbault *et al.*, 1999b; Ward, 1985), and probably in shallow estuaries, coastal plumes and shallow shelf waters (Filippino *et al.*, in revision; Morse *et al.*, in preparation). Consequently, we must revisit this assumption if we hope to correctly parameterize models of new production (e.g., Martin and Pondaven, 2006; Mongin *et al.*, 2003). Clearly, our understanding of NO_2^- cycling in the ocean is far from complete.

3.1.4. N_2

The special case of N_2 fixation is addressed by Carpenter and Capone (Chapter 4, this volume) and will not be discussed in detail here, other than to say that, like NO_3^- uptake, this is the ultimate source of new N to the ocean and it is an energetically costly way for cells to acquire N, requiring a large amount of ATP (Postgate, 1998). N_2 fixation is limited to cells that have the genetic capability to synthesize nitrogenase, the enzyme complex mediating the reduction of N_2. This limits the number of taxa that can fix N_2. Most of the identified organisms capable of N_2 fixation in the ocean are cyanobacteria, either free-living or in symbioses with some marine diatoms and dinoflagellates. There are also α- and γ-proteobacteria and archael N_2 fixers in estuarine, coastal, and open ocean systems (Mehta and Baross, 2006; Mulholland *et al.*, submitted for publication; Short *et al.*, 2004).

Because nitrogenase is inhibited by oxygen, many cyanobacteria synthesize heterocysts, armored cells, that retard oxygen diffusion in and that exclude oxygen evolving processes, in which they perform N_2 fixation (see Chapter 35 by Berman-Frank *et al.*, this volume). The marine non-heterocystous cyanobacteria *Trichodesmium* spp., are unusual in that they lack the ability to form heterocysts, but they do form diazocytes, groups of nitrogenase-containing cells that play similar roles but lack many of the protective mechanisms of heterocysts (Bergman and Carpenter, 1991; El-Shehawy *et al.*, 2003; Fredriksson and Bergman, 1997). These specialized cells are also under genetic control and are regulated by feedback from extracellular nutrients (Cai and Wolk, 1997; El-Shehawy *et al.*, 2003; Wolk, 2000). In the marine environment, N_2 fixation is thought to be limited by phosphorus (Karl *et al.*, 2001; Mills *et al.*, 2004; Sañudo-Wilhelmy *et al.*, 2001; Wu *et al.*, 2000; Zehr *et al.*, 2007). Until fairly recently, N_2 uptake was considered quantitatively insignificant and so generally excluded from N budgets and calculations of f-ratios in oceanic environments. To date, it is not included routinely in studies of N uptake (Mulholland *et al.*, submitted for publication), although rates of N_2 uptake can be significant (Table 7.2). Mapping the distribution of *nif* genes (genes encoding nitrogenase) in the environment and estimating rates of N_2 fixation have been the topics of intense research over the last two decades (see Chapter 4 by Carpenter

Table 7.2 Ranges of water column N$_2$ fixation rates

Date	Location	Depth	N$_2$ fixation (nmol N L^{-1}h^{-1})	Method	Reference
2001–2003	Gulf of Mexico	Surface	0.011–0.23	^{15}N$_2$	Mulholland et al. (2006)
2003	Gulf of Mexico	Pigment maximum	0.044–0.063	^{15}N$_2$	Mulholland et al. (2006)
2002	New Caledonia	Surface	0.23–0.85	^{15}N$_2$	Mulholland (2007)
2002	N Atlantic	25 m (nighttime)	∼ 0.147	AR	Falcon et al. (2004)
2001	N Atlantic	Upper 100 m	0.025–0.045	^{15}N$_2$	Falcon et al. (2004)
2002	N Pacific	Upper 100 m (nighttime)	∼ 0.003	AR	Falcon et al. (2004)
2000–2001	Station ALOHA & Kaneohe Bay	25 m & Surface	0.01 – 0.15	^{15}N$_2$	Montoya et al. (2004)
2002	Eastern North Pacific	Mixed layer & pigment maximum	0.047–1.85 (0.72)	^{15}N$_2$	Montoya et al. (2004)
1999	Arafura Sea	Pigment maximum	20–62	^{15}N$_2$	Montoya et al. (2004)
2000	Station ALOHA	25 m	0.010–0.016	^{15}N$_2$	Zehr et al. (2001)
2002	Tropical Atlantic	Upper 100 m	Up to 3.1	^{15}N$_2$	Voss et al. (2004)
2000–2001	Station ALOHA	Upper 100 m	0–0.09	^{15}N$_2$	Dore et al. (2002)[a]
2002	E Mediterranean	16 m	5.38	^{15}N$_2$	Rees et al. (2006)[b]
2003–2004	W Mediterranean	Upper 60 m	0.083–0.71	^{15}N$_2$	Garcia et al. (2006)[a]
2003–2004	S Atlantic 40°–11°S	Surface	0.032–1.37	^{15}N$_2$	Millward et al. (2005)[a]
2003–2004	Atlantic 10°S–10°N	Surface	0.22–0.30	^{15}N$_2$	Millward et al. (2005)[a]
2003–2004	N Atlantic 11°–40°N	Surface	0.012–0.033	^{15}N$_2$	Millward et al. (2005)[a]
2000–2001	ALOHA	25 m	0.014–0.095		Zehr et al. (2007)[c]
2001–2002	Kane'ohe Bay	Surface	0.029–0.048	^{15}N$_2$	Zehr et al. (2007)[c]
July 2006	Chesapeake Bay Plume	Surface	0.81–1.46	^{15}N$_2$	Mulholland et al. (submitted for publication)
July 2006	Coastal waters influenced by Gulf Stream	Surface	0.41–0.73	^{15}N$_2$	Mulholland et al. (submitted for publication)
July 2006	Coastal waters – non-Gulf Stream	Surface	0.07–0.88	^{15}N$_2$	Mulholland et al. (submitted for publication)

[a] Converted from daily rate assuming N$_2$ fixation persisted for 24 h per day.
[b] Report rates from outside the phosphate enriched area.
[c] Used averages from control incubations.
Rates are presented as hourly rates because it is unclear whether N$_2$ fixation by unicellular diazotrophs exhibits diel periodicity (updated from Mulholland et al., 2006). AR is acetylene reduction.

and Capone, this volume). It has now become apparent that organisms containing *nif* genes are widely distributed in the marine environment and that N_2 fixation can be regionally and globally significant; N_2 fixation by diazotrophic endosymbionts can contribute significantly to new production in some environments (Carpenter et al., 1999), N_2 fixation by *Trichodesmium* sp. can contribute up to 50% of the new production where they occur (Capone et al., 2005; Karl et al., 1997; Mahaffey et al., 2004), and unicellular diazotrophs may contribute up to 10% of global oceanic new production (Montoya et al., 2004).

The oceanic N inventory largely depends on the balance between N inputs from terrestrial systems and N_2 fixation versus N losses from denitrification. Based on current estimates, the oceanic N budget is far from balanced (e.g., oceanic denitrification far exceeds N_2 fixation; Codispoti, 2007; Codispoti et al., 2001; Chapter 1 by Gruber, this volume). A better understanding of the geographical distribution and limitations on marine N_2 fixation is required to understand N cycling and oceanic N budgets under present, past, and future oceanic scenarios.

3.2. Bulk organic nitrogen dissolved

DON can often be the largest pool of dissolved combined N but it is largely uncharacterized (see Chapter 3 by Aluwihare and Meador, this volume; Bronk, 2002). The marine DON pool consists of highly reactive and relatively recalcitrant fractions and, while largely uncharacterized, includes a variety of compounds such as urea, dissolved free and combined amino acids and a variety of cellular intermediates, and excretion and degradation products that can be readily taken up (Table 7.1) and metabolized by phytoplankton and bacteria in the environment. Unfortunately, rates of DON uptake (excluding urea) are rarely measured.

DON can be rendered more or less available through extracellular enzymatic reactions that degrade DON into usable components (e.g., Chapter 32 by Berges and Mulholland, this volume; Chrost, 1991; Hoppe, 1983; Hoppe et al., 2002; Mulholland et al., 1998; 2002a; Palenik and Morel, 1990a,b; Pantoja and Lee, 1999; Pantoja et al., 1997) and photochemical reactions (Bushaw-Newton and Moran, 1999). The components and characteristics of the DON pool are described by Aluwihare and Meador (Chapter 3, this volume) and will not be discussed further here.

As for inorganic N, bacteria and phytoplankton are both sources and sinks of DON in the environment. Over the last two decades there has been a growing realization that organic N sources can be important components of the total N uptake in most marine systems where labile DON compounds, including urea, are often more abundant than DIN (Berman and Bronk, 2003; Bronk et al., 2007). Two decades ago, Paul (1983) stated that it was difficult to draw conclusions about organic N uptake based on the limited available data. A later review (Antia et al., 1991), summarized organic N uptake by phytoplankton by compound and by species but, much of this review focused on the physiological capability of cultured organisms rather than the ability of natural populations to use organic N compounds. There has been a resurgence of interest in the role of DON, and dissolved organic matter (DOM) in general, in the nutrition of phytoplankton as it has become clear

that DOM-enrichment has contributed to the degradation of coastal waterways (Glibert et al., 2001; Paerl, 1988; Chapter 11 by Paerl and Piehler, this volume) and that DON can be a quantitatively important source of N to organisms in the environment.

The quality and quantity of DON relative to DIN can influence community structure either directly or indirectly (e.g., Berman and Chava, 1999; Lewitus et al., 2000; Zubkov et al., 2003, 2004). While direct uptake of DOM by mixed communities of microalgae has been demonstrated (see Lewitus, 2006; Nilsson and Sundback, 1996; Paerl, 1991; Paerl et al., 1993; Wheeler et al., 1977), the number of studies examining this phenomenon has decreased since culture studies in the 1960s and 1970s. The recent resurgence in studies examining uptake of DON (Antia et al., 1991; Berman and Bronk, 2003; Bronk, 2002; Flynn and Butler, 1986) has been largely focused on potentially harmful algal bloom species (primarily dinoflagellates; Table 7.3; Berg et al., 1997, 2003a; Doblin et al., 1999; Fan et al., 2003b; Mulholland et al., 2002a; Smayda, 1997). Because amino acid uptake studies were previously biased towards diatoms and are currently biased towards potentially harmful taxa, little is known about DOM uptake by many flagellates (including non–toxic dinoflagellates) and picoplanktonic cyanobacteria. In addition, these taxonomic groups are difficult to culture axenically, culture collections have fewer representatives of these taxa (Graneli et al., 1997; John and Flynn, 1999; Zubkov et al., 2003), and monospecific populations of these organisms are rarely encountered in nature.

Despite these limitations, field studies suggest that many taxa use components of the DON pool for their nutrition. Chrysophytes, cryptophytes and dinoflagellates have notoriously versatile metabolisms (Antia, 1980; Gaines and Elbrachter, 1987) and Kristiansen (1990) thought it doubtful that there were any strictly photautotrophic crysophytes. Paerl (1991) demonstrated amino acid uptake by axenic Synechococcus and linked this to their high productivity in DIN-limited waters. High rates of DON uptake have been observed in populations dominated by Prochlorococcus, who take up methionine and leucine competitively with bacteria (Zubkov et al., 2003, 2004). Moreover, this ability may give Prochlorococcus a competitive advantage over other phytoplankton in the oligotrophic ocean gyres.

3.2.1. Urea

Measurements of urea concentrations and uptake rates in marine systems are now almost as common as measurements of the more 'traditional' N sources NO_3^- and NH_4^+ (Table 7.1). Urea is one of the simplest organic compounds, and its importance in supporting marine primary productivity has been recognized for several decades (e.g., McCarthy, 1972; McCarthy et al., 1977; Remsen et al., 1972a,b), despite the fact that it constitutes a small fraction (5.2% ± 3.4%) of the bulk DON pool in most marine systems (Bronk, 2002). Indeed, urea contributes on average 18.8% ± 15.4% of the total measured N uptake across a wide range of marine systems (Bronk, 2002). Moreover, urea uptake can contribute over 50% of the total measured N uptake in some coastal systems (Glibert et al., 1991; Harrison et al., 1985; Kokkinakis and Wheeler, 1988; Kudela and Cochlan, 2000; Mulholland et al., 2004d, unpublished data; Rees et al., 2002) and urea turnover times can be as short as

Table 7.3 Summary of taxon-specific nitrogen (NO_3^-, NO_2^-, NH_4^+, urea, dissolved free amino acids [DFAA], peptides) uptake rates made in natural systems

Species/Taxon	Location	Method	Incubation conditions	NO_3^- (h^{-1})	NO_2^- (h^{-1})	NH_4^+ (h^{-1})	Uptake Urea (h^{-1})	DFAA (h^{-1})	Peptides (h^{-1})	N_2 (h^{-1})	Reference
DIATOMS											
Diatoms (unspecified)	Norwegian Fjords	Natural bloom	Simulated *in situ*	0.00530– 0.0250		0.0140– 0.0460	0.0044– 0.0186				Fernandez et al., 1996
Diatoms (unspecified)	Gulf of Riga	Natural bloom	Simulated *in situ*	0.0030		0.0150	0.0030	0.0025– 0.0030			Berg et al., 2003a
CYANOBACTERIA											
Prochlorococcus	Arabian Sea	FCM	Dark					0.0006– 0.0054			Zubkov et al., 2003
Prochlorococcus	South Atlantic tropical gyre	FCM	Dark					0.0002– 0.0003			Zubkov et al., 2004
Prochlorococcus	Sargasso Sea	FCM	Simulated *in situ*	0.001– 0.006	0.001– 0.009	0.004– 0.012	0.008– 0.019				Casey et al., 2007
Synechococcus	Arabian Sea	FCM	Dark					0–0.0021			Zubkov et al., 2003
Synechococcus	Sargasso Sea	FCM	Simulated *in situ*	0.0160– 0.0480		0.0210– 0.1130					Casey et al., 2007
Cyanobacteria (filamentous)	Gulf of Riga	Natural bloom	Simulated *in situ*	0.0030– 0.0050		0.0180– 0.0350	0.0050– 0.0170	0.0012– 0.0030			Berg et al., 2003a
Trichodesmium	Caribbean Sea	Natural bloom	Simulated *in situ*	ND– 0.000005		0.0005– 0.02	ND–0.002	0.0003			Mulholland and Capone, 1999
Trichodesmium	Sargasso Sea	Natural Bloom	Simulated *in situ*	0.01–39.8[a]		0.01–5.43[a]	0.05–0.29[a]	0.12–6.5[a]		32.3– 796.2[¥]	Orcutt et al., 2001
Nodularia spumigena	Baltic Sea	Natural Bloom	Simulated In Situ	0.0003– 0.0026		0.0017– 0.0129	0.0010– 0.0068				Sorensson and Sahlsten, 1987

(*Continued*)

Table 7.3 Summary of taxon-specific nitrogen (NO_3^-, NO_2^-, NH_4^+, urea, dissolved free amino acids [DFAA], peptides) uptake rates made in natural systems (*continued*)

Species/Taxon	Location	Method	Incubation conditions	NO_3^- (h^{-1})	NO_2^- (h^{-1})	NH_4^+ (h^{-1})	Uptake Urea (h^{-1})	DFAA (h^{-1})	Peptides (h^{-1})	N_2 (h^{-1})	Reference
DINOFLAGELLATES											
Prorocentrum minimum	Chesapeake Bay	Natural bloom	60% Incident light	2.02–30.35[b]		6.20–250.5[b]	0.98–21.22[b]	0.67–6.82[b]			Fan et al., 2003b
Prorocentrum minimum	Nucse River	Natural bloom	60% Incident light	3.27[b]		4.97[b]	5.33[b]	0.34[b]			Fan et al., 2003b
Lingulodinium polyedrum	Baja California	Natural bloom	Simulated *in situ*	480[b]		1010[b]	1321[b]				Kudela and Cochlan, 2000
Gonyaulax polyedra	Baja California	Natural bloom	Simulated *in situ*	0.0010–0.0100		0.0060–0.0120					MacIsaac, 1978
Alexandrium minutum	Penze Estuary, France	Natural bloom	Simulated *in situ*	0.0100–0.0400		0.0013–0.0080					Maguer et al., 2004
Prorocentrum minimum	Chesapeake Bay tributary	Natural bloom/ diel cycle	Simulated *in situ*	0.0052–0.0673		0.0004–0.0292	0.0138–0.0908	0.0014–0.0084	0.00041–0.0012		Mulholland and Lee, in revision
Akashiwo sanguinea	Chesapeake Bay tributary	Natural bloom/ diel cycle	Simulated *in situ*	0.0013–0.092		0.0004–0.06	0.003–0.017	0.0019–0.0186	0.00022–0.0065		Mulholland and Lee, in revision
Cochlodinium sp.	Chesapeake Bay tributary	Natural bloom	Simulated *in situ*				0.0013–0.0220				Mulholland and Lee, in revision
Heterocapsa triquetra	Chesapeake Bay tributary	Natural bloom	Simulated *in situ*	0.0053		0.0092	0.0013	0.0090			Mulholland and Lee, in revision
Gyrodinium cf. aureolum	Ushant frontal region	Natural bloom	Simulated *in situ*	0–0.02		0–0.2					Le Corre et al., 1993
COCCOLITHOPHORIDS											
Emiliania huxleyi	N North Sea	Natural bloom	Simulated *in situ*	0.0040–0.0620		0.05–0.12	0.2600–0.5200				Rees et al., 2002
Emiliania huxleyi	Norwegian Fjords	Natural bloom	Simulated *in situ*	0.0030–0.0450+		0.0270–0.0740+	0.002–0.0480[c]				Fernandez et al. 1996

OTHER AUTOTROPHS									
Phaeocystis	North Sea	Natural bloom	340µE m^{-2} g^{-1}; ambient temp.	0.003–0.005	0.001–0.002	0.0007–0.0014			Tungaraza *et al.*, 2003b
Aureococcus anophagefferens	Quantuck Bay–June	Natural bloom	Simulated *in situ*	0.0071	0.0805	0.0137	0.0222		Mulholland *et al.*, 2002a,b
Aureococcus anophagefferens	Quantuck Bay–Sept	Natural bloom	Simulated *in situ*	0.0075	0.0227	0.0756	0.1274		Mulholland *et al.*, 2002a,b
Aureococcus anophagefferens	Shinnecock Bay	Natural bloom	Simulated *in situ*	0.0150–0.0190	0.0750–0.0980	0.0820–0.0830			Lomas *et al.*, 1996
Aureococcus anophagefferens	Shinnecock Bay	Natural bloom	Simulated *in situ*				0.0098–0.0196		Berg *et al.*, 1997
Aureococcus anophagefferens	Chincoteague Bay 2002	Natural bloom	Simulated *in situ*	0.0016	0.0059	0.6056	0.0228		Mulholland *et al.*, 2004; Boneillo and Mulholland, in preparation
Aureococcus anophagefferens	Chincoteague Bay 2003–diel	Natural bloom	Simulated *in situ*	0.0029–0.0264	0.0006–0.0041	0.0500–0.1699	0.0030–0.0233		Mulholland *et al.*, 2004; Boneillo and Mulholland, in preparation
Autotrophic picoeukaryotes	Sargasso Sea	FCM	Simulated *in situ*	0.0360–0.0400	0.0350–0.0440				Casey *et al.*, 2007
Cryptophytes	Gulf of Riga	Natural bloom	Simulated *in situ*	0.0025	0.0150–0.0200	0.0075–0.0125	0.00015–0.0025		Berg *et al.*, 2003a
BACTERIA									
Natural bacteria	Sargasso Sea	Natural bloom	Dark ambient temp					0.1000–2.0000[d]	Keil and Kirchman, 1999
CILIATE									
Mesodinium rubrum	Peru upwelling	Natural bloom	Dark	0.0006–0.0030	0.0094–0.0142				Wilkerson and Grunseich, 1990

(*Continued*)

Table 7.3 Summary of taxon-specific nitrogen (NO_3^-, NO_2^-, NH_4^+, urea, dissolved free amino acids [DFAA], peptides) uptake rates made in natural systems (*continued*)

Species/Taxon	Location	Method	Incubation conditions	NO_3^- (h^{-1})	NO_2^- (h^{-1})	NH_4^+ (h^{-1})	Uptake Urea (h^{-1})	DFAA (h^{-1})	Peptides (h^{-1})	N_2 (h^{-1})	Reference
Mesodinium rubrum	Peru upwelling	Natural bloom	30 or 50% LD	0.0106–0.0474		0.0114–1.827					Wilkerson and Grunseich, 1990
SIZE-FRACTIONATED											
1µm (bacterial)	Morlaix Bay	Natural bloom	50% Incident light	0.0009	0.0000	0.0034	0.0009				Wafer *et al.*, 2004
1–15µm (pico/ nanoplankton)	Morlaix Bay	Natural bloom	50% Incident light	0.0079	0.0024	0.0160	0.0044				Wafer *et al.*, 2004
3–10µm cells (pico/ nanoplankton)	BoothBay Harbor, ME	FCM	Light	0.0040		0.0370					Lipschultz, 1995
3–10µm cells (pico/ nanoplankton)	BoothBay Harbor, ME	FCM	Dark	0.0010		0.0046					Lipschultz, 1995
10–53µm cells (microplankton)	BoothBay Harbor, ME	FCM	Light	0.0071		0.0170					Lipschultz, 1995
10–53µm cells (microplankton)	BoothBay Harbor, ME	FCM	Dark	0.0010		0.0033					Lipschultz, 1995
15–200µm (microplankton)	Morlaix Bay	Natural bloom	50% Incident light	0.0120[e]	0.0037[++]	0.0010[e]	0.0034[e]				Wafer *et al.*, 2004

[a] pmol colony^{-1} h^{-1}.
[b] fg at nitrogen cell^{-1} h^{-1}.
[c] converted using a C:N ratio of 6.626.
[d] mM h^{-1}.
[e] nmol l^{-1} h^{-1}.
[¥] pmol colony^{-1} h^{-1}.
[+] converted using a C:N ratio of 6.626.
[++] mM h^{-1}.
Measurements reflect data collected when the autotropic community was clearly dominated by a single functional group (e.g., diatoms) or a specifc bloom-forming organism (e.g., *Prorocentrum minimum*) as indicated by ''natural bloom'' in the Methods column. More recently, flow cytometry has been used as a means to separate sub-populations from istopic incubations ('FCM' in Methods column).

several hours (Lomas *et al.*, 2002; Tamminen and Irmisch, 1996). As for NH_4^+, rapid recycling and subsequent isotope dilution can result in underestimates of urea uptake (Bronk *et al.*, 1998; Hansell and Goering, 1989; see also Chapter 31 by Lipschultz, this volume), making even more striking the dichotomy between the low concentrations of urea (and low contribution to total DON pools) and the high uptake rates (Lomas *et al.*, 2002). However, urea isotope dilution measurements are rare and it is not entirely clear when or where isotope dilution would be most important.

Many field studies have presented data suggesting that phytoplankton are the dominant urea consumers in marine systems (e.g., Cho and Azam, 1995; Cho *et al.*, 1996a; Kokkinakis and Wheeler, 1988; Remsen *et al.*, 1972a; Savidge and Hutley, 1977; Savidge and Johnston, 1987; Tamminen and Irmisch, 1996) and that bacteria are the predominant urea producers (Cho *et al.*, 1996a; Jørgensen *et al.*, 1999a). However, we now know that bacteria are both sources and sinks of urea (Cho *et al.*, 1996a; Middelburg and Nieuwenhuize, 2000; Tungaraza *et al.,* 2003a,b). Urea uptake was higher in the bacteria than phytoplankton size fraction in the Thames Estuary (Middelberg and Nieuwenhuize, 2000) and in the North Sea (Tungaraza *et al.*, 2003a; Gilbert *et al.*, 2005). This could be due to the turbid nature of these systems, as urea uptake by phytoplankton has been shown to be light dependent (Lomas, 2004a; Mitamura, 1986; Mitamura and Saijo, 1975, 1980). However, light limitation of urea uptake does not appear to be universal because urea uptake rates in the phytoplankton size fractions were comparable during the day and night during blooms of *Aureococcus anophagefferens* (Boneillo and Mulholland, unpublished data), *Prorocentrum minimum*, and *Akashiwo sanguinea* (Mulholland *et al.*, unpublished data), and natural populations from the Sargasso Sea (Price and Harrison, 1988b). In *P. minimum* cultures, nighttime urea uptake was negligible in nutrient replete cultures but increased when cultures were grown on media with only 10 μM nitrogen (Mulholland *et al.*, unpublished data). Due to the nickel dependence of urease, the enzyme necessary for urea degradation in cells, this element is also thought to limit urea uptake and assimilation in the environment (Milligan and Harrison, 2000; Oliveira and Antia, 1986; Price and Morel, 1991; Rees and Bekheet, 1982; Syrett and Peplinska, 1988).

Although a variety of algal taxa can take up urea, recent work has focused on urea uptake during harmful algal blooms (HABs). The primary motivation behind this has been the long-term changes in organic to inorganic nutrient loading (e.g., Anderson *et al.*, 2002; Gilbert *et al.*, 2005) and a desire to test the hypothesis that this change has favored the selective growth of HABs. However, results have been conflicting. For example, in axenic cultures of *A. anophagefferens*, urea uptake rates were only ~50% of those found in natural bacterial associated with this organism (Berg *et al.*, 2002), consistent with the high bacterial urea uptake rates observed by Middelburg and Nieuwenhuize (2000). In contrast, in natural communities dominated by *A. anophagefferens*, urea uptake in the phyto–plankton size fraction was greater than that measured in the bacterial size fraction (Lomas, unpublished data; Mulholland *et al.*, 2002b, 2004a). During a red tide bloom of *Lingulodinium polyedra* on the California coast urea uptake rates were

twice those of NH_4^+ or NO_3^- uptake (Kudela and Cochlan, 2000). Urea and NH_4^+ uptake rates were comparable during mixed dinoflagellate blooms in the Neuse and Choptank River estuaries (Fan *et al.*, 2003b), while urea uptake rates far exceeded those of other N sources in populations dominated by *Karenia brevis* in the Gulf of Mexico (Mulholland *et al.*, 2006). These findings support the hypothesis that the ability to take up urea and other organic N at enhanced rates may favor the selective growth of some harmful algal species (Anderson *et al.*, 2002).

3.2.2. Amino acids

Uptake of DFAA was formerly thought to be the purview of heterotrophic bacteria, despite early observations that phytoplankton could take up DFAA (North and Stephens, 1967; Wheeler *et al.*, 1974, 1977). Although uptake of DFAA by bacteria can contribute substantially to this group's N demand, uptake of amino acids by phytoplankton was thought to be low because low affinities or uptake capacities for these compounds was demonstrated in culture systems (e.g, high K_s values compared with environmental concentrations; Antia *et al.*, 1991) and theoretically, bacteria have more favorable surface area to volume ratios. The latter, however, has not been evaluated extensively (see Kirchman, 2000) and we now know that uptake is not due to simple diffusion processes. Most studies demonstrating a low affinity for amino acid uptake in phytoplankton were done in cultures where nutrient interactions, preconditioning, and unrealistically high concentrations may have confounded the ability to estimate kinetic parameters in a way that would be representative of natural systems. An increase in the affinity for amino acids has indeed been observed when cells are N-deprived (Antia *et al.,* 1991; Flynn and Syrett, 1985, 1986a; Flynn *et al.*, 1987; North and Stephens, 1972) but, it is difficult to maintain cultures under N deprivation. Few studies have investigated amino acid uptake at concentrations representative of natural waters. In one such study, larger diatoms were able to take up amino acids at lower concentrations than smaller species (Flynn, 1990).

Because of the general perception that amino acids are taken up primarily by bacteria, in nature, uptake of amino acids is generally attributed to bacteria without adequate evaluation of just "who" is actually taking up these compounds. Uptake of organic compounds by bacteria is generally assessed using radio-labeled (^{14}C and ^3H) compounds. In general, phytoplankton ecologists have employed ^{15}N tracer techniques while microbial ecologists employ radio-tracer techniques. Consequently, the bias for organic compounds has been to attribute their uptake to bacteria, regardless of the experimental design and community composition. In contrast to prevailing views, microalgae accounted for more than 50% of glycine uptake in natural populations from coastal waters in the Pacific dominated by nanoplankton (Wheeler *et al.*, 1977). In estuarine environments along the Atlantic coast dominated by pelagophytes and dinoflagellates, the phytoplankton size fraction was responsible for a significant portion of the amino acid N (and C) uptake (Table 7.3; Mulholland, unpublished data; Mulholland *et al.*, 2002b, 2003). Amino acid uptake rates are high in communities dominated by phytoplankton mixotrophs (Mulholland *et al.*, 2002b, 2003, 2004d). In fact, the majority of leucine N and C was taken up by

the >20 μm size fraction during a bloom of the dinoflagellate *Prorocentrum minimum* (Rocha *et al.*, 2004) and this has important implications for evaluating bacterial productivity in natural systems by leucine incorporation (Mulholland *et al.*, in review). Similar results have been observed in the open ocean. Using a combination of radiotracers and flow cytometry, the marine cyanobacteria *Prochlorococcus* (the same size as heterotrophic bacteria and therefore negates the surface area to volume ratio argument) has been shown to account for 25–33% of the total microbial uptake of methionine and leucine (Zubkov *et al.*, 2003, 2004).

Although DFAA concentrations are fairly low in marine and estuarine systems, as for NH_4^+ and urea, their turnover can be quite rapid (Keil and Kirchman, 1991; Middelboe *et al.*, 1995a), and their production has been correlated with *in situ* primary productivity (Bronk *et al.*, 1998; Sellner and Nealley, 1997). As for NH_4^+, uptake and release of DFAA appear to be tightly coupled (Fuhrman, 1987), but not all amino acids are used to the same degree as indicated by the relative differences in the compositions of the intracellular free and combined amino acids and extracellular amino acid pools. Also, microalgal uptake of amino acids may not occur simultaneously with photosynthesis (e.g., Flynn and Butler, 1986). Because the turnover of DFAA can at times exceed estimated bacterial N demand (Fuhrman, 1990; Gardner *et al.*, 1993; Keil and Kirchman, 1991; Kirchman *et al.*, 1989) it is likely that there is either extracellular degradation of DFAA, regeneration of DFAA nitrogen as NH_4^+ (Cotner and Gardner, 1993), or the uptake of DFAA by organisms other than bacteria.

A variety of phytoplankton are known to possess cell surface oxidases (Palenik and Morel, 1990a,b) and extracellular oxidation of amino acids has been shown to occur in nature (Pantoja and Lee, 1994; Mulholland *et al.*, 1998, 2002a). Direct uptake of amino acid-derived N from this process represented up to 4% of the observed NH_4^+ uptake in a mid-Atlantic estuary (Mulholland *et al.*, 2003). Recently, a cell surface protein expressed under N-limitation was identified as a deaminase suggesting that these enzymes are regulated by cellular N status as for other pathways of N uptake and metabolism (Palenik and Koke, 1995). Failure to account for alternative pathways for mobilization of DON might result in underestimates of its utilization in nature.

3.2.3. Peptides/proteins

Together DFAA and DCAA are thought to account for about 50% of the bacterial N demand (Coffin, 1989; Keil and Kirchman, 1991; Kirchman, 2000; Middelboe *et al.*, 1995a) and about 25% of the bacterial C demand in estuarine and coastal systems (Middelboe *et al.*, 1995a). DCAA concentrations are higher than those of DFAA, and estuarine DCAA can account for up to 13% of total DON (Keil and Kirchman, 1991). DCAA lability can vary greatly since they are comprised of a mixture of peptides, proteins and other amino acids that require acid hydrolysis to release them from their chemical or physical matrix. Moreover, many amino acids may be trapped in refractory matrices preventing their mobilization in natural environments.

In living phytoplankton and zooplankton cells, proteins account for a major fraction of the cellular C and most of the N (Dortch *et al.*, 1984; Kirchman, 2000;

Nguyen and Harvey, 1994). We therefore expect organic matter released from living organisms to contain protein; the predominance of amide N in the high molecular weight (HMW) DOM pool seems to confirm this, and in fact, proteins have now been identified in seawater (Suzuki *et al.*, 1997; Tanoue, 1995; Tanoue *et al.*, 1996; Yamada and Tanoue, 2003). The relatively low contributions of combined amino acids to the total N pool suggest that: (1) protein N is not the dominant form of HMW-DON, (2) protein and peptide N is rapidly degraded and consumed in the environment, and/or (3) that analytical methods currently being employed, other than NMR, underestimate the contribution of proteins to this fraction of the DOM pool (see Chapter 3 by Aluwihare and Meador, this volume). In seawater, most microbes and phytoplankton can take up only inorganic or small organic compounds (Antia *et al.*, 1991; Nikaido and Vaara, 1985). The use of larger organic compounds, such as peptides and proteins, by microorganisms is often limited by the size of the compound. However, a variety of microorganisms including phytoplankton (Berg *et al.*, 2002; Mulholland *et al.*, 2002a, 2003; Stoecker and Gustafson, 2003) and bacteria (Chrost, 1991; Hoppe, 1983; Hoppe *et al.*, 2002) have extracellular proteases and hydrolases (Chapter 32 by Berges and Mulholland, this volume). For example, axenic *Aureococcus anophagefferens* (pelagophyceae) cultures hydrolyzed aminopeptides and chitobiose at rates that are significant relevant to bacterial extracellular hydrolysis (Berg *et al.*, 2002). There do not appear to be any studies of the direct uptake (by diffusion) of polypeptides by phytoplankton or microalgae (Antia *et al.*, 1991), although a dipeptide, glycylglycine was reported to be a good N source for the growth of three marine prymnesiophytes (Turner, 1979). Recent work also suggests that uptake of dialanine can be significant in populations dominated by a variety of bloom-forming mixotrophic phytoplankton (Mulholland and Lee, in revision).

3.2.4. Other organic compounds

Only 5% to 18% of bulk DON has been identified as urea, DFAA or DCAA, proteins/peptides, however, it is estimated that anywhere from 12% to 72% of DON is bioavailable in marine and estuarine systems (Bronk, 2002 and references therein). Consequently, compounds other than those discussed above (e.g., amino sugars, purines, pyrimidines, and nucleic acids) are likely available to bacteria and/or phytoplankton, either directly or indirectly through bacterial regeneration of these substrates to NH_4^+, urea or amino acids, however, little is known about their uptake characteristics (Antia *et al.*, 1991). Recent discoveries of the genes encoding all enzymes for a complete urea cycle in a diatom (Armbrust *et al.*, 2004), genes encoding proteins capable of cyanate metabolism in cyanobacteria (Garcia–Fernandez *et al.*, 2004), and the ability of *Synechococcus* to grow on cyanate as a sole source of N (Palenik *et al.*, 2003) suggests that we are only beginning to understand the capacity of marine microbes to utilize DOM in nature.

Bulk DON uptake by microorganisms has been examined using a bioassay approach (Stepanauskas *et al.*, 1999a,b; Berg *et al.*, 2003b) as well as by synthesizing ^{15}N-labeled DON (Bronk and Glibert, 1993). Berg *et al.* (2003b) observed that 25–36% of the available N in HMW-DON, isolated from shallow eutrophic sediments, was depleted during growth of axenic cultures of *Aureococcus anophagefferens*. Bulk

DON uptake by natural populations of Chesepeake Bay phytoplankton often exceeded rates of combined NH_4^+ and NO_3^- uptake during spring and summer with uncharacterized DON uptake (i.e., not urea) equivalent to about 12% of the NH_4^+ uptake rates (Bronk and Glibert, 1993). In addition, high molecular weight humic material is thought to represent a significant source of N in some systems (Carlsson et al., 1998; Legrand and Carlsson, 1998; Stolte et al., 2002). To date, culture and field studies have primarily investigated DOM uptake by microplankton and so there are very few studies examining DOM uptake by nanoflagellates or picoplankton (Lewitus, 2006).

Besides supplying N, other advantages of DOM uptake might also include access to organically-bound trace metals, augmentation of autotrophic C acquisition, and access to P. Heterotrophic capabilities appear to be widespread in pennate but not centric diatoms (Lewitus, in press), dinoflagellates, chrysophytes, cryptophytes, and other algal taxa. Osmotrophy, the uptake of DOM, has not been widely assessed in marine phytoplankton (but see Stoecker, 1999), however, among dinoflagellates, phagotrophy is common (Anderson et al., 2002; Burkholder et al., 1997; Carlsson et al., 1998; Graneli and Moreira, 1990; Lewitus, in press; Mahoney and Mclaughlin, 1977). Indeed, direct uptake of HMW-DOM (>1 kD) has been hypothesized as a "niche" for photo- and heterotrophic flagellates in direct competition with bacteria (Legrand and Carlsson, 1998; Marchant and Scott, 1993; Schuster et al., 1998; Sherr, 1988; Tranvik et al., 1993). Although there is still only a limited amount of information regarding the use of "uncharacterized" DOM by natural communities, this may be a significant source of N to phytoplankton and bacteria in nature.

3.3. Nutrient interactions

3.3.1. Nutrient/nutrient interactions

The interaction between NH_4^+ and NO_3^- was reviewed by Dortch (1990). Several conclusions were drawn that continue to be valid more than a decade later: (1) ambient concentrations of NH_4^+ can reduce, but do not completely inhibit, NO_3^- uptake and, (2) the concentration of NH_4^+ at which NO_3^- uptake is depressed varies greatly between phytoplankton species (for example, compare Collos et al., 2004; Dortch et al., 1991a) and with nutrient prehistory (Dortch et al., 1991a). Furthermore, the bulk of the evidence suggests that the repression in NO_3^- uptake rates by NH_4^+ is not direct, but rather mediated by organic metabolites, likely glutamine or the glutamine:glutamate or glutamine:α-ketoglutarate ratio, which are ultimately related to the overall N status of the cell (e.g., Flynn, 1990; Flynn et al., 1989; Page et al., 1999; Stephens et al., 2003).

Field studies continue to support the conclusion of Dortch (1990), that the effect of NH_4^+ on NO_3^- uptake is highly variable. For example, NO_3^- uptake rates in natural assemblages dominated by the coccolithophorid Emiliania huxleyi from the subarctic NE Pacific Ocean were reduced by 50% at NH_4^+ concentrations of 0.24 μM with nearly complete inhibition at 2.2 μM (Varela and Harrison, 1999a). Based on the average ambient NH_4^+ concentrations during these blooms, this translates into a 38–70% reduction in NO_3^- uptake rates, the majority of which occurred

during the daylight period. Similar reductions in NO_3^- uptake were negatively correlated with NH_4^+ concentration in the subarctic Pacific (Wheeler and Kokkinakis, 1990). Likewise, for the picoplankter, *Micromonas pusilla*, NH_4^+ concentrations of 1–10 μM inhibited NO_3^- uptake (Cochlan and Harrison, 1991a). A more extreme example comes from the Tagas estuary (Portugal) where NH_4^+ concentrations in excess of 10 μM only reduced NO_3^- uptake rates by 24%, with lower NH_4^+ concentrations exhibiting no significant impact on NO_3^- uptake (Cabrita *et al.*, 1999). These results attest to the variability in the response of populations to the dominant source of combined N.

Other environmental variables such as temperature, light, and availability of trace metals and other macro- and micronutrients (see Chapter 37 by Kudela, this volume and Chapter 38 by Hutchins and Fu, this volume) also affect nutrient interactions and the effects vary depending on the dominant species. As growth temperature decreased, so to did the extent to which NH_4^+ inhibited NO_3^- uptake for the diatoms, *Chaetoceros* sp. and *Thalassiosira weissflogii*, and the dinoflagellates, *Prorocentrum minimum* and *Gyrodinium uncatenum* (Lomas and Glibert, 1999a,b). This may explain the higher than expected rates of NO_3^- uptake during the winter/spring period when NH_4^+ concentrations are high in estuarine and coastal systems (e.g., Chesapeake Bay). The may not hold true for summer assemblages in Chesapeake Bay. During a bloom of the dinoflagellate, *Prorocentrum minimum*, there was a positive relationship between temperature and the affinity for NH_4^+ and amino acids and a negative relationship between temperature and affinity for NO_3^- (Fan *et al.*, 2003b). In addition to temperature, Yin *et al.* (1998) showed that NH_4^+ inhibition of NO_3^- uptake was light dependent. In light–limited cultures, there was no short-term NH_4^+ inhibition of NO_3^- uptake by the diatom, *T. pseudonana*, while under saturating and moderate light NO_3^- uptake was reduced by 70% and 62%, respectively, by NH_4^+. Confounding effects from multiple environmental variables and stressors complicate our understanding of nutrient interactions in the natural environment.

Like NO_3^- uptake, N_2 fixation (and the development of heterocysts) is thought to be inhibited by NH_4^+. This conjecture is based on a variety of field and laboratory studies. However, most laboratory studies employ unrealistically high NH_4^+ concentrations (>100 μM) (e.g., El-Shehawy *et al.*, 2003; Ohki *et al.*, 1991). In recent studies conducted under oligotrophic conditions (in terms of N), N_2 fixation by *Trichodesmium* spp., proceeded simultaneously with the uptake of NH_4^+ (Mulholland and Capone, 1999, 2000, 2001; Mulholland *et al.*, 2001, 2004b) suggesting that this view needs to be revisited in open ocean environments where inorganic N concentrations are rarely above 1 μM, and most often at or near the analytical detection limit, and there is tight coupling between NH_4^+ production and uptake (Mulholland *et al.*, 2001, Sunda personal communication).

Several studies have also been conducted to explore interactions between urea and uptake of other N substrates. In N sufficient cultures of the diatom *Thalassiosira pseudonana* and *T. weissflogii* (growing in NO_3^- replete media) the addition of urea partially inhibited NO_3^- uptake (Lomas, 2004b; Price and Harrison, 1988a) and urea additions of 5 μM resulted in 28% reduction of NO_3^- uptake by *Micromonas pusilla* (Cochlan and Harrison, 1991a). However, for *T. weissflogii*, the reduction in

NO_3^- uptake by urea was different than that observed upon the addition of NH_4^+ (Lomas, 2004b). Addition of NH_4^+ to urea-grown cultures of the diatoms *T. weissflogii, T. pseudonana* and *Skeletonema costatum* decreased urea uptake rates by more than 30% (Horrigan and McCarthy, 1982; Lomas, 2004b). Short-term additions of either NH_4^+ or NO_3^- did not alter urea uptake rates in natural phytoplankton populations from the Baltic Sea, although inhibition of urea uptake by NH_4^+ was observed after one day (Tamminen and Irmisch, 1996). In the Oslofjord, measured urea uptake rates were inversely related to ambient NH_4^+ concentrations (Kristiansen, 1983), while in the northeast Pacific Ocean, high NO_3^- concentrations ($>20\ \mu M$) led to higher urea uptake rates than under low NO_3^- conditions in the 'diatom' fraction but not in the nanoplankton fraction (Kokkinakis and Wheeler, 1988). Lund (1987) observed that for *S. costatum* the following combinations of N sources showed mutual interference on uptake rates: NH_4^+ on NO_3^-, NH_4^+ + urea on NO_3^-, urea on NH_4^+, NO_3^- + urea on NH_4^+, NO_3^- on urea, NH_4^+ on urea, and NH_4^+ + NO_3^- on urea, whereas there was no mutual interference of NO_3^- on NH_4^+ and of urea on NO_3^-. Moreover, summed uptake rates for oxidized (NO_3^-) and reduced N sources (urea, NH_4^+) greatly exceeded the rate necessary to maintain growth rates suggesting N uptake in excess of that necessary for growth. In studies of the marine N_2 fixer, *Trichodesmium*, uptake of N_2 could account for net growth although NH_4^+ uptake accounted for up to 80% of the N turnover in culture vessels (Mulholland and Capone, 1999, 2001). This finding is consistent with a number of field studies where summed N uptake greatly exceeds the *in situ* growth rate and suggests that gross N productivity exceeds net community growth.

As for other forms of N, NH_4^+ was thought to inhibit uptake of DFAA but this does not appear to be universally true (see Antia *et al.*, 1991 and references therein). Amino acid uptake by phytoplankton was not inhibited by the presence of NH_4^+, NO_3^-, NO_2^-, urea or other amino acids in several studies (Flynn and Syrett, 1986b; Flynn and Wright, 1986; Flynn *et al.*, 1987). Similarly, Goldman and Dennett (2000) found that the presence of NH_4^+ did not inhibit amino acid uptake by bacteria. In fact, the relative assimilation of DFAA, DCAA and NH_4^+ varied between C and N limited cultures and during exponential versus stationary phase growth suggesting that physiological status plays a role in determining nutrient uptake capacity (Middelboe *et al.*, 1995a).

In nature, a diverse suite of N compounds are often available and can be taken up. Clearly nutrient interactions are complex and vary between environments and the organisms inhabiting them. Nitrogen uptake varies as a result of physiological adaptations of particular organisms to the environment, their physiological state, and the degree to which they are acclimated to their changing environment. One could argue that organisms are adapted to the nutrient environment in which they live and the timescales of natural variations characteristic of that environment.

3.3.2. Environmental interactions
Light is a key environmental factor having an impact on nearly all phytoplankton physiological processes; N uptake is no different. Nutrient uptake versus irradiance curves often show a hyperbolic relationship that have been mathematically fit by

both a Michaelis-Menten type model and Jassby-Platt type photosynthesis model (e.g., Cochlan et al., 1991b; Hu and Smith, 1998; Kudela and Cochlan, 2000; Kudela et al., 1997; Lim et al., 2006; Muggli and Smith, 1993; Paasche et al., 1984; Priscu et al., 1991). Photoinhibition of N uptake has been observed (e.g., Hu and Smith, 1998; Planas et al., 1999), but it is far from universal. What is universal, however, is the need to include a term for dark N uptake. Unlike, dark photosynthetic C fixation, dark N uptake can be anywhere from 10–100% of the uptake rate measured in the light, and this varies with physiological condition of the resident population, N source, and phytoplankton species. Indeed, the ratio of dark uptake to light uptake ($V_D:V_L$) is often used as a proxy for nutrient status. Values close to unity suggest that the studied population is N stressed, and therefore assimilating all N that is encountered. For example, Gyrodinium aureolum did not take up NO_3^- in the dark under replete growth conditions, but when NO_3^- starved, $V_D:V_L$ values approached 0.5, with no significant increase in light uptake rates (Paasche et al., 1984). Consistent with laboratory studies on Prorocentrum minimum (Paasche et al., 1984), natural bloom populations of Prorocentrum minimum displayed a $V_D:V_L$ ratio of ~0.5 (Fan and Glibert, 2005). This is not always the case however, as MacIsaac (1978) observed strong light dependence of NO_3^- and NH_4^+ uptake in Gonyaulax polyedra ($V_D:V_L$ range 0–0.3), whereas Kudela and Cochlan (2000) found $V_D:V_L$ values >0.5 for NO_3^-, NH_4^+ and urea.

In addition to diel variability in N uptake rates, there are also differences between N substrates in the irradiance level where uptake saturates. Interestingly, Kudela and Cochlan (2000) observed that NO_3^- uptake by Gonyaulax polyedra saturated at the lowest irradiance (~3% of incident irradiance), whereas NH_4^+ (7%) and urea (12%) uptake saturated as much higher irradiances. Natural phytoplankton populations along an upwelling gradient displayed similar values for NO_3^- (5%) and NH_4^+ (2%) uptake (Kudela et al., 1997). The light dependence of measured N uptake response is also related to the pre-conditioning of the population being studied. For example, populations collected from the base of the euphotic zone, and therefore likely acclimated to low light, display much less light dependence of NO_3^- and urea uptake, presumably due to reduced capacity for uptake at higher irradiances (e.g, Cochlan et al., 1991b).

One characteristic of all of these previous studies is that samples were collected during the light period and then incubated in the dark to determine the dark N uptake rates. Fundamentally, dark N uptake relies on previously stored photosynthate and therefore it is possible that the dark uptake rates measured in the above studies are higher than if N uptake were measured during night time. Some of the earliest studies on nighttime NO_3^- uptake measured very low rates (e.g., MacIsaac, 1978; MacIsaac and Dugdale, 1969, 1972). Recent work in more oligotrophic environments has shown that nighttime NO_3^-, NH_4^+ and urea uptake rates, like daytime populations incubated in the dark, show $V_D:V_L$ ratios of 0.3–1.0 (e.g., Lipschultz, 2001; Price and Harrison, 1988b; Tremblay et al., 2000). As expected, some of this variability is related to N limitation. Natural picoplankton populations in the Sargasso Sea have $V_D:V_L$ values for NO_3^- uptake >0.5 when ambient NO_3^- concentrations are below 30 nM, but <0.1 when ambient NO_3^- concentrations exceed 500 nM (Lomas et al., in review). It is apparent that the

interaction between light and N uptake is very complicated and unifying rules for all phytoplankton at all growth states are not yet developed.

3.4. Phytoplankton diversity and nitrogen uptake

There is a diverse and growing body of results from culture studies indicating that phytoplankton diversity is an important variable when it comes to understanding N cycling in the marine environment. There is also quite a bit of information on N uptake from natural systems during monospecific blooms or from flow cytometrically sorted populations (Table 7.3). Studies have indicated that there is preferential utilization of NO_3^- by diatoms in coastal and estuarine environments (Lomas and Glibert, 1999a,b, and references therein), that *Prochlorococcus* strains are unable to grow on NO_3^- (Moore *et al.*, 2002b), and that low DIN and high DON selectively favors the growth of harmful algal species (Anderson *et al.*, 2002). The latter observation has received the most attention and is discussed further.

Harmful algal blooms (HABs) appear to be increasing in their frequency and duration in coastal systems around the world as a result of cultural eutrophication (GEOHAB, 2001; Glibert *et al.*, 2005a,b; Chapter 11 by Paerl and Piehler, this volume). The special case of HABs has been the subject of much recent research on N cycling in coastal systems (see review by Anderson *et al.*, 2002 for further details). In particular, DON has been implicated as a nutrient source that may preferentially stimulate harmful algal blooms (HABs) (e.g., Anderson *et al.*, 2002; Graneli *et al.*, 1999). Many HABs are known mixotrophs and can acquire C both autotrophically and heterotrophically through the assimilation of organic molecules. Because DON also contains C, using DON as an N source can offer a metabolic supplement to strictly autotrophic C assimilation.

Many harmful algal taxa are not strong competitors for inorganic N sources in nature and their uptake kinetics for inorganic N sources are 'average'. For example, *Alexandrium catenella* was not a strong competitor for NO_3^-, NO_2^-, NH_4^+, or urea at low concentrations ($<10\ \mu M$ N) compared with other phytoplankton, but labile organic N compounds could be important in triggering blooms (Collos *et al.*, 2004). The brown tide organism, *Aureococcus anophagefferens*, has been shown to grow on organic substrates, in cultures, and to take up and grow on organic compounds, including urea, in nature (Berg *et al.*, 1997; Gobler and Sanudo-Wilhelmy, 2001; Gobler *et al.*, 2002; Lomas *et al.*, 2004; Mulholland *et al.*, 2002b, 2004a). In contrast to *A. catenella*, *A. anophagefferens* has demonstrated equally high affinity for NH_4^+ and urea in culture and natural systems (Lomas *et al.*, 1996; Mulholland *et al.*, 2002b).

Growth of the red tide dinoflagellate, *Karenia brevis*, has been linked to N compounds, including DON, released from the marine N_2 fixer, *Trichodesmium* (Bronk *et al.*, 2004; Mulholland *et al.*, 2004c). Further, *Prorocentrum minimum* and *Pfiesteria* sp. are thought to consume DON and have been linked to systems with high DOC:DON ratios (Glibert *et al.*, 2001). However, both of these species can use a range of N sources in cultured and natural systems (Fan *et al.*, 2003b; Lewitus *et al.*, 1999). In a seasonal study of N utilization by bloom-forming

species in a lower Chesapeake Bay subtributary, the dominant N source varied seasonally as the bloom species changed, over the course of individual blooms, and over diel cycles suggesting substantial metabolic flexibility in N acquisition (Mulholland *et al.*, in preparation). So, although organisms appear to be adapted to where they live, the overall metabolic flexibility of organisms may be important when a diverse array of nutrients are delivered at a variety of temporal and spatial scales.

4. Pathways of Nitrogen Uptake and Assimilation

Up to this point, we have discussed primarily uptake of N with respect to the extracellular environment. However, N uptake and assimilation are stepwise processes that can result in uncoupling between uptake and growth (e.g., Wheeler, 1983) and, as alluded to above, feedback from intracellular metabolites may be important regulators of N uptake from the environment. In addition to feedback regulation, most of the enzymes involved in the uptake and assimilation of N are tied to energy sources and so are affected by light, the presence of oxygen, and the supply of enzyme co-factors and metabolic substrates. For example, uptake and reduction of NO_3^-, NO_2^-, and urea have been linked to the light supply in phytoplankton due to the necessity for ATP and NADPH from photophosphorylation. While uptake and reduction of these compounds is thought to proceed at maximum rates only in the light under nutrient replete conditions, when N is depleted, active uptake of these compounds may occur even in the dark (Antia *et al.*, 1991).

4.1. Nitrogen transport and assimilation

Nitrogen can enter cells via passive diffusion, but under low nutrient, oligotrophic conditions concentration gradients across the cell membrane might actually result in a net efflux of nutrients from the cell. Because most N sources are present at low concentrations in marine environments, they are generally taken up via active membrane transport systems and permeases. A variety of transporters, transporter complexes, and permeases have been identified that facilitate the uptake of inorganic and organic N and its concentration in cells (see Chapter 32 by Berges and Mulholland, this volume).

Generally, there are several transporters and uptake systems for common N compounds. For example, permeases or other transporters seem to be necessary for uptake of NH_4^+ at low concentrations (<1 μM; most oligotrophic environments) (Herrero *et al.*, 2001). Genes encoding permeases have been found and one (*amt1*) seems responsible for most transport when there are micromolar concentrations (Herrero *et al.*, 2001; Montesinos *et al.*, 1998). More recent evidence in the marine diatom *Cylindrotheca fusiformis* suggest that there are two subgroups of NH_4^+ transporters, *amt1* and *amt2*, with multiple copies of each (Hildebrand, 2005).

Expression of both transporters are increased during growth on NO_3^-, although differentially, and it is suggested that *amt2*, might actually function as an N sensor rather than an efficient transporter. Research into NO_3^- transporters (NAT) in marine phytoplankton have shown that they are induced under both N starvation and growth on NO_3^- enriched medium (Hildebrand and Dahlin, 2000b). In cyanobacteria an inducible ABC-type permease, encoded by the gene cluster *urtABCDE*, is required for the uptake of urea at low concentrations (<1 μM) even though the internal enzyme to metabolize urea is constitutively expressed (Valladares *et al.*, 2002). More detailed information on the genes encoding for N transporters in marine phytoplankton can be found in Chapter 30 by Jenkins and Zehr in this volume.

Although active transport is energetically costly, uptake is more rapid than passive diffusion (Neame and Richards, 1972), the result being that many metabolites are stored internally. The diatoms are perhaps the best known for this due to their large internal vacuole space. Estimated intracellular concentrations of many N substrates (e.g., NH_4^+, NO_3^-, NO_2^-, urea, amino acids) can be in excess of millimolar concentrations (e.g., Dortch *et al.*, 1984; Lomas and Glibert, 2000; Lourenco *et al.*, 1998). It has been hypothesized that unicellular cyanobacteria, *Synechococcus*, may use its N-rich phycobilipigments as a storage pool during times of 'plenty' (Barlow and Alberte, 1985; Wyman *et al.*, 1985), although the existence of a photosynthetic and a non-photosynthetic phycoerythrin pool has been debated (Kana and Glibert, 1987a,b) along with the ability of *Synechococcus* to mobilize photosynthetically coupled phycoerythrin upon N starvation (Glibert *et al.*, 1986).

Because common intermediates can accumulate intracellularly, uptake of N is sensitive to both intracellular nitrogen pools and the composition and concentrations of extracellular N sources. As discussed above, the presence of NH_4^+ in the growth medium can differentially inhibit the uptake of different N compounds, and although this is likely a result of the different energetic demands for their reduction, the inhibitory effect of NH_4^+ is more often thought to be exerted through feedback from intracellular pools resulting from its assimilation (e.g., Flores and Herrero, 2005). NH_4^+ is a common intermediate to inorganic N reduction and glutamine (gln) and glutamate (glu) are the primary products of N assimilation. These common intermediates may be important in regulating N uptake and assimilation through feedback from product pools and the glutamine:glutamate ratio (Flynn, 1991; Chapter 30 by Jenkins and Zehr, this volume).

Inorganic N is taken up by marine microorganisms, primarily via active membrane transport, reduced to NH_4^+, and then assimilated by microorganisms into amino acids through the glutamine synthetase (GS)/glutamate synthase (GOGAT) pathway (Bressler and Ahmed, 1984; Miflin and Lea, 1980; Fig. 7.2). Glutamate dehydrogenase (GDH) does not represent a significant pathway for NH_4^+ assimilation in most plants (e.g., Suzuki and Knaff, 2005), but is an important enzyme involved in regeneration of N from zooplankton (see Chapter 8 by Bronk and Steinberg, this volume). Because of the added energy and reductant necessary to reduce NO_3^-, NO_2^-, and N_2 to NH_4^+, NH_4^+ was thought to be the N source

preferentially assimilated by phytoplankton (e.g., Eppley and Thomas, 1969, but see Thompson *et al.*, 1989). Stepwise reduction of NO_3^- to NH_4^+ requires nitrate (NR) and nitrite (NiR) reductases and reduction of N_2 to NH_4^+ requires the enzyme complex dinitrogenase reductase (NA). The reduction of NO_3^- to NO_2^- is mediated by NR while the reduction of NO_2^- to NH_4^+ is mediated by NiR, the former is thought to be the rate-limiting step of the enzyme pair and therefore, intracellular accumulation of NO_2^- during NO_3^- assimilation is thought to be minimal. NR appears to be both constitutive and inducible in marine microbes. In addition, there appears to be NH_4^+ promoted repression of NR both at the level of gene expression and enzyme activity (Berges, 1997; Flores and Herrero, 2005).

   Intracellular hydrolysis of urea and its ultimate assimilation occurs through one of two enzymatic pathways: urease or ATP:urea amidolyase. The later has been demonstrated to exist exclusively in the chlorophyte algae (Leftley and Syrett, 1973) and involve the ATP-dependent catalysis of urea to allophanate and urea carboxylase (Bekheet and Syrett, 1977). Urease is widespread in chromophytic algae (Oliveira and Antia, 1986) and involves the nickel-dependent hydrolysis of urea into two ammonia molecules and carbon dioxide. Urease activity appears to be constitutive in a number of marine diatoms such as *Cyclotella cryptica* (Oliveira and Antia, 1986), *Thalassiosira pseudonana* (Peers *et al.*, 2000), and *T. weissflogii* (Lomas, 2004b; Milligan and Harrison, 2000; Peers *et al.*, 2000) but appears to be N-regulated in other cases. There does appear to be species differences in the degree of constitutive expression versus upregulation of urease activity depend-ing upon the form of N in the growth medium. For example, in *T. pseudonana* but not *T. weissflogii* urease activity was upregulated 4- and 8-fold in cultures grown on NO_3^- and urea relative to those grown on NH_4^+ (Peers *et al.*, 2000). In contrast, cell-specific urease activities were higher in *Synechococcus* WH7805 growing on NO_3^- relative to cells grown on either NH_4^+ or urea (Collier *et al.*, 1999), while *Aureococcus anophagefferens* and *Prorocentrum minimum* exhibited high urease activities regardless of the N source (Fan *et al.*, 2003a). The dinoflagellate *Alexandrium fundyense* displayed inducible urease activity, although no urease activity was observed in NO_3^--replete cultures, and only very low activity was observed when grown on NH_4^+; once cultures became N limited, urease activity increased (Dyhrman and Anderson, 2003).

   Microalgae that use DFAA require active transport mechanisms because passive diffusion at environmentally relevant concentrations is theoretically unlikely (Antia *et al.*, 1991; Raven, 1980). Most algae that take up amino acids use them as N sources after catabolizing them or incorporate them directly into proteins (Antia *et al.*, 1991). There appear to be at least three different transport systems for amino acid uptake corresponding to the net charge of the amino acids well as "general" amino acid transport systems (see review by Antia *et al.*, 1991). The ability to take up multiple amino acids through common pathways and the range of apparent turnover times for individual amino acids complicates our interpretation of uptake studies that employ amino acids either singly or in combination. Amino acid uptake by microalgae can be induced by both N deprivation (Flynn and Syrett, 1985; North and Stephens, 1971, 1972; Wheeler *et al.*, 1974) and C deprivation

(e.g., during the dark period; Flynn and Butler, 1986), and the induction time appears to be influenced by the size of the intracellular amino acid pool. Despite the resurgence of interest in amino acid uptake by phytoplankton, little is known about its regulation.

Once taken up, amino acids appear to be stored in two separate intracellular pools: a storage pool and a metabolic pool. Intracellular and extracellular amino acid pools differ in their composition suggesting that amino acids are not taken up directly into the storage pool; rather they are first metabolized by cells. In fact, the composition of the intracellular amino acid pool varies with physiological status and the relative sizes of the glutamine and glutamate pools have been used to evaluate the physiological status of microalgae (Flynn, 1990; Flynn and Al-Amoudi, 1988; Mulholland and Capone, 1999; Mulholland et al., 1999), with higher intracellular gln:glu ratios characteristic of N replete cells.

4.2. Nitrogen uptake kinetics

The kinetics of N uptake by marine phytoplankton have been thoroughly reviewed (McCarthy, 1981), and differences between internally (Droop model) and externally controlled nutrient uptake models articulated (Goldman and Glibert, 1983). However, until recently, methodological constraints have limited the ability to accurately determine half-saturation concentrations in the open ocean where concentrations of most N compounds are nearly an order of magnitude lower than analytical capabilities (e.g., Goldman and Glibert, 1983). Analytical techniques have improved over the past 20 years (e.g., Garside, 1985; Zhang, 2000), but relatively few new studies have used these techniques to reevaluate half-saturation concentrations in the open ocean. Harrison et al. (1996) determined half-saturation constants (K_s) for natural phytoplankton assemblages for NO_3^- and NH_4^+ uptake on several cruises throughout the North Atlantic, and confirmed that K_s values in the open ocean were commonly <40–60 nM for both substrates. A similar conclusion was reached for natural populations in the Sargasso Sea (Lipschultz, 2001), and in several culture studies (Garside, 1991; Raimbault et al., 1990; Sunda, unpublished data) corroborating field studies.

Perhaps the most significant advance in the study of phytoplankton uptake kinetics is not that there are more data available, but rather a change in 'philosophy' with respect to NO_3^- uptake at environmentally relevant concentrations (Collos et al., 2005). Nearly all aquatic biogeochemical models use a 'classical' kinetics formulation to describe N uptake (e.g., Fasham et al., 1990; Moore et al., 2002a), but recent data for coastal systems suggests that this may not be entirely appropriate, and indeed may result in significant underestimates of NO_3^- - assimilation. Estuarine and coastal NO_3^- concentrations far exceed those in the open ocean, and not surprisingly, it appears that the characteristics of NO_3^- uptake kinetics differ between these two regions. When NO_3^- uptake kinetic experiments are conducted over the range of ambient NO_3^- concentrations, linear and biphasic uptake kinetics, in addition to classical uptake kinetics, have been observed. Half-saturation concentrations are difficult to estimate when linear and biphasic uptake kinetics are observed, but have generally fallen in the range of 1–10 μM (Cochlan and Bronk,

2001; Lomas and Glibert, 2000). During an annual study of NO_3^- uptake kinetics in a coastal Mediterranean lagoon Collos et al. (Collos et al., 1997) observed a shift from classical uptake kinetics to linear kinetics as the population shifted from being dominated by the smaller diatom *Chaetoceros* spp. to dominace by the larger diatom *Thalassiosira*. It has been hypothesized that linear uptake rates are driven by an active uptake mechanism to fill storage vacuoles (Collos et al., 1997; Lomas and Glibert, 2000). Winter/spring diatom populations in Chesapeake and Delaware Bays, dominated by *Rhizoselenia* and *Skeletonema* displayed biphasic kinetic patterns (Lomas and Glibert, 1999) and this may be an adaptation to a patchy or sporadic nutrient supply (e.g., Serra et al., 1978). Cultures (Collos et al., 1982; Lomas and Glibert, 2000) and natural estuarine populations of flagellates and dinoflagellates (Fan et al., 2003b) have also been shown to display biphasic and linear uptake kinetics. Collos et al. (2005) have summarized all of the existing data on multiphasic NO_3^- uptake and present a very convincing argument that there is indeed some degree of half-saturation concentration acclimation to elevated ambient NO_3^- concentrations.

Because kinetic parameters are not simply due to the inherent capabilities of cells, but also reflect the physiological status and nutrient prehistory it is often difficult to relate kinetic measurements to growth rates in the field where these things are unknown at the time of sampling. Consequently, determination of intra- versus extracellular controls on uptake and the taxa-specific differences in uptake capabilities have come largely from culture studies, which lack the complexities of natural systems. For example, because cultured dinoflagellates have generally higher K_s values for DIN than diatoms, it is assumed that they are poor competitors under DIN-rich conditions (e.g., Smayda, 1997). But, this is not always observed in nature. Many organisms can modulate their internal nutrient quotas and their short-term nutrient uptake rates in response to external nutrient concentrations (Droop, 1973; Morel, 1987). Large differences in half-saturation concentrations for growth versus short-term uptake may allow organisms to be more competitive under a wider range of nutrient conditions. So, even though kinetic data provide useful snapshots of cellular capabilities in the field and meaningful ''yardsticks'' of taxa-specific capabilities in culture systems, it is important to keep in mind the limitations of these data for making broad inferences in natural systems.

There is little information on uptake kinetics for organic N compounds and what is available is largely from culture systems where growth conditions may be unrepresentative of natural systems (Antia et al., 1991). The kinetics of urea uptake have been examined in natural systems but K_s values vary widely. Low K_s values (<2 μM) were observed in a range of environments from the Ross Sea (Cochlan and Bronk, 2001), to the mid-Atlantic Neuse estuary experiencing a mixed dinoflagellate bloom (Fan et al., 2003a,b), to a northeastern US coastal bay experiencing a bloom of *Aureococcus anophagefferens* (Lomas et al., 1996), and a mixed autotrophic community from the Baltic Sea (Tamminen and Irmisch, 1996). Much higher K_s values (5 to >30 μM) were observed in natural populations dominated by *P. minimum* in the Choptank River estuary (Fan et al., 2003b) and in *Trichodesmium* spp. collected from the tropical North Atlantic Ocean (Mulholland and Capone, 1999). Cultures of *P. minimum* and *Trichodesmium* species had much lower K_s values for urea uptake.

Although the number of representative species is limited, the available data suggests that autotrophic phytoplankton have consistently lower K_m values for urease than do bacteria (cf. Fan *et al.*, 2003a; Jahns, 1992; Mobley and Hausinger, 1989; Syrett and Al-Houty, 1984). Interestingly, studies on urea biogeochemistry commonly observe that bacteria are predominantly urea regenerators and phytoplankton are predominantly urea consumers (e.g., Cho *et al.*, 1996a; McCarthy, 1972; Radach *et al.*, 1990; Remsen *et al.*, 1972a). One could pose the hypothesis that functional differences in the ureases of bacteria versus phytoplankton contribute to view that urea uptake is more common in phytoplankton than in bacteria. Similarly, the early kinetic observations of amino acid and DON uptake by phytoplankton may have contributed to the view that amino acid uptake is more common in bacteria than in phytoplankton. In some initial studies bacteria exhibited biphasic uptake kinetics with both a high affinity active uptake system and a low affinity diffusion transport mechanism. However, in early studies with microalgae only low affinity DOM uptake systems were observed (see Lewitus, 2006).

Uptake kinetics for dissolved free amino acids have been measured in culture systems for a variety of organisms and the observed kinetic parameters have been used to argue against significant amino acid uptake by phytoplankton in nature (Antia *et al.*, 1991). Efforts to describe amino acid uptake kinetics in nature have yielded confusing results. Populations dominated by *A. anophagefferens* and *Karenia brevis* did not exhibit Michaelis-Menten type kinetics for amino acids; rather high uptake rates were measured at low amino acid concentrations and uptake rates decreased with increasing amino acid concentrations (Bronk *et al.*, 2004; Mulholland *et al.*, 2002b). As for amino acids, K_s concentrations for bulk DOM uptake were higher for microalgae than bacteria and microalgae K_s concentrations of particular DOM components were higher than concentrations in nature suggesting that DOM uptake in nature was not significiant (see Lewitus, 2006). However, complex DOM uptake kinetics, including an active uptake system at low concentrations, have been described for *Chlaymdomonas* (Bennett and Hobbie, 1972 and for other microalgae (Flynn and Butler, 1986, Hellebust, 1978; Lewitus, 2006). Clearly, there is need to examine uptake kinetics for dissolved organic N compounds (particularly amino acids) more rigorously in nature.

4.3. Transient or surge uptake

Many cultured marine phytoplankton possess the capacity to take up N substrates at rates that far exceed instantaneous growth rates in nature and those predicted using simple Michaelis–Menten kinetics (McCarthy and Goldman, 1979; Glibert and Goldman, 1981; Collos, 1982). These short-lived 'transient' uptake phenomena are uncoupled from cellular growth and have been termed surge uptake to distinguish this from internally and externally controlled N uptake (Conway *et al.*, 1976; Goldman and Glibert, 1983). Enhanced N uptake capabilities appear to be induced by N limitation. Nitrogen perturbation experiments demonstrate that N-limited or starved cultures display surge uptake and that the pattern varies depending on the algal taxa, the N source, the duration of the starvation period, and the nutrient history prior to starvation (Cochlan and Harrison, 1991b; Collos, 1983, 1984; Dortch *et al.*,

1982, 1991b; Horrigan and McCarthy, 1982; Raimbault and Gentilhomme, 1990; Raimbault *et al.*, 1990). Further, N-starved cells appear to adjust their metabolism to retain the capacity to exploit nutrients when they are resupplied. N-starved phytoplankton had similar NH_4^+ uptake capacities as exponentially growing cells; however incorporation of this NH_4^+ into macromolecules was reduced under N-starvation (Kanda and Hattori, 1988). In exponentially growing cells, uptake is tightly coupled with growth and synthesis of cellular macromolecules. Uncoupling between nutrient uptake and growth is characteristic of nutrient stress and allows cells to prioritize nutrient acquisition so that they can recover and later translate this into growth. During an acclimation experiment, *Isochrysis galbana* cultures increased their uptake efficiency in response to a pulsed NH_4^+ delivery system (e.g., increasingly high and rapid uptake during pulses and decreased excretion of N between pulses; Gentilhomme and Rich, 2001).

Nutrient interactions also affect the capacity for surge uptake. For example, NH_4^+ can reduce the capacity for surge uptake of NO_3^- or urea (Horrigan and McCarthy, 1982). In addition, surge uptake can result in the release of primary metabolites (e.g., NH_4^+ release following surge uptake of urea; Price and Harrison, 1988a). It is intriguing to think of the physiological controls where excess N might be taken up only to be released again once the cell 'realizes' that it has indeed taken up more than it can metabolize.

5. Regulation of Nitrogen Uptake and Assimilation

What we know about the regulation of N uptake and assimilation has increased tremendously over the last two decades. A variety of factors regulating uptake and assimilation of N have long been recognized, however, recent advances in genomic and proteomic research have put these into a new context and have expanded our understanding of cellular regulation in general. For example, enzyme activity has been studied for decades but only recently have we begun to isolate genes and develop gene probes from marine phytoplankton and bacteria (see Zehr and Ward, 2002). Various mechanisms are involved in the regulation of enzyme proteins and gene expression and these include enzyme induction or repression, feedback regulation, post-translational modification and regulatory circuit control. For inducible enzymes, there is potential for using gene expression (presence or absence) or enzyme activity as markers for enzymatically mediated uptake and assimilation. However, interpreting results can be complicated because many genes are constitutively expressed or their expression and abundance are entrained in the cell cycle or other cellular rhythms (Chapter 32 by Berges and Mulholland, this volume; Herrero *et al.*, 2001; Hildebrand and Dahlin, 2000; Song and Ward, 2004). Many older studies can be reinterpreted given our current understanding of genes and gene expression. Induction of enzyme synthesis can be in response to depletion of an intracellular product pool or the availability of an inducer compound in the extracellular environment, such as an enzyme substrate. For example, *Dunaliella tertiolecta* NR transcription is induced

by NO_3^- and repressed by NH_4^+ but is not induced under N depletion (Song and Ward, 2004). The accumulation of intracellular product pools can result in the feedback inhibition of downstream processes such as uptake and assimilation. Post-translational modification of enzymes can regulate uptake and assimilation by modulating the number of active sites available for catalyzing specific reactions. Studies examining the transcriptional activation of enzymes involved in uptake and assimilation of N have begun to demonstrate clear functional relationships between genomes and ecological capabilities. For example, the presence of NH_4^+ (or a metabolic product of NH_4^+ assimilation) represses protein expression (i.e., enzymes) involved in the assimilation of alternative N sources (e.g., NO_3^- and N_2) in some organisms (e.g., Eppley, 1978; Eppley et al., 1969). However, this inhibition is not caused by the NH_4^+ itself, rather, through the feedback of some product of its incorporation through GS/GOGAT (e.g., a nitrogenous metabolite; Flores and Herrero, 1994) or possibly the intracellular balance of C and N (e.g., Flynn, 1991).

Nutrient preferences and interactions can also be described in the context of cellular N control systems. While enzyme systems are often shown to be regulated independently, regulatory systems wherein groups of enzymes are regulated in concert have been described for N control in microbes. Nitrogen control through the nitrogen regulatory system (Ntr) is well-characterized in enteric and some other proteobacteria. In the marine realm, N control is probably best described for cyanobacteria. In cyanobacteria N control consists of repression of selected N pathways when other, more easily assimilated or preferred N sources are available (Herrero et al., 2001). Nitrogen control in cyanobacteria is mediated by *ntcA*, a transcriptional regulator and so differs from the Ntr system, although there are some similarities (e.g., in the signal transduction protein) (Flores and Herrero, 2005; Herrero et al., 2001). The *ntcA* genes are thought to regulate genes associated with NO_3^- uptake (Lindell et al., 1998; Suzuki et al., 1995), N_2 fixation (Herrero et al., 2001), and urea uptake (Collier et al., 1999; Flores and Herrero, 2005; Rocap et al., 2003). The N control gene, *ntcA*, is widely distributed in many cyanobacteria (Frias et al., 1993) but not in all cyanobacteria (Garcia-Fernandez and Diez, 2004). There may be important species or strain-specific differences in the genes that are controlled as part of a regulatory system. For example, transcripts of the high affinity NH_4^+ transporter (*amt1*) and the transcriptional activator of genes involved in N metabolism (*ntcA*) were not correlated in axenic *Prochlorococcus* strain PCC 9511 suggesting that *amt1* is not N-regulated in this strain (Lindell et al., 2002) even though it appeared to be regulated by N in other strains of *Prochlorococcus* (Rocap et al., 2003). In addition, glutamate synthase and some urease enzymes do not appear to be under N control in some organisms (Herrero et al., 2001; Lomas, 2004b; Peers et al., 2000; Valladares et al., 2002).

Genes for a variety of other N transporters have now been identified (Dufresne et al., 2003; Hildebrand and Dahlin, 2000) and appear to be subject to different types of control (Hildebrand and Dahlin, 2000; Lindell et al., 2002; Sakamoto et al., 1999; Suzuki et al., 1995; Wang et al., 2000). In addition, groups of genes (forming operons) appear to be regulated in concert. For example, transporter-encoding genes for NO_3^- uptake in *Synechococcus* (Suzuki et al., 1995) are

clustered with the structural genes for NR and NiR. The entire operon is expressed only when NH_4^+ is not present in the growth medium with the presence of NO_3^- or NO_2^- apparently having an inducer effect (Herrero *et al.*, 2001). This is similar to the dual control of assimilatory NR in bacterial *ntr* systems (Lin and Stewart, 1998; Moreno-Vivián *et al.*, 1999). Nitrogenase genes have also been identified (*nif*) and are clustered together (Herrero *et al.*, 2001). Many enzyme sytems are subject to multiple levels of control. So, in addition to nitrogen regulation at the level of gene expression, there are also pathways responsible for post-translational regulation of enzyme systems that include modification, reversible inactivation, and short-term inhibition (Herrero *et al.*, 2001, see also Chapter 30 by Jenkins and Zehr, this volume).

5.1. Whole genome analyses

Most species of picoplanktonic marine cyanobacteria currently identified belong to two genera, *Synechococcus* and *Prochlorococcus*. Together, these groups are thought to comprise 20–40% of chlorophyll biomass and carbon fixation in the oceans (Partensky *et al.*, 1999). Genomic analyses of *Prochlorococcus* (Dufresne *et al.*, 2003, Rocap *et al.*, 2003) and *Synechococcus* (Palenik *et al.*, 2003) have revealed an extensive array of information on N utilization by these important genera and provide an excellent example of how genomic analysis can yield insights into ecological capabilities. Examining their genetic capabilities, *Synechococcus* strain WH8102 can use NH_4^+, NO_2^-, NO_3^-, urea, cyanate, amino acids and peptides; *Prochlorococccus* MIT9313 can use all except NO_3^- and cyanate; *Prochlorococccus* MED4 only NH_4^+, urea, cyanate and peptides; and *Prochlorococccus* SS120 only NH_4^+ and amino acids (Dufresne *et al.*, 2003; Fuhrman, 2003; Palenik *et al.*, 2003; Rocap *et al.*, 2003). Virtually all isolated marine *Synechococcus* strains except *Synechococcus* sp. MIT9220, isolated from the equatorial Pacific, are capable of using NO_3^- as an N source for growth (Scanlan and West, 2002). Even the strain that can't use NO_3^- as an N source can use NO_2^-, NH_4^+ and urea as N sources. It appears that during the evolution of *Prochlorococcus*, genes for NO_3^- / NO_2^- transport and reduction (NR and NiR) were lost while the genes for nitrite reductase were retained or reacquired in some cases and lost in others (Dufresne *et al.*, 2003; Garcia-Fernandez *et al.*, 2004; Rocap *et al.*, 2003).

It has been hypothesized that each *Prochlorococcus* ecotype uses the N species that is most prevalent at the light levels to which they are adapted; NH_4^+ in the surface waters and NO_2^- at depth (Rocap *et al.*, 2003). *Synechococcus* on the other hand has retained NR and so is able to bloom during NO_3^- upwelling events (Dufresne *et al.*, 2003; Glover *et al.*, 1988; Rocap *et al.*, 2003) and this may contribute to its relative abundance in coastal systems. For example, *Synechococcus* is usually less abundant than *Prochlorococcus* in extremely oligotrophic environments, where NO_3^- concentrations are generally below the limits of analytical detection, but has a broader global distribution (Palenik *et al.*, 2003; Scanlon and West, 2002). *Prochlorococcus* strains also appear less versatile in their ability to use DON than *Synechococcus* (Palenik *et al.*, 2003; Rocap *et al.*, 2003). The *Synechococcus* genome also contains amino acid and oligopeptide transporters as well as

cyanate transporters and cyanase (degradation enzyme) suggesting that these can also be used as N sources. In fact, *Synechococcus* strain WH8102 can grow in culture on cyanate as a sole N source although cyanate use in nature has not been characterized (Palenik *et al.*, 2003).

Thalassiosira pseudonana, a diatom, has a genome that encodes multiple transporters for NO_3^-, NH_4^+ and as well as putative genes for uptake and catabolism of organic N including amino acids, purines and urea (Armbrust *et al.*, 2004). Two major intermediates of the urea cycle, ornithine and arginine are integrated into cell metabolism (Armbrust *et al.*, 2004). Other N-regulated proteins have been identified on the cell surfaces of *Emiliania huxleyi* and these are present under N-limitation or during growth on urea (Palenik and Koke, 1995). As more genomes are analyzed, we will learn more and more about the genetic capabilities of phytoplankton taxa and groups. Yet while genetic information can be invaluable for determining ultimate capacity, experimental evidence is often lacking to demonstrate that particular genes are actually expressed and active.

6. What Does the Future Hold?

While our knowledge of the marine N cycle has certainly increased over the last two decades, much remains to be learned. The number of N uptake measurements has increased significantly as has the diversity of N compounds used in these studies, but our understanding of the spatial, temporal and biological variability of N uptake in nature remains limited. Perhaps our understanding of the controls on N uptake in natural systems is still hindered by a lack of adequate methods. We know that a variety of compounds are used simultaneously from uptake studies done on bulk communities and that these confound our ability to calculate f-ratios. Maybe the f-ratio is no longer a useful metric for estimating new production. We also know that there are inherent differences in the capabilities of organisms and their flexibility in acquiring N from forms available in the environment, but know little about cell-specific and taxa-specific uptake of particular N substrates.

We've just begun to scratch the surface of answering questions regarding the 'preferences' of particular microbes for different N substrates in their natural environment by applying single-cell technologies (e.g., micro-FISH, CARD-FISH and flow cytometry) to N cycling studies (see also Chapter 31 by Lipschultz, this volume). For example, flow cytometric sorting has been shown to be effective for directly assessing assimilation of a variety of nutrients by marine phytoplankton versus bacteria in natural water samples (e.g., Boneillo *et al.*, unpublished data; Casey *et al.*, 2007; Zubkov and Tarran, 2005; Zubkov *et al.*, 2001, 2003, 2004; see Table 7.3). Flow cytometric sorting protocols have traditionally used inherent pigment signatures and DNA stains to separate populations and enable inferences between broad phylogenetic groups. However, these are general markers and do not allow one to investigate diversity and functions within broad phylogenetic categories such as bacteria and picoeukaryotes (e.g., Worden, 2006; Worden *et al.*, 2004).

With the advance of new molecular methods that combine the genetic specificity of FISH techniques with tyramide signal amplication (CARD-FISH), we can use flow cytometry to assess N uptake by phylogenetically distinct populations of bacteria (e.g., Pernthaler et al., 2002a,b; Sekar et al., 2004) and picoautotrophs (e.g,. Biegala et al., 2003; Pernthaler et al., 2003). Now the primary limitation to these studies has shifted to the choice of isotopic tracer. Obviously ^{15}N is the tracer of choice for N uptake studies, but this is a stable isotope and therefore requires substantial masses for sample analysis. For example, to study N uptake by a bacterial clade that comprises 10% of the total oceanic bacterial community, one would need to sort ~10 L of seawater to get ~4 µg N sample to analyze. Even with a high-speed cell sorter, without some form of concentration protocol (e.g., Casey et al., 2007), this is not practical. Radioisotope tracers improve sensitivity but then one must assume some stoichiometric conversion to units of N. Although still in their infancy these new methods have promise for the study of bacteria and phytoplankton physiology in mixed assemblages and are a vast improvement over crude size-fractionation techniques.

These new methods are particularly useful for the study of microbial groups that are not amenable to culturing. For example, many taxa, particularly many known mixotrophs, are difficult to culture or do not grow well in unialgal cultures, suggesting that some sort of mutualism exists between certain phytoplankton and associated bacterial assemblages (e.g., Doucette, 1995; Rooney-Varga et al., 2005). Application of new techniques will be necessary to provide direct evidence for nutritional linkages and flows of N between microbial consortia and groups. Moreover, the degree to which mixotrophy occurs in the environment is relevant for ocean biogeochemical models, which commonly parameterize heterotrophic and autotrophic organisms separately and into several ecologically functional groups (e.g., N_2 fixers, cyanobacteria, picoeukaryotes) based on what is known about their physiology. Much of what is known about algal physiology arises from culture studies that fail to represent the complexities characterizing biologically-mediated elemental cycling in natural systems.

The natural environment is undergoing significant and important changes. We have seen the anthropogenic increase in atmospheric carbon dioxide (CO_2) translate into increases in the CO_2 concentrations in the surface ocean (Bates, 2001) with an associated decrease in ocean pH. It has been hypothesized that elevated pCO_2 may impair the competitive ability of coccolithorids (Tortell et al., 2002); a very important species with respect to N cycling globally, or differentially impact phytoplankton growth rates, even within the same taxonomic group (e.g., diatoms; Kim et al., 2006). It has also been hypothesized that the shift to a high CO_2/low pH ocean, may significantly affect N cycling in anticipated and unanticipated ways such as the balance between nitrification/denitrification (Huesemann et al., 2002) and N inputs due to N_2 fixation (Hutchins et al., 2007). Another change, closer to home for those of us living in coastal communities, is a shift in forms of N being released into coastal systems. The past few decades have seen a significant change from inorganic nutrient loading to organic nutrient loading (Chapter 11 by Paerl and Piehler, this volume), and it has been suggested that this change in the primary N agents leading to eutrophication may be selecting for harmful and nuisance algal species (e.g.,

Anderson *et al.*, 2002). However, much work remains to verify this observation across a range of environments. For example, harmful algal blooms also occur in upwelling systems (Kudela *et al.*, 2005) where there is abundant NO_3^-.

The marine N cycle is exciting and over the past two decades, since the first edition of Nitrogen in the Marine Environment, there has been an explosion in our knowledge of temporal and spatial variability of N assimilation as well as a broader appreciation for the N substrates that are assimilated. With new methods in hand and an environment changing at unprecedented rates in response to anthropogenic forcing, the next two decades will also be exciting as we further explore the physiological plasticity of microbial populations and directly test ecological hypotheses in the environment.

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NITROGEN REGENERATION

Deborah A. Bronk *and* Deborah K. Steinberg

Contents

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1. INTRODUCTION

Primary producers in oceanic surface waters have the chemical characteristics of healthy robust cells in regions where the concentrations of dissolved inorganic nitrogen (DIN) is often below the limit of detection (i.e., 0.03 μM). This apparent paradox is possible because of the process of nitrogen (N) regeneration and its tight coupling to N uptake. The objectives of this chapter are to review the forms and sources of regenerated N, both inorganic and organic, to describe the various processes that result in N regeneration and to survey N regeneration rates in

estuarine, coastal, and open ocean systems. The focus of the chapter is on water column N regeneration. Sediments are discussed from the perspective that they serve as a source of N to the water column. The chapter concludes with a brief survey of methods and recommendations for future research.

2. Types of Regenerated Nitrogen

Nitrogen is regenerated in both inorganic and organic forms. The inorganic forms include ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-). Organic forms include urea, dissolved free amino acids (DFAA), nucleic acids, and a broad suite of dissolved combined amino acids (DCAA) and proteins, as well as other largely unidentified components of the dissolved organic nitrogen (DON) pool including enzymes (Chapter 3 by Aluwihare and Meador, this volume).

2.1. Inorganic

Ammonium is released during bacterial remineralization of dissolved organic matter (DOM) in the water column (e.g., Goldman *et al.*, 1987) and in sediments (Chapter 19 by Joye and Anderson, this volume), is an excretory product of zooplankton (Chapter 26 by Steinberg and Saba, this volume), and can be produced during the photochemical breakdown of DOM (Chapter 10 by Gryzbowski and Tranvik, this volume). The most prevalent oxidized form of inorganic N, NO_3^-, is the end product of nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$; Chapter 5 by Ward, this volume). The other oxidized form, NO_2^- is produced as an intermediate during the process of nitrification and denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$; Chapter 6 by Devol, this volume). Nitrite is also released photochemically from the reduction of NO_3^- (Zafiriou and True, 1979).

Vertical profiles of NH_4^+ are generally highly variable due to the large number of processes that can produce and consume NH_4^+. In contrast, the classic water column nutrient profile, low concentrations in surface water and higher concentrations at depth, is epitomized by NO_3^-. The NO_3^- is incorporated into particulate organic matter (POM) in surface waters, driving the concentration to near zero. As POM sinks through the water column, the N is remineralized to NH_4^+, which is ultimately nitrified to NO_3^-. As a result, concentrations of NO_3^- in the deep ocean are many times higher (i.e., >30 µM) than those commonly observed in surface waters. Profiles of NO_2^- concentrations often have subsurface maxima generally the result of phytoplankton excretion or an imbalance between NH_4^+ oxidation and NO_2^- oxidation (Lomas and Lipschultz, 2006; Chapter 5 by Ward, this volume). This review will focus on regeneration of reduced N (NH_4^+ and DON) and readers are directed to the other chapters noted above for information on the cycling of NO_3^- and NO_2^-.

2.2. Organic

Of the organic forms, urea and DFAA have received the most attention. Urea is an excretory product of zooplankton and fish. Bacteria also release it during the degradation of some N compounds, such as purines (Jørgensen, 2006).

Concentrations of urea in marine systems tend to be less than 1 μM in most systems though in some circumstances much higher concentrations have been observed (e.g., 10 μM in the Resolute Passage, Conover *et al.*, 1999). DFAAs are produced intracellularly by phytoplankton and bacteria and are released during such processes as bactivory and grazing. Concentrations of DFAA tend to be lower than urea at less than 0.5 μM (Bronk, 2002).

The nucleic acid pool is composed of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Viruses, which are basically little packets of DNA, are so small that they are not removed by standard filtration. As a result, a significant fraction of the measured nucleic acid pool is composed of DNA contained within viruses. Accordingly, the DNA pool can be divided into three fractions—free or enzymatically hydrolysable dissolved DNA (ehD-DNA), dissolved DNA (D-DNA) within viruses, and uncharacterized bound DNA (Brum, 2005; Jiang and Paul, 1995). In the North Pacific Subtropical gyre 49–63% of the measured total D-DNA is contained within viruses with the remainder found within the ehD-DNA fraction (Brum, 2005). In the Gulf of Mexico, however, only 8–15% of D-DNA is found within viruses, 50% is in the ehD-DNA fraction, and 35–42% is in the unidentified bound fraction (Jiang and Paul, 1995). Turnover times of the ehD-DNA pool are likely shorter than those of the D-DNA bound in viruses, which is protected to some extent from hydrolytic enzyme activity (Siuda and Chrost, 2000; Weinbauer *et al.*, 1993). Proteins and DCAA are also produced by phytoplankton and bacteria and are likely released during processes that destroy cells such as grazing and viral infection and lysis. Another organic N fraction is composed of the extracellular enzymes that are released primarily by bacteria and function to break down DOM into smaller moieties that can then be taken up (Chapter 32 by Berges and Mulholland, this volume). In one bacterial culture study, the bacteria *Psuedoalteromonas atlantica* produced a wide array of extracellular enzymes, some of which retained activity for as long as 15 days after secretion (Hoffman and Decho, 2000).

3. Sources of Regenerated Nitrogen

Biological sources of N include: direct release, either active or passive, from phytoplankton, N_2 fixers, bacteria, and macroalgae (Branch and Griffiths, 1988; Mann, 1982); viral infection and subsequent lysis of both autotrophs and heterotrophs; fecal pellet dissolution, excretion, and grazing (i.e., feeding on phytoplankton) or bactivory (i.e., feeding on bacteria) by micro- and macrozooplankton and other macroorganisms (Tupas and Koike, 1990); and release from corals, detritus, and sediments. At any given time, many processes likely contribute to release. As a result, the magnitude of the different release processes is difficult to quantify and teasing them apart is an active area of ongoing research.

3.1. Phytoplankton

Nitrogen release from phytoplankton can occur two ways. First is direct release, which includes active release, often termed exudation, or passive diffusion from the cell (Fogg, 1983). The second is mediated release, where the release occurs when phytoplankton

cells are grazed, commonly called "sloppy feeding" (e.g., Lampert, 1978; Møller, 2007), or infected with a virus, the final stage of which results in cell rupture (e.g., Bratbak et al., 1993; Fuhrman, 1999; Gobler et al., 1997; Murray, 1995; Suttle, 1994).

3.1.1. Active and passive models of direct release

Two models have been proposed to account for direct release of N from cells—the active release outflow model (Fogg, 1966) and the passive diffusion model (Bjørnsen, 1988; Bratbak and Thingstad, 1985; Fogg, 1966). The outflow model is a viable explanation for DOC release but is unsatisfying as an explanation for release of DON. In the outflow model, release results when excess photosynthates accumulate within the cell when carbon (C) fixation exceeds assimilation as a result of nutrient limitation (Fogg, 1983; reviewed in Carlson, 2002). The cell dumps the C because it does not have the N available to make cellular constituents such as proteins or enzymes. This apparent excess of DOC, relative to DON production, has been observed in a number of environments (e.g., Alvarez-Salgado et al., 1999; Doval et al., 1997; Søndergaard et al., 2000; Williams, 1995). It is highly unlikely that cells would have an excess of N over C, however, so there is no similar rationale for dumping N. Murray (1995) used a modeling approach to suggest that direct DOM release can be a cost-effective, indirect means of reducing viral infection in phytoplankton. By releasing DOM the phytoplankton are supporting bacterial populations that can kill viruses, which the bacteria contact with much greater frequency than phytoplankton because of their small size. If DOM release can facilitate a decrease in the number of viruses able to infect phytoplankton, than the cost of the release may be energy well spent.

In the passive diffusion model, low molecular weight (LMW) compounds diffuse through the cell membrane as a result of the large concentration gradient between internal (i.e., mM) and external (i.e., nM to μM) N pools. Passive release likely varies based on physiological stress, exposure to UV radiation, and changes in temperature or light levels. One theory suggests that bacteria are ectoparasites on phytoplankton by taking up DOM, which keeps the concentration of extracellular DOM low resulting in a larger concentration gradient between organic pools inside and outside of the cell (Bjørnsen, 1988). If this theory is correct, smaller cells should have higher release rates per unit biomass, relative to larger cells, because they have a larger surface to volume ratio. In one ^{15}N study, Hasegawa et al. (2000a) found that smaller plankton did indeed release more DON compared to larger cells.

3.1.2. Magnitude of direct release

The magnitude of direct release has been a topic of much debate (Bronk and Ward, 2000; Bronk et al., 1994; Slawyk et al., 1998; Slawyk et al., 2000). A similar debate took place around DOC release in the 1960s and 1970s (Sharp, 1977). Cultures are the tool of choice for the study of direct release because they remove the effect of other trophic levels, such as grazing or, under axenic conditions, bacteria (reviewed in Antia et al., 1991; Bronk and Flynn, 2006). Nitrogen release has been studied extensively in culture and there is abundant literature on uptake and release, particularly of DON, from work done in the 1950s–1970s (reviewed in Antia

et al., 1991). On average, 19.3 ± 16.2% of gross N uptake of a number of different substrates is released as DON in the culture work reviewed in Table 8.1. This percent release is generally lower than the mean percent release observed in the field and discussed later in the chapter, which suggest the other trophic levels (i.e., grazers) are important in release of DON. From cultures we know that direct release is generally highest immediately after cells are transferred to new media (Collos *et al.*, 1992; Jones and Stewart, 1969; Slawyk and Raimbault, 1995). If the increased direct release is a response to a change in environmental conditions, and not a result of stress during transfer, then the amount of extracellular release in the ever-changing natural environment may be much higher than indicated by long-term culture studies (e.g., Bronk, 1999).

Direct release also likely changes at different phases of the cell cycle—exponential, stationary, and senescent. One common observation in cultures is an increase in direct release during senescence. For example, the highest rates of DCAA release occur during stationary phase while release during active growth is low (Flynn and Berry, 1999). The decline of phytoplankton blooms is another time period characterized by significant DOM release from phytoplankton due to such processes as cell stress or death, high rates of viral infection, or high grazing pressure (e.g., Carlson *et al.*, 1994; Jenkinson and Biddanda, 1995). There seems to be a pattern of low rates of release in actively growing cells, such as those found at the beginning of a bloom, and high rates of release when cells are stressed by nutrient limitation, light inhibition, etc., such as at the end of a bloom (Larsson and Hagström, 1979).

Nutrient status would also likely affect measured release rates and here the reverse trend is seen. *Scenedesmus quadricauda* cultures grown under N–replete conditions had higher DON release rates than cultures that had been grown under N–limited conditions suggesting that cells may suppress release to meet their cellular N demand (Nagao and Miyazaki, 2002). They also found evidence that *Scenedesmus quadricauda* released more of the DON that was stored intracellularly prior to the substrate addition than the other culture tested, *Microcystis novacekii*. In a suite of different batch cultures most DOM production occurred during nutrient replete growth (Biddanda and Benner, 1997). In another study with batch cultures of the cyano–bacterium *Synechococcus,* DOC release increased when cells went from N sufficient to N deficient conditions (Bronk, 1999). In contrast, DON release measured using ^{15}N tracers and short-term incubations of subsamples of the culture, was highest when cells were N sufficient as was the percentage of NH_4^+ uptake released as DON (Bronk, 1999). When NH_4^+ concentrations fell below the level of detection, release rates decreased by a factor of four to seven (Bronk, 1999), consistent with Biddanda and Benner (1997).

When considering release under N-limited conditions it is important to remember that the commonly measured DON release rates are net rates such that reincorporation of recently released DON would result in an underestimate of the true DON release rate. Many phytoplankters can use organic N to fulfill their nutritional requirements such that reincorporation is likely, particularly under N-limiting conditions (reviewed in Bronk *et al.*, 2007). An example of this was seen in a culture study with *Alexandrium catenella* where DON release was observed early in the

Table 8.1 The percentage of gross nitrogen (N) uptake released as dissolved organic nitrogen (DON) or dissolved free or combined amino acids (DFAA, DCAA) measured using changes in concentrations (CC) or ^{15}N techniques (^{15}N) where the DON was isolated with ion retardation columns (IRC) or wet chemical (WC) methods

Culture	Substrate	N Form Released	N Release: Gross N uptake (%)	Method	Reference
Dunaliella tertiolecta	NO_3^-	DON	6–21	^{15}N–WC	Slawyk *et al.* (1998)
Emiliana huxleyi	DIN	DON	20.5	CC	Biddanda and Benner (1997)
Heterosigma carterae	NH_4^+	DCAA	37.7[a]	CC	Flynn and Berry (1999)
Heterosigma carterae	NH_4^+	DFAA	15.9[a]	CC	Flynn and Berry (1999)
Mantoniella squamata	NH_4^+	DCAA	32.9[a]	CC	Flynn and Berry (1999)
Mantoniella squamata	NH_4^+	DFAA	3.8[a]	CC	Flynn and Berry (1999)
Microcystis novacekii	NH_4^+	DON	20.9 ± 3.7[b]	^{15}N–IRC	Nagao and Miyazaki (1999)
Microcystis novacekii	NH_4^+	DON	4.7 ± 1.3[c]	^{15}N–WC	Nagao and Miyazaki (2002)
Microcystis novacekii	NO_3^-	DON	5.1 ± 5.8[c]	^{15}N–WC	Nagao and Miyazaki (2002)
Phaeocystis sp.	DIN	DON	27.7[d]	CC	Biddanda and Benner (1997)
Phaeodactylum tricornutum and *Dunaliella tertiolecta*	NO_3^-	DON	3.9[e]	^{15}N–WC	Pujo-Pa *et al.* (1997)
Senedesmus quadricauda	NH_4^+	DON	36.8 ± 9.6[c]	^{15}N–WC	Nagao and Miyazaki (2002)
Senedesmus quadricauda	NO_3^-	DON	15.8 ± 3.4[c]	^{15}N–WC	Nagao and Miyazaki (2002)
Skeletonema costatum	DIN	DON	19.3[d]	CC	Biddanda and Benner (1997)
Synechococcus bacillaris	DIN	DON	18.2[d]	CC	Biddanda and Benner (1997)
Synechococcus WH7803	NH_4^+	DON	11.3 ± 6.3	^{15}N–IRC	Bronk (1999)
Synechococcus WH8018	NH_4^+	DON	12.0 ± 5.7	^{15}N–IRC	Bronk (1999)
Synedra planctonica	NO_3^-	DON	36.0	CC	Collos *et al.* (1992)
Thalassiosira psuedonana	NH_4^+	DCAA	18.5[a]	CC	Flynn and Berry (1999)
Thalassiosira psuedonana	NH_4^+	DFAA	7.4[a]	CC	Flynn and Berry (1999)
Trichodesmium IMS101	N_2	DON	5 ± 6	^{15}N–IRC	Mulholland *et al.* (2004)
		Mean	19.3[f] ± 16.2		

[a] Percent release equals release divided by uptake + DCAA release + DFAA release; the uptake rate was calculated as the increase in algal N over the first day.
[b] Cells preserved with formalin prior to filtering.
[c] Mean in light 3 h incubations.
[d] Calculated by converting the change in DON concentrations over 14 days into a per hour rate.
[e] Estimated using ^{15}N mass balance not actual uptake and release rates.
[f] When both DFAA and DCAA release was measured the two rates were combined in calculating the mean % release.
Data are presented as the mean $\pm$ standard deviation. When ranges are presented, the mean % release was calculated using the median value in the range; the standard deviation was calculated by propogating the available error terms.

culture but DON reincorporation was measured later in the experiment (Collos et al., 2004).

3.1.3. Programmed cell death

Death and subsequent lysis of phytoplankton cells can result from attack by viruses (e.g., Brussaard, 2004; Proctor and Fuhrman, 1990) or attack by fungi or other parasites (e.g., Holfeld, 1998). These processes result in the cells bursting open and releasing their cellular contents; viral release will be covered in more detail in a later section of this chapter. Another more direct form of release that is gaining attention in oceanography is programmed cell death (PCD), also known as autocatalytic cell death. Bidle and Falkowski (2004) define PCD as a form of autocatalytic cell suicide in which an endogenous biochemical pathway leads to morphological changes and ultimately, cellular dissolution. Proteases exist within cells or at the cells' surface and perform a number of functions including metabolic regulation through enzyme degradation and protein breakdown. Actual cell lysis is believed to involve a caspace-like compound. Programmed cell death can occur due to advanced age or in response to environmental stress such as nutrient deprivation, exposure to intense light (e.g., Llabrés and Agusti, 2006) or excessive salt concentrations or oxidation stress (Bidle and Falkowski, 2004).

There have been a number of studies that provide evidence that PCD occurs in cultures including cultures of the diatom *Ditylum brightwellii* in response to N and phosphorus (P) limitation (Brussaard et al., 1997), in N-depleted *Thalassiosira weissflogii* (Berges and Falkowski, 1996), in *Peridinium gatunese* in response to CO_2 limitation (Vardi et al., 1999), in *Dunaliella tertiolecta* in response to light deprivation (Segovia et al., 2003), in *Trichodesmium* IMS101 in response to P and iron starvation and excess light (Berman-Frank et al., 2004), and in freshwater *Anabaena* spp. in response to osmotic stress (Ning et al., 2002).

Though PCD no doubt occurs, it is a challenge methodologically to quantify it independent of other processes like viral infection. It is important to quantify the contribution of PCD to DOM release, however, because different routes of cell lysis likely generate DOM of different lability. If a cell lyses from viral infection, DOM within the host cell is shunted towards the production of more viruses until the cell eventually bursts. In PCD, however, the cell is lysed via the action of proteolytic enzymes resulting in DOM and enzymes entering the environment. From a bacteria's perspective, the DOM around cells that had lysed via PCD would likely be more labile and of better quality than around cells that had burst due to viral infection. One approach to quantifying PCD is to measure the concentration of cytoplasmic esterases (Brussaard et al., 1995; van Boekel et al., 1992) and then to make the following assumptions: (1) esterases enter the water during lysis, (2) only phytoplankton release substantial levels of esterases (Agusti et al., 1998), and (3) grazing processes, such as sloppy feeding, do not result in esterase release. The last of these assumptions seems unlikely and there has been considerable debate as to how to quantify the many assumptions (Agusti and Duarte, 2002; Riegman et al., 2002). Although there may be reason to question the exact magnitude of the rates measured, the general patterns in lysis rates discussed below appear robust.

In the northwestern Mediterranean Sea, rates of phytoplankton lysis were highest at the surface and declined sharply with depth. Lysis rates ranged from 0.026 day^{-1} to 1.0 day^{-1} such that lysis rates were $\sim$50% of the gross phytoplankton growth rate at the surface but only 7% at the deep chlorophyll maximum (Agusti *et al.*, 1998). Another study in the northwestern Mediterranean found that phytoplankton cell lysis was highest in the summer (0.41 $\pm$ 0.049 day^{-1}) and lowest in the winter (0.061 $\pm$ 0.005 day^{-1}) with rates strongly correlated with temperature ($r^2 = 0.71$, $p < 0.00001$, Agusti and Duarte, 2000). There was also a one to two month lag between maximum average monthly gross primary production and the maximum average lysis rate (Agusti and Duarte, 2000). Brussaard *et al.* (1995) estimated that 75% of phytoplankton death during the crash of the spring bloom in the North Sea was due to lysis although it was relatively low at other times of the year. The current understanding is that lysis rates can be high, particularly when sinking rates and grazing rates are low. If cells do not rapidly sink or are grazed their extended time in the water column provides greater opportunity for viral attack or to be confronted by some other negative environmental variable.

3.1.4. Mediated release

Sloppy feeding, by zooplankton, and viral infection, with eventual cell rupture, are two important processes that can result in N regeneration (discussed in more detail in Sections 3.4 and 3.5). Though the classic example of sloppy feeding is a diatom being fed on by a copepod, the importance of sloppy feeding by smaller heterotrophic flagellates has also been suggested in a number of studies (e.g., Glibert, 1998; Legendre and Rassoulzadegan, 1995; Varela *et al.*, 2003a). DON release mediated by copepods increased when cells were N-limited, suggesting a possible additive effect of direct release by N stress and mediated release via grazing (Miller and Glibert, 1998). In a culture study with *Scenedesmus quadricauda* and *Microcystis novacekii*, however, only minimal DON release was observed when cells were N-limited (Nagao and Miyazaki, 2002). A similar observation was made during a culture study with two clones of marine *Synechococcus* (Bronk, 1999); rates of DON release, as well as the percentage of NH_4^+ uptake released as DON decreased when the cells entered N-limitation. In the case of viral infection, the phage can increase to such numbers within the cell that they burst the cell. In the process, they release any dissolved intracellular components into the environment.

3.1.5. Other release mechanisms

Direct release can also include release due to osmotic changes. Organisms are generally adapted to a fairly narrow salinity range. When cells are outside of that range some can protect themselves through the synthesis and accumulation of osmoprotective compounds, some of which contain N such as glycine betaine (e.g., Keller *et al.*, 1999). Phytoplankton can also release reduced organic (dissolved primary amines, DPA) and inorganic N (NH_4^+ or NO_2^-) as a protective mechanism against high light levels (Lomas and Glibert, 1999, see Section 5.2.3 below). Another understudied form of release is the lysis of protoplasm during spermatogenesis brought on by N starvation (Sakshaug and Holm-Hansen, 1977).

Ammonification is another process that can result in N release. The simplest definition of the process is the release of NH_4^+ from organic matter (e.g., Herbert, 1999). It can occur by a number of different processes including remineralization by bacteria in the water column and sediments. Photochemical ammonification occurs abiotically when NH_4^+ is released from organic matter as a result of exposure to UV radiation (reviewed in Bronk, 2002 and Chapter 10 by Gryzbowski and Tranvik, this volume). Ammonium efflux from cells has also been observed following urea uptake in a number of culture experiments (e.g., Price and Harrison, 1988; Rees and Bekheet, 1982; Uchida, 1976). The release may be due to passive diffusion through the cell membrane and is likely unavoidable because NH_3 is lipid soluble.

3.1.6. Release by different phytoplankton groups

In the field, cells that possess large intracellular vacuoles might be expected to be particularly important in dissolved N release but the reverse is often observed. As noted above, in a ^{15}N study, smaller phytoplankton cells released more $DO^{15}N$ than larger cells (Hasegawa et al., 2000a). In upwelling regions of the central Atlantic dominated by large phytoplankton (>2 μm) the percent of total N uptake released as DON was $<30\%$ (Varela et al., 2005). In oligotrophic regions dominated by smaller phytoplankton, however, the percent of total N released as DON was often $>50\%$, although it was highly variable (Varela et al., 2005). In another study, low rates of DON release occurred where small cells (<2 μm) dominated total biomass and primary production (Varela et al., 2003a). However, the highest DON release rates were measured when smaller cells contributed more to total biomass than to primary production. The smaller cells were largely flagellates that, due to the small contribution this size class made to primary production, were likely heterotrophs. As such, they would have likely contributed to DON release via direct release, enhanced by their small surface to volume ratio, as well as via sloppy feeding.

One group of algae that has received considerable attention is the colonial, mat-forming diatom *Rhizosolenia* (Villareal et al., 1993). *Rhizosolenia* persist in oligotrophic regions generally dominated by small picoplankton. They persist in these regions because of their ability to migrate vertically into the nitracline where they fill large internal vacuoles with NO_3^- (Villareal et al., 1999). Higher C:N and carbohydrate to protein ratios have been observed in negatively buoyant mats suggesting that cellular N levels play a role in buoyancy (Villareal et al., 1996). Once at the surface, the cells can release NO_3^-, NO_2^-, NH_4^+ and presumably DON directly or when grazed during sloppy feeding. Rates of inorganic N release were measured in vertically migrating mats collected in the oligotrophic North Pacific gyre. Average rates of release were 27.1 ± 8.3 and 3.6 ± 1.9 nmol N (μg Chl a)$^{-1}$ h^{-1} for NO_3^- and NH_4^+ respectively (Singler and Villareal, 2005); the magnitude of DON release in the ocean via this mechanism has yet to be quantified. It has been estimated that the equivalent of 3–35% of the new NO_3^- consumed in the surface mixed layer in the central Pacific gyre could be transported via this vertical migration (Villareal et al., 1996). A number of other large phytoplankton may employ similar vertical migrations as part of their N acquisition strategies

including the dinoflagellate *Pyrocystis* and the diatom *Ethmodiscus* (Villareal and Lipschultz, 1995; Villareal *et al.*, 1996).

3.2. N₂ fixers

Estimates of N_2 fixation rates in the global ocean continue to rise as results emerge from studies with the main N_2 fixer in the ocean *Trichodesmium*, the heterocystous endosymbiont *Richelia*, as well as more recently discovered N_2 fixers including unicellular diazotrophic cyanobacteria and bacterioplankton (Capone *et al.*, 1997; Hansell and Feely 2000; Karl *et al.*, 1997; Lipschultz and Owens, 1996; Montoya *et al.*, 2004; Zehr *et al.*, 1998 and 2001). *Trichodesmium* is involved in N release directly, through release of amino acids, DON, and NH_4^+ (reviewed in Table 8.2). *Trichodesmium* is also a source of NH_4^+ and DON as a result of remineralization by associated bacteria, sloppy feeding and excretion by grazers (Sellner, 1992; Sheridan *et al.*, 2002).

In natural populations of *Trichodesmium* up to 50% of the recently fixed N_2 is released directly as DON during growth (Glibert and Bronk, 1994), primarily as DFAA (Capone *et al.*, 1994). Other evidence for N release by *Trichodesmium* in the field includes high concentrations of NH_4^+, NO_3^-, and DON seen within *Trichodesmium* blooms at the subtropical North Pacific station ALOHA (Karl *et al.*, 1992; Letelier and Karl, 1996). In natural populations from the Caribbean Sea and cultured populations of *Trichodesmium* NIBB1067, lysogenic phages have been identified that can infect cells resulting in lysis and the release of cellular contents (Ohki, 1999). An estimated 0.3–6.5% of *Trichodesmium* trichomes were lysed by viruses per day in the Tropical North Pacific Ocean (Hewson *et al.*, 2004). This viral lysis estimate translates to an estimated N release rate between 3 and 65% of *Trichodesmium* production. An estimated 0.32–15 nmol colony^{-1} h^{-1} was released due to peptidase and b–glucosamidase activity associated with bacteria residing among *Trichodesmium* colonies in the subtropical Atlantic Ocean (Nausch, 1996). *Trichodesmium* appears to have few grazers. One exception is the copepod *Macrosetella gracilis*, which regularly associates with and consumes *Trichodesmium* colonies, releasing NH_4^+ (and likely DON) through excretion and sloppy feeding to surface waters where *Trichodesmium* occur (O'Neil and Roman, 1992; O'Neil *et al.*, 1996). *Trichodesmium* is also known to migrate vertically (up to 70 m) in the water column, using carbohydrate ballasting, as a means of acquiring P deeper in the water column (Villareal and Carpenter, 2003). The recent discovery of many unicellular N_2 fixers raises interesting questions about the fate of the recently fixed N_2 by the group. If the smaller unicellular forms can be grazed, then the fate of the N from unicellular N_2 fixers may be very different from that of the large colonial forms (Montoya *et al.*, 2004).

Two other N_2 fixers that have been studied are *Aphanizomenon* and *Nodularia*. In a study in the Baltic, $7.7 \pm 2.1\%$ and $6.7 \pm 2.1\%$ of the $^{15}N_2$ gas recently fixed by *Aphanizomenon* and *Nodularia* respectively appeared in the picoplankton fraction, which indicates N_2 fixation and subsequent N release and reincorporation.

Table 8.2 Summary of published rates of ammonium (NH_4^+) regeneration and dissolved organic nitrogen (DON) release from N_2 fixers in the field in the light. The standard deviations were calculated using propogation of error.

Organism	Location	Date/ Culture	Rate Measured	N release rate pmol N colony^{-1}h^{-1}	N release: N_2 fixation (%)	Reference
Trichodesmium thiebautii	Caribbean	Sept 1991	$N_2 \rightarrow$ glutamate (light)[a]	88 ± 112	34 ± 38	Capone *et al.* (1994)
Trichodesmium thiebautii	Caribbean	Sept 1991	$N_2 \rightarrow$ glutamate (dark)[a]	30 ± 21	3 ± 1	Capone *et al.* (1994)
Trichodesmium thiebautii	Caribbean	Sept 1991	$N_2 \rightarrow$ glutamine (light)[a]	18 ± 29	10 ± 17	Capone *et al.* (1994)
Trichodesmium thiebautii	Caribbean	Jan 1992	$N_2 \rightarrow$ glutamate (light)[a]	73 ± 49	44 ± 40	Capone *et al.* (1994)
Trichodesmium thiebautii	Caribbean	Jan 1992	$N_2 \rightarrow$ glutamine (light)[a]	32 ± 67	12 ± 10	Capone *et al.* (1994)
Trichodesmium thiebautii	Caribbean	Jan 1992	$^{15}N_2 \rightarrow$ DON	114 ± 148	44 ± 32	Glibert and Bronk (1994)
Trichodesmium thiebautii	Caribbean	Jan 1992	$^{15}N_2 \rightarrow$ LMW DON[b]	63 ± 81	25 ± 16	Glibert and Bronk (1994)
Trichodesmium sp.	Gulf of Mexico	July 2001 and 2002	$^{15}N_2 \rightarrow$ Total N[c]	515 ± 348	52 ± 22	Mulholland *et al.* (2006)
Trichodesmium sp.	Gulf of Mexico	June 2003	$^{15}N_2 \rightarrow$ Total N[c]	364 ± 201	40 ± 18	Mulholland *et al.* (2006)
Trichodesmium sp.	Gulf of Mexico	Nov 2003	$^{15}N_2 \rightarrow$ Total N[c]	680 ± 450	51 ± 30	Mulholland *et al.* (2006)
Trichodesmium sp.	Gulf of Mexico	June 2003	$^{15}N_2 \rightarrow NH_4^+$[d]	154 ± 143	37 ± 57	Mulholland *et al.* (2006)
Trichodesmium sp.	Gulf of Mexico	Nov 2003	$^{15}N_2 \rightarrow NH_4^+$[d]	155 ± 87	75 ± 34	Mulholland *et al.* (2006)

[a] Rates were determined by net changes in dissolved free amino acid concentrations as determined with HPLC.
[b] The sample was passed through a Centriprep ultrafilter with a 10,000 MW cutoff.
[c] Total release, including DON and NH_4^+ release, was estimated as the difference between gross acetylene reduction and net $^{15}N_2$ uptake.
[d] Release of NH_4^+ was measured with ^{15}N isotope dilution.

3.3. Bacteria

Bacteria contribute to NH_4^+ regeneration and DON release during remineraliza-tion of POM (e.g., Smith *et al.*, 1992), by bactivory or as a result of viral lysis (e.g., Alonso *et al.*, 2000). It has also been suggested that bacteria can release ammonia (NH_3) via simple diffusion because intracellular concentrations are so much higher than extracellular concentrations (Hoch *et al.*, 1992). Considering the controversy over the percentage of bacteria that are actively growing (e.g., Smith and del Giorgio, 2003), the role of bacteria in NH_4^+ regeneration is still largely unknown.

With respect to NH_4^+ regeneration, bacteria differ from phytoplankton in that bacteria can serve as both sources and sinks. Whether bacteria are a source or a sink for NH_4^+ depends on their nutritional state, the availability and quality of N substrates (e.g., C:N ratio), and their gross growth efficiency (Goldman and Dennett, 1991; Goldman *et al.*, 1987; Jørgensen *et al.*, 1993). When the C:N ratio of bacteria is greater than the C:N ratio of the substrate, bacteria will regenerate NH_4^+ (Billen, 1984; Goldman *et al.*, 1987). With a bacterial C:N ratio of 4–5 (Goldman and Dennett, 1991) this means that bacteria would only regenerate NH_4^+ when they were consuming very low C:N substrates such as amino acids or peptides (e.g., Kirchman *et al.*, 1989, 1990). Kirchman *et al.* suggest that NH_4^+ utilization may be repressed by amino acid uptake. In this way bacteria also differ from other heterotrophs, such as protozoa, which rely on particulates and so are only a source of dissolved N, not a sink (Kirchman, 2000). The role of bacteria in N regeneration can be described by a simple model (Parnas, 1975).

According to this model, the rate of N excretion (E_N) is:

$$E_N = C_T \frac{1}{C{:}N_S} - \frac{GGE}{C{:}N_B \times 1000} \qquad (8.1)$$

with C_T ($= C_B + C_R$) where C_B is the C that goes into biomass and C_R is the C that is respired. $C{:}N_S$ and $C{:}N_B$ are the C:N ratios of the organic substrate and a bacteria cell respectively and GGE is gross growth efficiency (= $C_B C_T^{-1} \times 100$, Goldman *et al.*, 1987). When E_N is positive, N is excreted. When E_N is negative, net N is consumed. The relationship between bacterial growth efficiency and the C:N ratio of a substrate is given in Fig. 8.1 and described in detail in Kirchman (2000). In the range of growth efficiencies and $C{:}N_S$ ratios likely found in nature, the model predicts that bacteria should excrete NH_4^+. Based on a suite of culture experiments, however, Goldman *et al.* (1987) speculate that bacterial substrates in marine systems will have a $C{:}N_S$ ratio greater than 10 such that actively growing bacteria are unlikely to be efficient NH_4^+ remineralizers. Bacteria are also efficient competitors with phytoplankton for NH_4^+ (Kirchman, 2000). When bacteria are utilizing NH_4^+, any release of NH_4^+ would likely be an indirect effect of a process such as bactivory. In a series of incubations done with surface water collected in Sugami Bay, Japan, variable amounts of either glucose (C only) or glutamate (C and N) were added as the C source. Rates of NH_4^+ regeneration, measured with isotope dilution, were up to 4.5 times higher in the whole water glutamate treatments with 67% of the NH_4^+ regeneration occurring in the <1 μm size fraction (Hasegawa *et al.*, 2005).
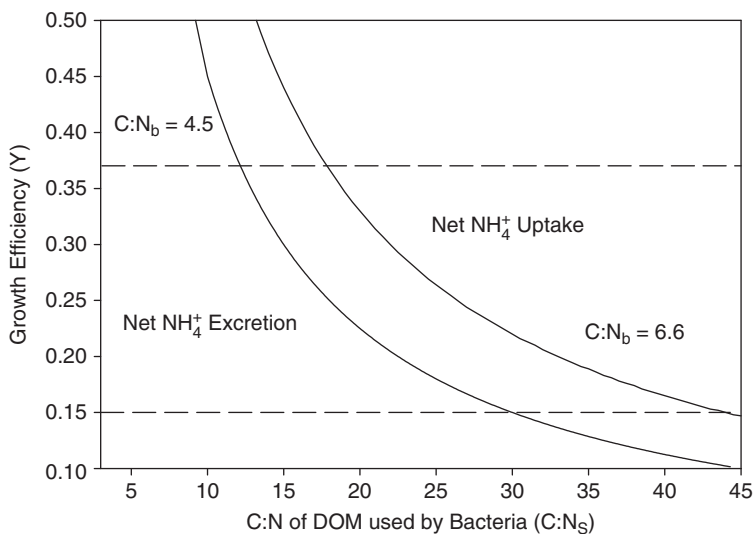
Figure 8.1 The relationship between bacterial growth efficiency and the C:N ratio of the dissolved organic matter (DOM) substrate ($C:N_s$). The curved indicate zero flux for a given bacterial C:N ($C:N_b$). Taken from Kirchman (2000).

A weakness of the model discussed above is its reliance on detailed information about C:N ratios of bacterial substrates that is currently unavailable. A later study modeled bacterial growth by optimizing electron flow, substrate uptake kinetics, and bacterial C:N ratio (Vallino *et al.*, 1996). This study found that the $C:N_S$ ratio is not always an accurate predictor of whether bacteria will remineralize NH_4^+.

With respect to DON, urea appears to be the most important form released. In two cultures of commercially available marine bacteria, urea production was highest when cell growth declined and at the start of stationary phase (Therkildsen *et al.*, 1997), which suggests that intracellular purines and pyrimidines were likely sources of the urea. Some portion of the intracellular RNA pool is likely degraded to urea and NH_4^+, particularly during exponential growth (Mason and Engli, 1993; Therkildsen *et al.*, 1997). In another study, production of NH_4^+ and urea by natural bacterial populations was observed in Lake Kinneret, Israel, the Charente Estuary, France, and the French coast of the Atlantic (Berman *et al.*, 1999). In these studies, guanine, hypoxanthine, and arginine additions resulted in increases in urea concentrations over time. The significance of the finding to natural waters is unknown, however, because the DON compounds tested were added at concentrations much higher than those found in the environment (40 mM, Berman *et al.*, 1999). Urea release has also been documented during bioassays in the Gulf of Riga (Jørgensen *et al.*, 1999). In a series of bacterial culture experiments, the concentration of D-DNA increased in the medium as a result of viral infection and bactivory (Alonso *et al.*, 2000). Finally, in another study the depletion of a number of nucleotide bases (guanine, hypoxanthine, adenine, xanthine, and pyrimidines) was followed by the production of urea in dark bioassays with bacteria collected off the coast of Denmark (Berg and Jørgensen, 2006).

In addition to urea, bacteria produce a suite of extracellular enzymes. These enzymes become components of the DON pool itself, as well as important agents that alter the characteristics of the DON itself (e.g., Martinez *et al.*, 1996). Bacteria also release amino acids. There is an abundant literature of bacterial amino acid secretion related to the biotechnology field (e.g., Krämer, 1994) but much less is known in marine systems.

3.4. Viruses and other parasites

A potentially large but unquantified mechanism for N regeneration is viruses. The role of viruses in N regeneration is three-fold. First, after a virus infects an autotrophic (including phytoplankton and macroalgae) or heterotrophic host, the phage increase to such numbers that the cells burst releasing the dissolved materials inside. As a result viral lysis is a highly efficient mechanism for moving cellular contents to the DOM pool. Second, viruses are the most abundant fraction of the plankton with concentrations ranging from 10^9 to 10^{11} viral particles L^{-1} (Suttle, 2005; Wommack and Colwell, 2000). Due to their small size (20–200 nm, Brussaard, 2004), viruses are themselves components of the DOM pool, which can make up a significant fraction of "dissolved" DNA. Third, viruses are important determinants of autotrophic and heterotrophic community structure (see reviews by Fuhrman, 1999; 2000; Suttle, 2005; Wommack and Colwell, 2000). As a result, viruses can, for example, act to select for a phytoplankton community that is more or less susceptible to a process such as sloppy feeding. Viruses that infect marine bacteria and cyanobacteria appear to be very common while viruses infecting eukaryotic marine phytoplankton are much less so. Relative to viruses that infect bacteria, the number of viruses isolated that infect eukaryotic phytoplankton are few (reviewed in Bidle and Falkowski, 2004 and Brussaard, 2004).

Viral abundance tends to be positively correlated with both chlorophyll concentrations and bacterial abundance (Maranger and Bird, 1995). Data on viral-mediated mortality are highly variable. A number of different approaches have been used to quantify phage-induced mortality of bacteria and phytoplankton but the task has proved challenging. Best estimates are that viruses kill an estimated ~20–40% of bacteria daily (Suttle, 2005). There are even fewer estimates of phytoplankton mortality but these too cluster in the ~10–40% range with extreme examples where 100% of the mortality of a phytoplankton bloom was attributed to viruses (reviewed in Brussaard, 2004).

A conceptual model of the incorporation of viruses and viral lysis into the aquatic food web was offered by Wommack and Colwell (2000). Each time a cell lyses the contents of the host cell are released including macromolecules, cell organelles, and viral particles. Theoretically, the end result of viral lysis is to divert C and N, fixed by phytoplankton and transformed into bacterial biomass, away from zooplankton consumers into the DOM pool (Bratbak *et al.*, 1994; Fuhrman, 1992; Fuhrman and Noble, 1995; Murray and Eldridge, 1994). More recently it has been demonstrated that viral infection can increase the sinking rate of a phytoplankton cell (*Heterosigma akashiwo*) likely resulting in more particulate material leaving surface waters (Lawrence and Suttle, 2004). This is an important variable to consider with respect to N release. In waters with a deep mixed layer, viral lysis would likely occur

in the mixed layer and the N released would be retained in the surface. In waters with a shallow mixed layer, however, the acceleration in sinking rates due to viral infection could result in transport of the cells out of the mixed layer, such that the N released when cells lyse would not be available to support surface production (Lawrence and Suttle, 2004).

A number of studies have examined the effects of viral lysis on the flux and composition of the DOM pool. Changing the abundance of viruses within experiments results in a change of bacterial abundance (Proctor and Fuhrman, 1991) and a change in the composition and concentration of DOM (Peduzzi and Weinbauer, 1993; Weinbauer and Peduzzi, 1995). The magnitude of the increase in regeneration caused by viruses is likely a function of the abundance of viruses and hosts, the burst size, and viral production rates (e.g., Miki and Yamamura, 2005). In a model of the flux of C through a microbial food web with and without viruses, the presence of viruses resulted in a 27% increase in bacterial respiration and production and a 37% decrease in C transfer to protozoan grazers (Fuhrman, 1992). Experimental evidence has since supported the model results with respect to increased bacterial respiration (Middelboe et al., 1996) and the presence of viruses enhancing the transfer of bacterial production to the DOM pool (Middelboe and Lyck, 2002). In a number of experiments and field studies phage lysis products stimulated bacterial growth and metabolic activity, indicating that viral lysis can contribute to a loop in which a portion of available nutrients cycle between the DOM pool and bacterial biomass without contributing to higher trophic levels (e.g., Middelboe and Lyck, 2002; Middelboe et al., 1996). An estimated 6–26% of organic C production goes into the DOC pool as a result of viral lysis in a process termed the viral shunt (Wilhelm and Suttle, 1999). Finally, changes in DOM composition with viral lysis have been demonstrated in mesocosm experiments (Weinbauer and Peduzzi, 1995). In experiments conducted during a number of phytoplankton blooms bacterial abundance was found to increase significantly after viral infection increased and the bloom crashed (e.g., Bratbak et al., 1998; Castberg et al., 2001; Gobler et al., 1997). In similar experiments viral lysis was suggested to be responsible for the near complete conversion of lost Phaeocystis pouchetii biomass to bacterial biomass through production of excess DOC (Bratbak et al., 1998).

Though viruses have received the most attention, fungi are also a source of parasitic infection although most work on phytoplankton fungal infection has been done with freshwater species (e.g., Holfeld, 1998). Similar to viral infection, fungal infection can also result in N release. As with viruses, fungal infections have been linked with mass mortalities and have been implicated in controlling bloom formation and affecting phytoplankton community composition (Park et al., 2004). The apparent relationship between phytoplankton size and parasite infection differs between viral and fungal infection, however. There are a number of studies showing viral infection of smaller phytoplankton species, such as chrysophytes and cyanobacteria, but viral infection does not appear to be as common for the larger dinoflagellates and diatoms (Brussaard, 2004). In contrast, eukaryotic parasites, such as fungi, appear more common in these larger cells (Park et al., 2004).

Epibiotic association with zooplankton can also take a number of forms (reviewed in Carman and Dobbs, 1997). Parasitic infection can have a number of

negative effects including the development of lesions through which DOM can be lost (Park *et al.*, 2004). Similarly, if the parasitic infection is extreme, mass mortality can occur resulting in substantial DOM release (Gomez-Gutierrez *et al.*, 2003).

3.5. Zooplankton

Zooplankton play a key role in regeneration of N in marine systems through their grazing and metabolism. Microzooplankton (<200 μm, the majority of which are heterotrophic protozoans) contribute to regeneration mainly through excretion. The larger mesozooplankton (200 μm – ~2 cm, including the abundant crustaceans, such as copepods) and macrozooplankton (>2 cm, such as the larger gelatinous zooplankton) contribute to N regeneration via excretion, sloppy feeding, and dissolution of egested fecal pellets. The relative importance and rates of these metabolic processes to N regeneration are discussed in detail in Chapter 26 by Steinberg and Saba (this volume, summarized in Table 8.3). In the following section we focus on the ecological and environmental significance of N regeneration by marine zooplankton.

Table 8.3 Example ammonia excretion rates for a variety of zooplankton taxa summarized from Chapter 26 by Steinberg and Saba (this volume; see their Table 26.4 for details and references)

Taxa	Range of Excretion rates ($\mu mol \, N \, indiv^{-1} h^{-1}$)
Microzooplankton	
Ciliates	3.6×10^{-8} to 3.8×10^{-5}
Flagellates	2.3×10^{-9} to 7.1×10^{-8}
Crustacea	
Copepods	1.7×10^{-4} to 1.7×10^{-2}
Amphipods	4.2×10^{-3} to 2.2×10^{-1}
Euphausiids	8.3×10^{-3} to 8.6×10^{-1}
Ostracods	1.7×10^{-3}
Shrimp	6.3×10^{-2} to 1.3
Mysids	3.2×10^{-1} to 4.8
Gelatinous zooplankton	
Chaetognaths	1.7×10^{-3} to 6.7×10^{-3}
Ctenophores	2.5×10^{-3} to 4.6×10^{-1}
Heteropods	1.6×10^{-1}
Pteropods	3.2×10^{-2} to 2.0×10^{-1}
Salps	5.8×10^{-3} to 1.2
Scyphomedusae	2.3×10^{-2} to 45.5
Siphonophores	4.2×10^{-3} to 8.3×10^{-3}
Hydromedusae	1.7×10^{-3} to 4.6×10^{-2}
Polychaetes	2.9×10^{-2} to 3.5×10^{-1}

3.5.1. Release by different zooplankton groups

The principal regenerators of NH_4^+ in marine pelagic ecosystems are typically thought to be the protozoan microzooplankton, with mesozooplankton playing a more minor role (Bode et al., 2004a; Dam et al., 1993; Glibert et al., 1992; Miller and Glibert, 1998). For example, Verity (1985) estimated that N regeneration by tintinnid ciliates exceeded that by meso/macrozooplankton in Naragansett Bay by a factor of 2–3. In a more extreme case, microzooplankton remineralization in Kanehoe Bay, Hawaii exceeded that by meso/macrozooplankton by a factor of 30–40 (Caperon et al., 1979). Ammonium regeneration by copepods was only 3–19% of that by the <94 μm plankton fraction off the coast of Japan (Hasegawa et al., 2000a), and 0.9–10% of the total N regeneration by microplankton (note: the term microplankton includes bacteria as well as microzooplankton) from Chesapeake Bay (Miller and Glibert, 1998). Within the microplankton size range (<200 μm), the most important NH_4^+ regenerators are in the smaller size classes— with the 1–15 μm nanoflagellate and small ciliate fraction generally being the most important (Glibert, 1982; Le Corre et al., 1996; Maguer et al., 1999; Probyn, 1987), and the <1 μm bacteria fraction more important in some instances (e.g., Harrison et al., 1983; Probyn, 1987). This can vary by environment and season, however. For example, larger flagellates and ciliates in the 15–200 μm fraction accounted for 50% of the microplankton N regeneration in spring and summer in the western English Channel (Maguer et al., 1999), and copepods, rotifers, tintinnids, and heterotrophic dinoflagellates in the 45–200 μm fraction produced most of the regenerated NH_4^+ in summer in Oslofjord (Paasche and Kristiansen, 1982).

One difficulty encountered when trying to quantify the role of different size fractions to regeneration is the assumption that the different fractions measured will equal the sum of the parts. Studies have shown, however, that the sum of the rates measured in the different size fractions often exceeds that of the total rate in the unfractionated sample (Glibert et al., 1992).

Although bacteria and protozoan microzooplankton often account for the majority of N regeneration within a given system, mesozooplankton, through processes such as selective grazing and sloppy feeding, have a number of important modifying and indirect effects on N regeneration (Glibert et al., 1992; Miller et al., 1997). For example, in microcosm experiments with natural microplankton (<200 μm) amended with nutrients and copepod grazers, NH_4^+ concentration changes in treatments containing microplankton + copepods + nutrients compared to treatments containing microplankton + nutrients exceeded that expected from copepod release rates alone, suggesting that copepods increased nutrient regeneration by microzooplankton (Miller et al., 1997). A similar result was found in earlier mesocosm studies where the presence of copepods resulted in higher nutrient regeneration, primary production, and growth rates of bacteria and heterotrophic nanoflagellates (Roman et al., 1988). Miller et al. (1997) suggest that release of DON from zooplankton, via sloppy feeding or excretion, may stimulate NH_4^+ regeneration by bacteria by providing a relatively low C:N ratio substrate for bacterial growth.

Trophic cascade effects (top-down control of food webs by predators leading to alternating high/low abundance of each subsequent lower trophic level) can alter the amount and type of N released and regenerated. Copepods can control the

abundance of ciliates, thus releasing grazing pressure and indirectly stimulating NH_4^+ regeneration by flagellates (Glibert et al., 1992; Miller et al., 1995). In a study of an upwelling system off Spain, N regeneration by microplankton (bacteria, heterotrophic flagellates, and ciliates) was always higher than that of mesozooplankton, however, the relative contribution of each changed depending on the stage of the upwelling cycle (high production vs. low production) (Bode et al., 2004b), presumably due to trophic structure changes and resultant changes in N regeneration during and in between upwelling pulses.

Mesozooplankton alone can also contribute significantly to regeneration. For example, krill swarms in the Antarctic can be important regional sources of NH_4^+ (Atkinson and Whitehouse, 2000; Huntley and Nordhausen, 1995). As noted above, Hasegawa et al. (2000a) found that microplankton (<94 μm) were relatively more important in the release of DON than copepods in coastal waters off Japan, however, release of DFAAs by copepods was sometimes as large as release by microplankton (<202 μm) in Long Island Sound (Fuhrman, 1987). Many gelatinous zooplankton form blooms, which could also contribute substantially to NH_4^+ regeneration. In Kiel Bight during the summer, standing stock of the medusae *Aurelia aurita* outweighs that of micro- and the rest of the meso-macrozooplankton community by 1.5–4 times (Schneider, 1989). In years of high biomass, NH_4^+ regeneration by *Aurelia aurita* exceeds that of all other zooplankton. This medusae is the second most important supplier of regenerated nutrients in Kiel Bight (supplying 11% of phytoplankton N requirements), with bottom sediments being the largest regenerated N source (Schneider, 1989). In other systems, however, gelatinous zooplankton do not appear to be important N regenerators. In Chesapeake Bay the contribution of NH_4^+ regeneration from medusae and ctenophores to phytoplankton primary production was minor (supplying 0.6% of phytoplankton N requirements, Nemazie et al., 1993). Furthermore, the dominant copepod in Chesapeake Bay, *Acartia tonsa*, only could supply 0.4–1% of the NH_4^+ required by phytoplankton, and thus Nemazie et al. (1993) concluded microzooplankton must be the dominant regenerators of NH_4^+ in this estuary. Pitt et al. (2005) suggests gelatinous zooplankton such as scyphozoan medusae promote growth of phytoplankton both from the bottom up, by supplying NH_4^+ through metabolism, and via top-down control, by consuming other zooplankton that feed on phytoplankton.

Finally, a variety of zooplankton have recently been shown to produce colored (chromophoric) DOM (CDOM), a fraction of which is fluorescent (FDOM) (Steinberg et al., 2004; Urban-Rich et al., 2004, 2006). Although the nitrogenous composition of CDOM/FDOM is little known, fluorescent humic-like material (likely fulvic and humic acids) is produced during grazing, influencing the optical characteristics of coastal waters (Urban-Rich et al., 2006). A recent culture study shows that humic-N can be a source of N for coastal phytoplankton (See et al., 2006).

3.5.2. Contribution of zooplankton regeneration to primary production

The role of zooplankton in the regeneration of NH_4^+ (and in only a handful of studies, DON) to fuel phytoplankton productivity has been determined in a number of studies, examples of which are shown in Table 8.4. The amount of primary production that can be supported by zooplankton NH_4^+ excretion depends on environment, taxa studied,

Table 8.4 Percent contribution of zooplankton ammonia excretion (or ammonia + urea[a]) to nitrogen requirements of phytoplankton primary production in a variety of environments (after Bidigare, 1983; LeBorgne, 1986; Hernández-León *et al.*, 2008)

Location	Taxa	% Contribution (day^{-1})	Reference
Inshore/Estuarine			
Long Island Sound	Mixed zooplankton >200 μm	43–66	Harris (1959)
Coastal lagoon, New South Wales, Australia	*Catostylus mosaicus* scyphozoan medusae	8	Pitt *et al.* (2005)
Chesapeake Bay	*Chrysaora quinquecirrha* scyphozoan medusae and *Mnemiopsis leidyi* ctenophores	0.6	Nemazie *et al.* (1993)
Great South Bay, NY	*Mnemiopsis leidyi* ctenophores and mixed zooplankton >200 μm	1–3 (summer) 39 (spring)	Park and Carpenter (1987)
Narragansett Bay	*Mnemiopsis leidyi* ctenophores	3–15	Kremer (1975)
Narragansett Bay	Mixed zooplankton >200 μm	4 (annual)	Vargo (1979)
Narragansett Bay	*Tintinnopsis* spp. tintinnids	11 (annual), 18[a] 29 (Aug–Sept), 48[a]	Verity (1985)
Kaneohe Bay	*Sagitta enflata* chaetognaths	3	Szyper (1981)
Western English Channel	Mixed microplankton[b] <200 μm	60 (annual)	Maguer *et al.* (1999)
Coastal and Open ocean			
Kiel Bight, Western Baltic	*Aurelia aurita* scyphozoan medusae	17 (1982) 5 (1983–1984)	Schnieder (1989)
Mediterranean Sea	*Pelagic noctiluca* scyphozoan medusae	7	Malej (1991)
NW Mediterranean Sea	Mesozooplankton	43 (mean) 100–285 (coastal)[c] 9–189 (frontal)[c] 5–160 (offshore)[c]	Alcaraz *et al.* (1994)
Bransfield Strait, Antarctic	Salps Crustacean zooplankton	9 (mean) 0.4 (mean)	Alcaraz *et al.* (1998)

(Continued)

Table 8.4 Percent contribution of zooplankton ammonia excretion (or ammonia + urea[a]) to nitrogen requirements of phytoplankton primary production in a variety of environments (after Bidigare, 1983; LeBorgne, 1986; Hernández-León *et al.*, 2008) (*continued*)

Location	Taxa	% Contribution (day^{-1})	Reference
South Georgia Island, Antarctic	*Euphausia superba*	16–50 (Eastern area) 3–4 (Western area)	Atkinson and Whitehouse (2000)
South Georgia Island, Antarctic	Mesozooplankton (copepods and small euphausiids)	32 (Eastern area) 12 (Western area)	Atkinson and Whitehouse (2001)
Grand Canaria Islands coastal eddies	Mixed mesozooplankton	8	Hernández-León *et al.* (2001)
Gulf of Lions, NW Mediterranean	Mixed zooplankton	31–32	Gaudy *et al.* (2003)
Bay of Biscay	Mixed mesozooplankton	25 (outside eddy) 40 (edge of eddy) 44 (eddy center)	Isla *et al.* (2004a)
Western N. Atlantic	Mixed gelatinous zooplankton	39–63	Biggs (1977)
Western N. Atlantic	Mixed gelatinous zooplankton	4–6	Biggs *et al.* (1981, 1984)
N.E. Atlantic	Microzooplankton	0–94	Gaul *et al.* (1999)
Sargasso Sea	Diel vertical migrating mesozooplankton	2 (mean) 19 (max)	Steinberg *et al.* (2002)
N.W Iberian oceanic	Mixed mesozooplankton	30	Isla *et al.* (2004b)
Atlantic from 50°N-30°S	Mixed mesozooplankton	31–120	Isla *et al.* (2004c)
North Pacific central gyre	Mixed mesozooplankton	40–50	Eppley *et al.* (1973)
Central tropical Pacific	Mixed mesozooplankton	3–17	Zhang *et al.* (1995)
Upwelling			
N.W. Africa upwelling	Mixed mesozooplankton	44	Smith and Whitledge (1977)
Peru upwelling	3 large copepod species: *Calanus chilensis, Eucalanus inermis,* and *Centropages brachiatus*	4.3	Dagg *et al.* (1980)
N.W. Iberian upwelling	Mixed mesozooplankton	3.8[a]	Isla *et al.* (2004b)

[a] Ammonia + urea.
[b] The term microplankton includes bacteria as well as microzooplankton.
[c] Range of means is provided for several oceanic provinces.

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and season, and ranges widely from <1–285%, but is normally in the range of 3–50% (Table 8.4). Even individual species (e.g., tintinnids *Tintinnopsis* spp., ctenophores *Mnemiopsis leidyi*, and euphausiids *Euphausiia superba*, Table 8.4) that are abundant and key components of the planktonic food web at certain times of the year can have a large impact on supplying regenerated N to meet phytoplankton requirements. The contribution of zooplankton NH_4^+ excretion to phytoplankton N requirements might be expected to be lower in environments where phytoplankton rely primarily on new N (NO_3^-), such as upwelling or high latitude regions, and higher in areas that rely more heavily on recycled N (NH_4^+), such as oligotrophic, open–ocean regions. On a global scale in the open ocean, this appears to be true. Hernández-León *et al.* (2008) calculated the potential contribution of open-ocean mesozooplankton community excretion to fuel primary production by assigning to latitudinal range literature values of mesozooplankton specific NH_4^+ excretion (fraction of body N excreted d^{-1}) and rates of primary production by biogeochemical province. The largest contribution of NH_4^+ excretion to primary production was indeed in the tropics (mean of 47% of the primary production), decreasing in the subtropics and temorate regions (27–28%) to the polar areas (4–8%), with a global average of 23% (Hernández-León *et al.*, 2008). However, comparing individual studies of excretion by mixed mesozooplankton (i.e., excluding studies of individual species) in different environments shows there are no obvious distinctions between the importance of N regeneration by zooplankton to phytoplankton N demand in oligotrophic vs. upwelling regions (Table 8.4). For example, within upwelling regions, some studies indicate a minor contribution to phytoplankton N demand of around 4% (e.g., Isla *et al.*, 2004b) while others show a more major contribution of 44% (Smith and Whitledge, 1977). For comparison, in the oligotrophic Sargasso Sea, western N. Atlantic, and Mediterranean Sea, N regeneration by mixed mesozooplankton often accounts for as low as <6% of the phytoplankton demand, but can be as high as 160% (e.g., offshore station in Mediterranean Sea) (Table 8.4).

Similarly, it might be predicted that zooplankton N regeneration of NH_4^+ in coastal and inshore temperate environments would contribute least to phytoplankton primary production during the spring bloom, and become relatively more important in summer or other times of the year when phytoplankton typically rely more on recycled nutrients. This does appear to be true for a study in Narragansett Bay, where regeneration of NH_4^+ by tintinnids is more important during the summer than the rest of the year (Verity, 1985, Table 8.4). The opposite trend, however, is apparent in a study where NH_4^+ excretion by ctenophores and mixed mesozooplankton in Great South Bay, NY is more important to phytoplankton primary production in spring (contributing to 39% of phytoplankton N demand) than in summer (contributing to 1–3% of phytoplankton N demand) (Park and Carpenter, 1987, Table 8.4). In inshore waters, nutrient regeneration by the benthic community can be a more important source to phytoplankton than zooplankton regeneration (e.g., Schneider, 1989).

3.5.3. Vertical transport
While zooplankton fuel primary production in the surface waters, they can also contribute to N regeneration at depth. A process that has received attention in the last two decades is the transport of regenerated N below the euphotic zone by diel

vertically migrating mesozooplankton. Diel migrators consume organic particles in the surface waters at night and metabolize the ingested food below the mixed layer during the day resulting in excretion at depth (e.g., Longhurst and Harrison, 1988). This "active" vertical N and C flux can be significant compared to passive sedimentation of organic particles measured by sediment traps (Al-Mutairi and Landry, 2001; Dam et al., 1995; Le Borgne and Rodier, 1997; Longhurst et al., 1990; Steinberg et al., 2000; Steinberg et al., 2002; Zhang and Dam, 1997). The total active N flux is both excreted dissolved NH_4^+ and DON, as well as particulate organic N (PON) in fecal pellets produced at depth, which can subsequently leach DON (e.g., Jumars et al., 1989). On average, migratory dissolved N flux for a number of open ocean environments varies from 0.6 to 3.6 mg N m^{-2} day^{-1} and is equal to 9–45% of the sinking PON flux; maximum dissolved N fluxes can exceed the sinking PON flux (see Table 4 in Steinberg et al., 2002). Migratory active dissolved N flux on average could support a small proportion (2–10%) of phytoplankton N demand for primary production (Al-Mutairi and Landry, 2001; Dam et al., 1995; Longhurst et al., 1989; Rodier and Le Borgne, 1997—calculated from their Table 8; Steinberg et al., 2002). Zooplankton migratory N flux has implications for bacterial regeneration of zooplankton-derived DON in the mesopelagic zone, as a significant proportion of the total dissolved N (TDN) excreted by migrators at depth can be excreted as DON. In the equatorial Pacific, 46% (HNLC area) and 60% (oligotrophic site) of TDN excreted by vertical migrators was as DON (Le Borgne and Rodier, 1997) and 32% of the TDN excreted by migrators was as DON in the Sargasso Sea (Steinberg et al., 2002).

3.6. Nekton

The contribution of marine nekton (the stronger swimming pelagic macroorganisms) such as fish and decapod crustaceans (e.g., shrimp) to regeneration of N in marine systems has received considerably less attention than has zooplankton (Haertel-Borer et al., 2004). In addition, the quantification and significance of large consumer-driven nutrient recycling is much better known in freshwater systems (e.g., see review by Vanni, 2002). These larger motile organisms contribute to N regeneration via excretion or dissolution of egested feces (although the role of sloppy feeding has not been studied for these larger organisms and may also contribute to the dissolved N pool), transport of nutrients via migration, and release of sediment bound nutrients back into the water column by bioturbation. One discipline where regeneration of N by nekton has been studied intensely is aquaculture. High densities of farmed fish can lead to elevated NH_4^+ concentrations, which can be toxic to the fish, as well as lead to high sediment loading by fish feces, resulting in anoxic bottom sediments, a decrease in benthic diversity, and other problems (Ip et al., 2001; Wu, 1995). In this section we focus on the ecological and environmental significance of N regeneration by marine nekton. For a comprehensive review of the physiology of N metabolism and excretion by fish, see the volume of *Fish Physiology* dedicated to N excretion edited by Wright and Anderson (2001).

Like the zooplankton (see Chapter 26 by Steinberg and Saba, this volume), marine teleost fish are primarily ammonotelic (Wood, 2001), but also can excrete a significant proportion of their TDN as urea or other DON compounds (in both early

developmental stages and as adults) (Anderson, 2001; Torres *et al.*, 1996; Walsh *et al.*, 2001; Wood, 2001; Wright and Fyhn, 2001; Wright and Land, 1998). For example, in a survey of 16 marine fish species, Walsh *et al.* (2001) found urea excretion by teleosts constituted 1–24% of the combined NH_4^+ plus urea excreted. For one species for which TDN excretion was measured, DON (urea plus unknown compounds) constituted 4–31% of the TDN excreted. For the sardine *Sardinop sagax*, DON constituted 31% of the TDN excreted by unfed fish and 25% for fish fed mysids (van der Lingen, 1998). Marine elasmobranchs (sharks, skates, and rays) are primarily ureotelic but excretion rates have been measured on very few species (Wood, 2001), thus their contribution to N regeneration will not be considered here.

Diet can play an important role in N recycling by nekton, and may lead to trophic effects on N regeneration in a given ecosystem. For example, N assimilation efficiencies for omnivorous anchovies (*Engraulis capensis*) and sardines (*Sardinops sagax*) from upwelling regions fed phytoplankton are lower (83% and 79%, respectively) than those fed zooplankton (87% and 93%, respectively) (James *et al.*, 1989; van der Lingen, 1998). N assimilation efficiency for Atlantic menhaden (*Brevoortia tyrannus*), however, was similar (∼92%) for feeding on phytoplankton or zooplankton (Durbin and Durbin, 1981). The stoichiometry of consumer-driven nutrient recycling also has an important trophic impact, as the N:P ratio of excretion can have significant effects on phytoplankton physiology and community structure (e.g., Elser and Urabe, 1999).

The contribution of nekton NH_4^+ regeneration to phytoplankton N requirements for primary production has been determined in several studies. Based on their measured N excretion rates of anchovy (*Engraulis mordax*) and jack mackerel (*Trachurus symmetricus*), and fisheries data from the California Cooperative Oceanic Fisheries Investigations (CalCOFI) survey, McCarthy and Whitledge (1972) estimated regeneration of NH_4^+ and urea by pelagic fish off the coast of southern California accounts for 10% of the NH_4^+ and 2% of the urea required by phytoplankton for primary production. Omnivorous fish in a surf zone ecosystem supply <1% of the total phytoplankton N requirements (Cockcroft and Du Preez, 1990), with penaeid prawns supplying 12% (Cockcroft and McLachlan, 1987), and mysids 10% (Cockcroft *et al.*, 1988), of the phytoplankton N requirements in this same system.

Nekton represent an important local source of regenerated N in a number of habitats. The release of NH_4^+ and P by nekton (fish and shrimp) in intertidal creeks of a salt marsh estuary in South Carolina was quantified by Haertel-Borer *et al.* (2004). They found NH_4^+ excretion by nekton in early spring and summer (mean of 199 μmol NH_4^+ m^{-2} h^{-1}, in a water column depth of 0.4–0.7 m) was considerably higher than by meso- and macrozooplankton (approximately 2 μmol NH_4^+ m^{-2} h^{-1}, calculated from their Table 6) and higher than all other sources of NH_4^+ (e.g., atmospheric deposition, benthic remineralization, bacteria) with the exception of oyster reefs (oysters plus sediment and epifauna) (mean of 229 μmol NH_4^+ m^{-2} h^{-1}, in summer). Although nekton excretion was relatively more important in NH_4^+ release than bioturbation in their experiments, nekton excretion plus bioturbation was on average the largest source of NH_4^+ in this system (mean of 274 μmol NH_4^+ m^{-2} h^{-1}). Many of the nekton in the intertidal creeks were feeding on benthic invertebrates or detritus, and thus translocating benthic-bound nutrients into the water column via regeneration (see below). Fish are also a significant nutrient source,

via their defecation and excretion, to coral reef communities (Meyer and Schultz, 1985; Meyer *et al.*, 1983). For example, juvenile french and white grunts (*Haemulon flavolineatum and H. plumieri*) were capable of doubling the N supplied to corals from other sources and stimulating coral growth and likely biological activity in reef benthos (Meyer and Schultz, 1985). Diel periodicity in N excretion and defecation by reef-dwelling fish (Polunin and Koike, 1987) may also have important effects on nutrient cycling and phytoplankton communities in reef ecosystems.

The importance of fish as a vector for biotic nutrient transport (or translocation) of biomass or dissolved nutrients from one habitat or ecosystem to another has been explored in a several studies. The migration of gulf menhaden (*Brevoortia patronus*) results in the transport of N and P (as biomass) from an estuary into the Gulf of Mexico that is of the same order of magnitude as passive export of nutrients by tides and freshwater flow (Deegan, 1993). Planktivorous fish (the blacksmith, *Chromis punctipinnis*) feeding in the water column on zooplankton excrete NH_4^+ within the subtidal rocky reef while sheltering at night (Bray *et al.*, 1986). The amount of NH_4^+ excreted by these sheltering fish is about one order of magnitude higher than the total amount excreted by macroinvertbrates and fish foraging mainly within the reef (Bray *et al.*, 1988). Hjerne and Hansson (2002) found the removal of nutrients in fish biomass by the fishery in the Baltic Sea was 1.4% of the total annual N load (and 7% of the P load). The fishery removed a more significant proportion of the estimated anthropogenic fraction of the total nutrient load that reaches the open Baltic Sea (2.5% of the anthropogenic N load and 18% of the P load), constituting a significant flux of nutrients from the ecosystem. They suggest that the fishery may ''counteract eutrophication in the open Baltic Sea by removing considerable amounts of nutrients through fish landings,'' although they caution that ''other effects of fishing may counterbalance this positive effect.'' Fish feeding on benthic prey (or on organic detritus and algae associated with sediments) but excreting metabolized nutrients into the open water column is another mode of N transport. In freshwater systems this release of sediment-bound nutrients into the water column can be an important source of nutrients compared to inputs from the watershed (e.g., Gido, 2002; Schaus *et al.*, 1997). This process has been little studied in marine systems (although see Haertel-Borer *et al.*, 2004, discussed above).

Finally, as described in the preceding section 3.5.3. for zooplankton, vertically migrating nekton in the open ocean may also actively transport regenerated N below the euphotic zone through diel vertical migration, although data are scarce for nekton active transport (Longhurst and Harrison, 1988; Longhurst *et al.*, 1989, and see Angel and Pugh, 2000, and Hidaka *et al.*, 2001 for C transport by micronekton). The fecal matter of vertically migrating myctophids in the western equatorial Pacific was found to be high in amino acids (equal to 37–42% of fecal organic C) and could provide a source of essential amino acids for mesopelagic animals (Hidaka *et al.*, 2001).

3.7. Corals

Corals are known to release relatively large amounts of particulate organic C (POC) and DOC largely in the form of mucus (e.g., Crossland, 1987). The release of DOC-rich mucus has been hypothesized to serve a number of roles including as an aid in

defense and feeding, a protection from dessication and sediment, and as a sunscreen. It has also been suggested that the high C:N ratio of mucus could result from a diet rich in C (Brown and Bythell, 2005).

A review of analyses of the composition of mucus indicated that protein makes up 4–49% of the coral organism with total N making up 0.04–5.2% of mucus (Brown and Bythell, 2005). The primary constituents of the mucus matrix are glycoproteins or mucins. In a recent model of the mucus layer, organic exudates and organic N are released from the coral surface and go on to support a dynamic microbial community within the mucopolysaccharide layer around the coral (Rohwer and Kelley, 2004).

In comparison, relatively little is known about N release. Two studies of DON release found that up to 50% of the total N requirement of the coral colony is released (Bythell, 1988). Rates of DON release were measured in the zooxanthellate coral *Galaxea fascicularis* over a 24-h cycle (Ferrier-Pagès *et al.*, 1998a). There was a diel periodicity inherent in DON release from both fed (on *Artemia*) and unfed organisms with large pulses of DON release observed in mid-morning and mid-afternoon. Daily release rates were 0.5–0.6 µmol DON (mg protein)$^{-1}$ for unfed organisms, 1–3 µmol DON (mg protein)$^{-1}$ for fed organisms, and 0.2–1.3 µmol DON (mg protein)$^{-1}$ for organisms supplemented with N and P. DON did not persist in the media, however, but was rapidly reincorporated, likely by free-living epibiotic and/or intracellular bacteria. The rapidity of measured reincorporation suggests that the release rates measured are likely significantly underestimated. During the pulses of DON release the C:N ratio of the material was very low (2.5–4.5) but reached as high as 13 in the non-peak times. A new chromatographic/mass spectrometric method of measuring DON release (see Chapter 28 by McCarthy and Bronk, this volume) was used to estimate DON release from the hermatypic coral *Porites cylindrica* (Miyajima *et al.*, 2005). Over the course of a four day experiment $^{15}NO_3^-$ was taken up by the coral endo-symbiont and DON was produced at a surface-area specific rate of 0.58 nmol N cm^{-2} day^{-1}.

3.8. Detritus and marine snow

One source of DOM release that is particularly difficult to quantify is release from detritus or marine snow. In an inverse modeling study of C and N in the South California Bight, Jackson and Eldridge (1992) found evidence that the detrital pool was a key intermediate between primary production and release of DOM. The problem with quantifying release from detritus in the field is defining what is meant by detritus. When a phytoplankter dies, does it instantly become part of the detrital pool? A suite of analytical techniques have been brought to bear on defining what constitutes a living cells versus what is detritus (e.g., Minor and Nallathamby, 2004 and references therein). Fecal material is another important component of the detrital pool, which raises questions as to whether one considers DON release from fecal pellets (see Chapter 26 by Steinberg and Saba, this volume) to come from zooplankton or from detritus.

The release of DOM by bacteria solubilizing sinking particles has been of considerable interest in investigations of particle flux to the ocean's interior. Smith *et al.* (1992) showed that amino acids associated with particulates were rapidly

hydrolyzed by exoenzymes secreted by bacteria associated with marine snow aggregates, suggesting that sinking particles may be more rapidly solubilized than previously thought. The hydrolyzed amino acids were not taken up by the attached bacteria, indicating release of DON into surrounding waters. Marine aggregates also have pore fluids that can be enriched (often orders of magnitude over surrounding seawater) with dissolved inorganic and organic nutrients such as NH_4^+ (Gotschalk and Alldredge, 1989; Shanks and Trent, 1979), silicic acid (Brzezinski *et al.*, 1997), and DOC (Alldredge, 2000) that could be released into the surrounding seawater when grazed or otherwise disrupted.

3.9. Sediments

Due to space limitations we confine our discussion of sediments to a review of their role as a source of regenerated N to the water column. For a more thorough treatment of N cycling in sediments see Chapter 19 by Joye and Anderson (this volume), for a review of DON in sediments see Burdige (2002), and for more detailed reviews of N regeneration from sediments see Burdige (2006), Klump and Martens (1983) and Nixon (1981).

In sediments there are three principle microbial processes that affect N regeneration—denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$; Chapter 6 by Devol, this volume), nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$; Chapter 5 by Ward, this volume) and ammonification. In addition to classical denitrification, the recently discovered anammox (anaerobic ammonium oxidation) is another denitrifying process (Chapter 6 by Devol, this volume). In the anammox reaction NH_4^+ is combined with NO_2^- to yield N_2 and water. The production of N_2 makes anammox another form of denitrification. Ammonification is the release of NH_4^+ from organic matter. The direct link with organic matter makes ammonification distinct from other processes that regenerate NH_4^+ such as grazing. Ammonification is difficult to quantify, however, because it is difficult to determine the source of NH_4^+. Though most ammonification is thought to originate with bulk DON, some individual compounds have been shown to be important. Urea, which can be quickly hydrolyzed to NH_4^+ by bacterial ureases, is an important source of NH_4^+ in sediments, particularly in those with high macrofaunal biomass. For example, in the sediments on the shelf of the Bering Sea up to 80% of the NH_4^+ production was attributed to urea hydrolysis (Lomstein *et al.*, 1989). To these microbial processes we must also add release from macroalgae, microphytobenthos, and benthic fauna in general (discussed below).

As in the water column one must also consider simultaneous N utilization when discussing N regeneration in sediments. Two sets of processes in particular can remove N from sediments, without transferring fixed N to the water column. The first is denitrification (e.g., Laursen and Seitzinger, 2002) and anammox, where N is lost from the system as N_2 gas., The second is chemical adsorption, including the binding of DON to particles, which protects it from microbial breakdown (e.g., Hedges and Keil, 1999), and NH_4^+ adsorption to clays through association with ion exchange sites (see Burdige, 2006; Klump and Martens, 1983).

A survey of rates of N release from sediments in ocean, coastal and estuarine environments is presented in Table 8.5; we note that this is not an exhaustive

Table 8.5 Rates of nitrogen (N) release from sediment. Rates were quantified by measuring concentration changes (CC) in either sediment cores (SC) or benthic chambers (BC)

Location	Date	N release rate mmol N m^{-2} day^{-1}	Chemical species	Method	Reference
Oceanic					
Arctic, offshore off Norway	June, July 1991	0.016 ± 0.070	NH_4^+	CC in SC	Blackburn *et al.* (1996)
Continental margin, eastern North Pacific	June–July 1988	0.372 ± 0.328	NH_4^+	CC in BC	Devol and Christensen (1993)
Continental margin, eastern North Pacific	June 1991	0.536 ± 0.510	NH_4^+	CC in BC	Devol and Christensen (1993)
Continental shelf, North Sea	July	0.262 ± 0.250	NH_4^+	CC in BC	Lohse *et al.* (1996)
Skagerrak, North Sea	Aug 1992, May 1993 and 1994	0.080 ± 0.111	NH_4^+	CC in SC	Landén and Hall (2000)
Continental margin, eastern North Pacific	June–July 1988	0.026 ± 0.035	NO_2^-	CC in BC	Devol and Christensen (1993)
Continental margin, eastern North Pacific	June 1991	0.138 ± 0.121	NO_2^-	CC in BC	Devol and Christensen (1993)
Continental margin, eastern North Pacific	June–July 1988	-1.210 ± 0.346	NO_3^-	CC in BC	Devol and Christensen (1993)
Continental margin, eastern North Pacific	June 1991	-1.210 ± 0.475	NO_3^-	CC in BC	Devol and Christensen (1993)
Continental shelf, North Sea	July	0.254 ± 0.180	NO_3^-	CC in BC	Lohse *et al.* (1996)

(*Continued*)

Table 8.5 Rates of nitrogen (N) release from sediment. Rates were quantified by measuring concentration changes (CC) in either sediment cores (SC) or benthic chambers (BC) (*continued*)

Location	Date	N release rate mmol N m^{-2} day^{-1}	Chemical species	Method	Reference
Arctic, offshore off Norway	June, July 1991	1.008 ± 1.091	DON	CC in SC	Blackburn *et al.* (1996)
Arctic, offshore off Norway	June, July 1991	0.014 ± 0.030	urea	CC in SC	Blackburn *et al.* (1996)
Continental margin off Peru		0.17	DFAA	Conc gradient	Henrichs *et al.* (1984)
Skagerrak, North Sea	Aug 1992, May 1993 and 1994	-0.0003 ± 0.007	DFAA	CC in SC	Landén and Hall (2000)
Coastal					
Arctic, nearshore off Norway	June, July 1991	0.054 ± 0.493	NH$_4^+$	CC in SC	Blackburn *et al.* (1996)
Concepción Bay, Chile	June, Nov 1998, Jan, Mar 1999	12.92 ± 10.1	NH$_4^+$	CC in SC	Graco *et al.* (2001)
Georgia Bight	Dec 1981, Mar, May, June 1982	1.38	NH$_4^+$	CC in SC	Hopkinson (1987)
Gray's Reef, GA	July 1985	0.53–21.5	NH$_4^+$	^{15}N ID-SE	Hopkinson *et al.* (1991)
Kaneohe Bay, HI reef flat, slope, and bottom	Jan 1994–Apr 1998	0.665 ± 1.069	NH$_4^+$	CC in BC	Stimson and Larned (2000)
Upwelling region off Chile	Mar 1999	1.2–5.6	NH$_4^+$	CC in SC	Molina *et al.* (2004)
Concepción Bay, Chile	June, Nov 1998, Jan, Mar 1999	-1.33 ± 2.10	NO$_3^-$	CC in SC	Graco *et al.* (2001)
Kaneohe Bay, HI reef flat, slope, and bottom	Jan 1994–Apr 1998	-0.028 ± 0.377	NO$_3^-$	CC in BC	Stimson and Larned (2000)
Upwelling region off Chile	Mar 1999	-0.6 to -4.3	NO$_3^-$	CC in SC	Molina *et al.* (2004)
Georgia Bight	Dec 1981, Mar, May, June 1982	0.262	NO$_3^-$/ NO$_2^-$	CC in SC	Hopkinson (1987)
Arctic, nearshore off Norway	June, July 1991	0.899 ± 3.166	DON	CC in SC	Blackburn *et al.* (1996)

Georgia Bight	Dec 1981, Mar, May, June 1982	0.197	DON	CC in SC	Hopkinson (1987)
Gray's Reef, GA	July 1985	−0.02 to 34.0	DON	[15]N ID-SE[a]	Hopkinson et al. (1991)
Kaneohe Bay, HI reef flat, slope, and bottom	Jan 1994–Apr 1998	1.701 ± 1.856	DON	CC in BC	Stimson and Larned (2000)
Upwelling region off Chile— coastal stations	Mar 1999	4.9–75	DON	CC in SC	Molina et al. (2004)
Upwelling region off Chile— offshore stations	Mar 1999	−9.7 to −20.5	DON	CC in SC	Molina et al. (2004)
Arctic, nearshore off Norway	June, July 1991	0.009 ± 0.171	Urea	CC in SC	Blackburn et al. (1996)
Upwelling region off Chile	Mar 1999	−0.03 to −2.77	Urea	CC in SC	Molina et al. (2004)
Cape Lookout Bight, NC	∼Mar 1985	0.05–3.56	DFAA	Conc gradient	Burdige and Martens (1990)

Estuarine

Aarhus Bay, Denmark	Nov 1994	0.020 ± 0.040	NH_4^+ in control	CC in BC[b]	Pedersen et al. (1999)
Aarhus Bay, Denmark	Nov 1994	1.389 ± 0.240[c]	NH_4^+ with eel grass	CC in BC[b]	Pedersen et al. (1999)
Baffin Bay, TX	Apr, Aug, Dec 1999, Apr 2000	3.25 ± 1.72	NH_4^+	CC in SC	Gardner et al. (2006)
Bay of Cadiz, Spain	March–Jan.	6.19–36.6	NH_4^+	CC in BC	Forja et al. (1994)
Chesapeake Bay	Dec 1988–Nov 1989	−0.035 to 0.506	NH_4^+	CC in SC	Cowan and Boynton (1996)
Chesapeake Bay—mesohaline	Mar 1995–Oct 1996	5.38 ± 0.47	NH_4^+	CC in BC	Burdige and Zheng (1998)
Chesapeake Bay—south bay	Mar 1995–Oct 1996	3.65 ± 1.73	NH_4^+	CC in BC	Burdige and Zheng (1998)
Coastal Virginia[d]	Oct 1998–Aug 1999[e]	0.099 ± 0.189	NH_4^+	CC in SC	Tyler et al. (2003)
Fourleague Bay, LA	Aug 1981–May 1982	−1.2 to 10.8	NH_4^+	CC in BC	Teague et al. (1988)
Gullmar Fjord, western Sweden	Apr 2001	−1.10 to 2.4	NH_4^+	CC in SC	Sundbäck et al. (2004)

(Continued)

Table 8.5 Rates of nitrogen (N) release from sediment. Rates were quantified by measuring concentration changes (CC) in either sediment cores (SC) or benthic chambers (BC) (*continued*)

Location	Date	N release rate mmol N m^{-2} day^{-1}	Chemical species	Method	Reference
Gullmar Fjord, western Sweden	Sept 2001	−0.312 to 0.552	NH_4^+	CC in SC	Sundbäck et al. (2004)
Huon Estuary, Tasmania[f]	Apr, June, Sept, Dec 2001	0.34 ± 1.38	NH_4^+	CC in SC	Cook et al. (2004)
Laguna Madre, TX	Apr, Aug, Dec 1999; Apr 2000	2.07 ± 1.39	NH_4^+	CC in SC	Gardner et al. (2006)
Limfjorden, Denmark		0.3	NH_4^{+q}	CC in SC	Sloth et al. (1995)
Mobile Bay, AL	1993 and 1994 (monthly)	−0.53 to 4.34	NH_4^+	CC in SC	Cowan et al. (1996)
Patuxent Estuary, VA	May–Dec 1978, Mar 1979[g]	−1.20 to 37.8	NH_4^+	CC in BC	Boynton et al. (1980)
Shallow warm-temperate lagoons, Australia[h]	Feb	−0.44 ± 3.60	NH_4^+	CC in SC	Eyre and Ferguson (2002)
Subtropical, east Australia	Dec 2000–Dec 2002	1.00 ± 6.17	NH_4^+	CC in SC	Eyre and Ferguson (2005)
Three estuaries in New South Wales, Australia[i]	1997–1999	−0.24 ± 6.10	NH_4^+	CC in SC	Ferguson et al. (2004)
Tomales Bay, CA	June 1987–May 1989[j]	1.96 ± 2.39	NH_4^+	CC in BC	Dollar et al. (1991)
Aarhus Bay, Denmark	Nov 1994	0.12 ± 0.02	NO_3^- in control	CC in BC[b]	Pedersen et al. (1999)
Aarhus Bay, Denmark	Nov 1994	0.06 ± 0.02	NO_3^- with eel grass	CC in BC[b]	Pedersen et al. (1999)
Chesapeake Bay	Dec 1988–Nov 1989	−0.120 to 0.035	NO_3^-	CC in SC	Cowan and Boynton (1996)
Fourleague Bay, LA	Aug 1981–May 1982	−16.32 to 14.9	NO_3^-	CC in BC	Teague et al. (1988)
Gullmar Fjord, western Sweden	Apr 2001	−1.824 to 0.48	NO_3^-/NO_2^-	CC in SC	Sundbäck et al. (2004)
Gullmar Fjord, western Sweden	Sept 2001	−2.208 to 1.68	NO_3^-/NO_2^-	CC in SC	Sundbäck et al. (2004)

Huon Estuary, Tasmania[f]	Apr, June, Sept, Dec 2001	-0.13 ± 0.22	NO_3^-	CC in SC	Cook et al. (2004)
Limfjorden, Denmark		0.7	NO_3^{-k}	CC in SC	Sloth et al. (1995)
Patuxent Estuary, VA	May–Dec 1978, Mar 1979[g]	-16.18 to 16.8	NO_3^-	CC in BC	Boynton et al. (1980)
Shallow warm-temperate lagoons, Australia[h]	Feb	-0.46 ± 1.60	NO_3^-	CC in SC	Eyre and Ferguson (2002)
Subtropical, east Australia	Dec 2000–Dec 2002	-0.95 ± 3.31	NO_3^-	CC in SC	Eyre and Ferguson (2002)
Three estuaries in New South Wales, Australia[i]	1997–1999	-16.27 ± 2.50	NO_3^-	CC in SC	Ferguson et al. (2004)
Coastal Virginia[l]	Oct 1997–Aug 1998[m]	-0.200 ± 0.181	DIN	CC in SC	Tyler et al. (2001)
Laholm Bay, Sweden	Sept 1984	0.240^n	DIN	CC in SC	Enoksson (1993)
Laholm Bay, Sweden	Sept 1984	0.168^o	DIN	CC in SC	Enoksson (1993)
Laholm Bay, Sweden	Sept 1984	1.176^p	DIN	CC in SC	Enoksson (1993)
Aarhus Bay, Denmark	Nov 1994	0.25–3.4	DON^m	CC in BC[b]	Pedersen et al. (1999)
Chesapeake Bay	Dec 1988–Nov 1989	-0.250 to 0.550	DON	CC in SC	Cowan and Boynton (1996)
Chesapeake Bay—mesohaline	Mar 1995–Oct 1996	0.18 ± 0.07	DON	CC in BC	Burdige and Zheng (1998)
Chesapeake Bay—mesohaline	Nov 1997	0.13 ± 0.04	DON	CC in BC	Burdige (2001)
Chesapeake Bay—south bay	Mar 1995–Oct 1996	0.11 ± 0.05	DON	CC in BC	Burdige and Zheng (1998)
Chesapeake Bay—south bay	Nov 1997	0.18 ± 0.05	DON	CC in BC	Burdige (2001)
Coastal Virginia[d]	Oct 1998–Aug 1999[e]	-0.166 ± 0.297	DON	CC in SC	Tyler et al. (2003)
Coastal Virginia[l]	Oct 1997–Aug 1998[m]	0.216 ± 0.361	DON	CC in SC	Tyler et al. (2001)

Table 8.5 Rates of nitrogen (N) release from sediment. Rates were quantified by measuring concentration changes (CC) in either sediment cores (SC) or benthic chambers (BC) (*continued*)

Location	Date	N release rate mmol N m^{-2} day^{-1}	Chemical species	Method	Reference
Coastal Virginia[l]	Oct 1997–Aug 1998[m]	0.066 ± 0.084	Urea	CC in SC	Tyler *et al.* (2001)
Fourleague Bay, LA	Aug 1981–May 1982	−116.4 to 107.6	DON	CC in BC	Teague *et al.* (1988)
Gullmar Fjord, western Sweden	Apr 2001	−1.7 to 1.3	DON	CC in SC	Sundbäck *et al.* (2004)
Gullmar Fjord, western Sweden	Sept 2001	1.4–1.7	DON	CC in SC	Sundbäck *et al.* (2004)
Huon Estuary, Tasmania[f]	Apr, June, Sept, Dec 2001	-0.18 ± 2.94	DON	CC in SC	Cook *et al.* (2004)
Laholm Bay, Sweden	Sept 1984	0.204[n]	DON	CC in SC	Enoksson (1993)
Laholm Bay, Sweden	Sept 1984	0.122[o]	DON	CC in SC	Enoksson (1993)
Laholm Bay, Sweden	Sept 1984	0.430[p]	DON	CC in SC	Enoksson (1993)
Limfjorden, Denmark		0.4	DON[k]	CC in SC	Sloth *et al.* (1995)
Patuxent Estuary, VA	May–Dec 1978, Mar 1979[g]	−10.27 to 5.2	DON	CC in BC	Boynton *et al.* (1980)
Shallow warm-temperate lagoons, Australia[h]	Feb	2.47 ± 1.76	DON	CC in SC	Eyre and Ferguson (2002)
Subtropical, east Australia	Dec 2000–Dec 2002	0.42 ± 5.53	DON	CC in SC	Eyre and Ferguson (2005)
Three estuaries in New South Wales, Australia[I]	1997-1999	0.05 ± 7.19	DON	CC in SC	Ferguson *et al.* (2004)
Tomales Bay, CA	June 1987–May 1989[j]	0.30 ± 0.93	DON	CC in BC	Dollar *et al.* (1991)
Aarhus Bay, Denmark	Nov 1994	0.039 ± 0.005	Urea in control	CC in BC[b]	Pedersen *et al.* (1999)
Aarhus Bay, Denmark	Nov 1994	0.130 ± 0.020	Urea with eel grass	CC in BC[b]	Pedersen *et al.* (1999)

Chesapeake Bay—mesohaline	Aug 1996–Nov 1997	0.057 ± 0.053[r]	Urea	CC in BC	Burdige (2001)
Chesapeake Bay—south bay	Nov 1997	0.004 ± 0.010[r]	Urea	CC in BC	Burdige (2001)
Coastal Virginia[d]	Oct 1998–Aug 1999[e]	0.054 ± 0.094	Urea	CC in SC	Tyler et al. (2003)
Limfjorden, Denmark		0.0	Urea[k]	CC in SC	Sloth et al. (1995)
Aarhus Bay, Denmark	Nov 1994	<0.1	DFAA[q]	CC in BC[b]	Pedersen et al. (1999)
Coastal Virginia[d]	Oct 1998–Aug 1999[e]	-0.019 ± 0.031	DFAA	CC in SC	Tyler et al. (2003)
Coastal Virginia[d]	Oct 1998–Aug 1999[e]	0.024 ± 0.082	DCAA	CC in SC	Tyler et al. (2003)
Aarhus Bay, Denmark	Nov 1994	0.050–0.610	THAA[q]	CC in BC[b]	Pedersen et al. (1999)

[a] Rates were measured using ^{15}N isotope dilution.
[b] Rates were measured in a flow through experimental chamber for 33 days in cores with eel grass added to the surface and in controls without eelgrass.
[c] Rates were measured over the first 17 days after the eel grass addition.
[d] Measurements were taken at a mainland tidal creek, mid-lagoon shoals, at Hog Island (a back barrier island) and Willis Wharf in Virginia
[e] Experiments were conducted in October 1998, and May, July, and August 1999.
[f] Rates measured at Castle Forbes and Port Cygnet; DON release rates only reported for Castle Forbes.
[g] Rates were measured during May, July, August, October, and December 1978 and March 1979.
[h] Study included flux measurements at sites characterized by six different sediment–plant types: Enteromorpha sp., phytoplankton detritus, seagrass + epiphytes, seagrass, Gracilaria sp., and benthic microalgae. Incubations were done in the light and dark; net release rates are reported.
[i] Measurements were taken in the upper estuary (fine grained), mid estuary (organic rich), and lower estuary (marine sands) of the Brunswick, Simpsons, and Sandon estuaries.
[j] Rates were measured twelve times between June 1987 and May 1989.
[k] Rate measured in the control sediment (sandy).
[l] Measurements were taken at a mainland tidal creek, mid-lagoon shoals, and Hog Island (a back barrier island) in Virginia.
[m] Experiments were conducted in October 1997, and May, July, and August 1998.
[n] Incubation days 0–10.
[o] Incubation days 10–29.
[p] Incubation days 10–29 after algal material was added to the top of the core to simulate deposition from an algal bloom.
[q] Data represent the range for cores with and without added eel grass.
[r] Rates were presented as mmol urea but were converted to mmol N for presentation here.
Data are presented as mean ± standard deviation. When data from a given site were reported with several averages and standard deviations, the data were pooled for an overall site average and a new estimate of the standard deviation was calculated using propogation of errors. Negative rates indicate uptake by the sediment. Rates are confined to those measured in situ in papers published since 1990; for a summary of earlier rates see Burdige (2006).

survey…though preparing it was exhausting. These rates were measured using a number of approaches. Generally some form of a chamber is placed over the sediment and then the change in N concentrations within the chamber is monitored over time (e.g., Burdige and Zheng, 1998). Similarly, sediment cores are collected and the change in N concentrations in the overlying water monitored over time (e.g., Tyler et al., 2003). Alternatively a flux is calculated based on the gradient in N concentrations in pore water down a core (e.g., Burdige and Martens, 1990; Landén and Hall, 2000). In some cases the calculated rates are underestimated relative to measured rates (e.g., Landén and Hall, 2000). One possible reason for the discrepancy could be that production or consumption of N is occurring right at the sediment surface rather than within the sediment (Burdige, 2001, 2006). We discuss sedimentary regeneration rates with respect to the types of N released and the variables that control these rates below.

3.9.1. Types of nitrogen regenerated from sediments

Both inorganic and organic N can be released from sediments with release of inorganic N having received considerably more attention. Of the inorganic forms, release of NH_4^+ is generally much larger than release of NO_3^- and NO_2^-. In fact, sediments are more commonly a sink for oxidized forms (e.g., Sundbäck et al., 2004; Tyler et al., 2001). In general, benthic DON fluxes are highly variable, can be into or out of the sediment, and are usually small when compared to total net N flux from sediments (Burdige, 2006; Burdige and Zheng, 1998; Devol and Christensen, 1993). Of the individual organic compounds, urea and amino acids have received the most attention. Urea was 36–70% of the TDN released from sediments in the north Bering Sea (Lomstein et al., 1989) and ~30% in a coastal bay in France (Boucher and Boucher-Rodoni, 1988). Some exceptions where rates of DON release from sediments rivaled or exceeded the release of inorganic N include work in Arctic sediments (Blackburn et al., 1996), a Swedish fjord (Sundbäck et al., 2004), continental margin sediments (Landén-Hillmeyr, 1998), and in a temperate Australian estuary (Cook et al., 2004).

Analogous to DON in the water column, DON in sediment pore water is a heterogeneous mixture of compounds, though we know relatively little about its composition. Total DON concentrations tend to increase with depth into sediments (Burdige, 2002). The bulk of porewater DON is <3 kDalton, representing 80–90% and 60–70% of total DON in estuarine and continental margin sediments, respectively (reviewed in Burdige, 2006). In describing the general composition of porewater DON, Burdige and Gardner (1998) proposed that POM in the sediments initially hydrolyzes to high MW (HMW) compounds and through continued remineralization results in pore water characterized by LMW refractory compounds; this idea builds on the size reactivity continuum model put forth to describe water column DOM (Amon and Benner, 1996). Patience et al. (1992) found evidence that DON in sediment pore waters has a higher proportion of heterocyclic components (N in aromatic rings), which are generally considered more refractory. While the presumably refractory LMW DON, believed to be largely humic in character, increases with depth, concentration of labile LMW material tends to decrease. This LMW material is composed of compounds such as total hydrolysable amino

acids (THAA), DFAA, and urea (e.g., Henrichs *et al.*, 1984; Landén and Hall, 2000). In one study off the coast of Denmark, pore water DON was 9.2 mmol m^{-2} and was composed of 26.8% THAA, 10.1% DFAA, and 1.8% urea, with the remainder uncharacterized (Lomstein *et al.*, 1998). Turnover times of these LMW compounds near the surface can be surprisingly short. In the Lomstein *et al.* (1998) study, turnover times were 7–52 h for THAA, 3–4 h for DFAA, and 1–3 h for urea.

3.9.2. Variables that affect sediment nitrogen regeneration

The direction and magnitude of net N flux is dependent on a number of variables including the type and amount of organic matter delivery, light, temperature and seasonality, sediment oxygen concentration, and the presence and type of vegetation, macroalgae and microphytobenthos, and benthic fauna. Rather than any single variable, however, the key to understanding sediment N regeneration often lies in the interactive feedback loops of one variable to another.

The amount and quality of the organic matter delivered to the sediment surface will determine the amount and form of the N remineralized from the sediment (e.g., Lomstein *et al.*, 1989). A number of studies have shown a positive correlation between increases in OM delivery to the sediment and N regeneration (Enoksson, 1993; Jensen *et al.*, 1990; Sloth *et al.*, 1995; Therkildsen *et al.*, 1996; Tyler *et al.*, 2001). The quality of organic matter is also important. For example, phytoplankton detritus would be expected to have a much lower C:N ratio than seagrass detritus or organic matter transported from the terrestrial environment (e.g., Enriquez *et al.*, 1993), such that more N regeneration would likely occur as a result of phytoplankton detrital deposition relative to deposition of seagrass detritus.

When material hits the sediment surface it has two basic fates in the short term. It can be incorporated into biomass, primarily of bacteria, or it can be remineralized. Evidence for this split is seen in studies where increases in benthic biomass are inversely proportional to DIN flux from the sediment (e.g., Van Duyl *et al.*, 1993). The rate at which N is transported from the sediment to the water column is further dependent on the processes of diffusion and bioturbation (see review by Herbert, 1999).

Sources of organic matter to the sediments include terrestrial organic matter and pelagic and benthic primary producers, including the secretion of organic matter by the roots and rhizomes of macrophytes (e.g., Jensen *et al.*, 1990). Once the organic matter is deposited at the sediment surface, there are several potential sources of N to the water column from the benthos including excretion by meiofauna, macrofauna, N$_2$ fixation, and benthic organic matter remineralization. The presence or absence of plants can also be an important determinant of the magnitude and direction of N flow (e.g., Caffrey and Kemp, 1992).

With respect to environmental variables, light and temperature have been shown to affect sedimentary regeneration (e.g., Eyre and Ferguson, 2002; Sundbäck *et al.*, 2004). The affect of light and temperature is partially responsible for the distinct seasonal patterns often observed in sedimentary processes. For example, in a study in Concepción Bay, Chile, benthic fluxes were negative in the winter but positive in the spring and summer (Graco *et al.*, 2001). In the Gullnar Fjord in Sweden, DIN flux was dominated by NH$_4^+$ in the spring and by NO$_3^-$ in the fall (Sundbäck *et al.*, 2004). Higher fluxes of DFAA were observed in coastal sediments from Cape

Lookout Bight, NC in the summer relative to winter (Burdige and Martens, 1990). Many other factors that affect release can also vary with the season, such as rainfall, which can also affect organic matter delivery.

Whether the sediment is oxic or anoxic is also an important determinant of sedimentary N fluxes as well as the forms of N released. In sediments with significant organic matter deposition, oxygen does not generally penetrate far into the sediments such that reduced compounds like NH_4^+ or N_2 tend to dominate remineralization (e.g., Pedersen *et al.*, 1999). Similarly, efflux of NO_3^- is also heavily dependent on oxygen penetration.

One important variable controlling oxygen levels in sediment is the presence and type of primary producers. Primary producers with low C:N ratios (i.e., phytoplankton and macroalgae) can fuel rapid bacterial decomposition, which can severely reduce the concentration of oxygen. The reduced oxygen can lower the rate of denitrification, via limitation of the source of available NO_3^- through nitrification, and ultimately result in an increase in NH_4^+ release from sediment. In contrast, seagrasses and benthic microalgae can oxygenate the upper sediments reversing the process (Caffrey and Kemp, 1990; Risgaard-Petersen and Jensen, 1997). In general, rates of ammonification and NH_4^+ release tend to be substantially higher in sediments colonized by seagrasses relative to bare sediments (e.g., Boon *et al.*, 1986; Caffrey and Kemp, 1992; Dennison *et al.*, 1987). Nitrification rates tend to be higher in vegetated sediments, likely as a result of the O_2 released by roots (Caffrey and Kemp, 1990). Likewise, denitrification rates also tend to be higher in vegetated areas (Caffrey and Kemp, 1990), likely as a result of increased substrate availability due to the higher rates of nitrification.

3.9.3. Sources of regenerated nitrogen associated with sediments

In addition to the microbial community present in all sediments other organisms can substantially alter the nature of N release from sediment, particularly macroalgae, microphytobenthos, and benthic fauna.

In some coastal systems, the most important primary producers are macroalgae (Peckol and Rivers, 1996). This is particularly true in some highly eutrophied areas (Peckol *et al.*, 1994) where macroalgae can often out-compete seagrasses under high nutrient conditions (Duarte, 1995). Most macroalgal production enters the detrital food web. Macroalgae tend to be high in labile biomass such that they rapidly decompose following senescence, which makes direct release a potentially large source of regenerated N during isolated periods (e.g., Duarte, 1995; Enriquez *et al.*, 1993). Macroalgae at the sediment surface can also limit N regeneration to the water column by taking up the N released from the sediment (e.g., McGlathery *et al.*, 1997; Valiela *et al.*, 1992). Macroalgae can release DOM directly or during grazing (e.g., Branch and Griffiths, 1988; Duggins *et al.*, 1989; Mann, 1988). Potential reasons for direct release include release of proteinaceous enzymes (e.g., Weich and Granéli, 1989), to attract epiphytes like N_2 fixers (e.g., Penhale and Capone, 1981; Thomsen *et al.*, 2006), and release of defensive allelopathic chemicals such as polyphenolic compounds to inhibit the growth of other algal species (e.g., Hay and Fenical, 1988; Lobban and Harrison, 1994). Grazing on macroalgal biomass can also result in N regeneration during sloppy feeding on the

macrophyte and excretion or fecal pellet dissolution after feeding. In general, an estimated 34% of macroalgal net primary production is grazed (Duarte and Cebrián, 1996). Presumably release can also result from parasitic or viral infection of macroalgae though we are unaware of any studies of these routes of release.

A number of studies have measured DOC production from macroalgae and an estimated 30–45% of macroalgal production is released as DOM (e.g., Mann, 1982; Sieburth, 1969). Relatively little is known, however, about release of N containing compounds specifically. Nitrogen release can take several forms including NH_4^+, urea, DCAA, and a suite of different free amino acids (Tyler et al., 2003). The high recovery of ^{15}N added, as either $^{15}NH_4^+$ (96% recovery) or $^{15}NO_3^-$ (99% recovery), in studies with Ulva fenestrata and Gracilaria pacifica, indicates that if substantial DON release occurs it is rapidly reincorporated or remineralized (Naldi and Wheeler, 2002). Branch and Griffiths (1988) found that DOM actively released by kelp consisted of only 5% protein with the remaining made up of C-rich moieties. One of the few studies to quantify N release from macroalgae was done in a shallow back-barrier lagoon in Virginia using Gracilaria vermiculophylla (Tyler and McGlathery, 2006, Table 8.6). In this system, an average of 67% of gross N uptake was released each day (Tyler and McGlathery, 2006); areal rates of N release range from 0.008 to 17 mmol m^{-2} day^{-1} (Table 8.6). Other studies found that release of DON and urea from macroalgae and sediments was higher in the light while NH_4^+ regeneration was higher in the dark (Tyler et al., 2001, 2003).

Different species of macroalgae would be expected to have different N release rates. For example, some macroalgae are known to have large intracellular inorganic N storage pools (e.g., McGlathery et al., 1996). Release of NH_4^+, as measured with isotope dilution, was observed in Ulva fenestrata (0.08–11.27 μmol N g^{-1} h^{-1}) and Gracilaria pacifica (0.12–0.77 μmol N g^{-1} h^{-1}) when $^{15}NH_4^+$ was added to the medium. However, no net NH_4^+ release, over uptake, was observed. Small pulses of free amino acids were released and then rapidly reincorporated (Naldi and Wheeler, 2002), but no protein release was detectable.

It is also known that desiccation is a main stressor for macroalgae and that DOM release increases after desiccation and reimmersion (Carlson and Carlson, 1984). Desiccation and reimmersion likely changes the suite of compounds that are released, resulting in release of more complex moieties such as polypeptides as a result of cell wall damage (Pregnell, 1983). Some algae are also known to release organic compounds as a form of allelopathic protection (Gross, 2003).

Microphytobenthos are another significant source of N from sediments that can also be an important barrier to sedimentary N release (e.g., McGlathery et al., 2001; Stimson and Larned, 2000). This is particularly true in northern microtidal regions where microphytobenthos are active all year long (e.g., Risgaard-Petersen, 2003; Sundbäck and Miles, 2000). In a Swedish fjord, there was a significant negative correlation between gross primary production of microphytobenthos and DIN fluxes, with microphytobenthos removing ∼35% of the remineralized N (Sundbäck et al., 2004); no correlation was observed with DON fluxes. In contrast, in another system with significant amounts of microphytobenthos, Cook et al. (2004) found that fluxes of urea and total dissolved primary amines were insignificant. Rates

Table 8.6 Rates of nitrogen (N) release from macroalgae measured using ^{15}N wet chemical techniques (^{15}N-WC) or changes in concentration (CC). Data are presented as the mean ± standard deviation. When data from a given site were reported with several averages and standard deviations, the data were pooled for an overall site average and a new estimate of the standard deviation was calculated using propogation of error. Negative rates indicate uptake by the plant or sediment

Location	Date	Plant species	N release rate mmol N m^{-2} day^{-1}	Chemical species	Method	Reference
Coastal Virginia[a]	Oct 1997– Aug 1998[b]	sediment + *Ulva lactuca*	0.540 ± 0.394	DON	CC	Tyler *et al.* (2001)
Coastal Virginia[a]	Oct 1997– Aug 1998[b]	Sediment + *Ulva lactuca*	−0.021 ± 0.093	Urea	CC	Tyler *et al.* (2001)
Coastal Virginia[a]	Oct 1997– Aug 1998[b]	sediment + *Ulva lactuca*	−0.400 ± 0.347	DIN	CC	Tyler *et al.* (2001)
Mainland tidal creek, Virginia	Oct 1998– Aug 1999[c]	sediment + *Ulva lactuca*	0.191 ± 0.036	DCAA[d]	CC	Tyler *et al.* (2003)
Mainland tidal creek, Virginia	Oct 1999– Aug 2000[e]	*Gracilaria vermiculophylla*	0.002–8.997	Total N	^{15}N-WC	Tyler and McGlathery (2006)
Mid-lagoon shoals, Virginia	Oct 1999– Aug 2000[e]	*Gracilaria vermiculophylla*	0.625–16.95	Total N	^{15}N-WC	Tyler and McGlathery (2006)
Hog Island (back-barrier), Virginia	Oct 1999– Aug 2000[e]	*Gracilaria vermiculophylla*	0.008–1.004	Total N	^{15}N-WC	Tyler and McGlathery (2006)

[a] Measurements were taken at a mainland tidal creek, mid-lagoon shoals, and Hog Island (a back barrier island) in Virginia; no net N release of NH_4^+, urea, or DCAA was observed at a fourth site, Willis Wharf.
[b] Experiments were conducted in October 1997, and May, July, and August 1998.
[c] Experiments were conducted in October 1998, and May, July, and August 1999.
[d] When all sites and dates were averaged, there was no net DIN, DON, urea or DFAA release measured.
[e] Experiments were conducted in October 1999, and February, April, and July 2000.

of DON dominated over DIN fluxes and were highest where the highest micro-phytobenthos biomass, production, and respiration occurred (Cook *et al.*, 2004). The link between high microphytobenthos and high DON release has also been shown in an Australian lagoon (Eyre and Ferguson, 2002).

In systems with high abundance of microphytobenthos and macroalgae, DON is often the major form of N released (Cook *et al.*, 2004; Eyre and Ferguson, 2002; Tyler *et al.*, 2001). One possible reason for this is uptake of DIN by the micro-phytobenthos and then release of DON through direct release or release mediated by grazing (Eyre and Ferguson, 2002). The production of DON occurs right at the surface, as pore water DON profiles do not indicate DON accumulation within the sediment (Cook *et al.*, 2004).

Although most NH_4^+ and DON release from sediments is attributed to bacterial remineralization, benthic fauna can also play an important but hard to quantify role (see reviews by Aller, 1988 and Burdige, 2002). Benthic infauna can contribute to N leaving the sediment through excretion or bioturbation (Aller, 1988; Gilbert *et al.*, 2003; Lomstein *et al.*, 1989). They also facilitate oxygen penetration far deeper into the sediments through irrigation and bioturbation thus potentially impacting the form, oxidized or reduced, of the N released (Aller, 1988). In sediments of the open Skagerrak, DFAA fluxes tended to be into the sediment when meio- and macro-fauna were in low abundance but tended to flux out of the sediment, along with NH_4^+, when sedimentary fauna were in higher abundance (Landén and Hall, 2000). Even in continental margin waters of greater than 100 m, macrobenthic irrigation is an important mechanism for transferring N into and out of the sediment (Devol and Christensen, 1993).

Beggiatoa is a large, sulfate-oxidizing bacteria that produces extensive mats at the sediment surface similar to another large marine bacteria, *Thioploca* (e.g., Schulz *et al.*, 1996). Both of these large bacteria are known to accumulate large stores of NO_3^- intracellularly (up to 500 mM) (Fossing *et al.*, 1995). *Beggiatoa* has been shown to release substantial amounts of NH_4^+, likely as a result of dissimilatory NO_3^- reduction to NH_4^+. In one study in Concepción Bay, Chile, NH_4^+ flux from *Beggiatoa* was estimated at 5 mmol m^{-2} day^{-1} or 17% of the total NH_4^+ release from the sediments in the summer (Graco *et al.*, 2001).

4. METHODS TO MEASURE NITROGEN REGENERATION

Regeneration rates are generally determined using bioassays or ^{15}N tracer methods (Chapter 31 by Lipschultz, this volume, Chapter 28 by McCarthy and Bronk, this volume). With the bioassay method, the accumulation of a compound is measured over time (e.g., Steinberg *et al.*, 2002). Bioassays have the advantage that they are relatively easy to perform. However, they can only provide net release rates, will only produce a rate at all if the release rate is greater than any uptake rates, and can suffer from bottle affects if long incubations are needed to see a signal.

The alternative to bioassays is to use tracer methods of which there are two general approaches. In the first, the dilution of an isotopically labeled pool is measured over time (NH_4^+ regeneration, Glibert *et al.*, 1982). In the second, the flow of ^{15}N is followed into a regenerated N pool directly (e.g., DON release, Bronk and Glibert, 1991).

Rates of NH_4^+ regeneration are most commonly measured using isotope dilution. In this approach, ^{15}N-labeled NH_4^+ is added to a sample at $\sim <10\%$ of the ambient concentration. At the end of the incubation the NH_4^+ pool is isolated via distillation (e.g., Glibert *et al.*, 1982), solid phase extraction (e.g., Selmer and Sorensson, 1986), or direct diffusion (Slawyk and Raimbault, 1995). The isotope dilution method assumes that any NH_4^+ released during the incubation is $^{14}NH_4^+$, which will dilute the $^{15}NH_4^+$ added at the start of the experiment. The rate of regeneration is then measured by monitoring the dilution of the labeled ^{15}N-labeled NH_4^+ pool over time (Blackburn, 1979; Caperon *et al.*, 1979). Urea regeneration can be measured in an analogous fashion (e.g., Slawyk *et al.*, 1990). Urea regeneration can also be measured using ^{14}C and ^{15}N-labeled urea with a method described by Hansell and Goering (1989).

Nitrogen regeneration can also be measured by monitoring the flow of a labeled compound from a source pool to a target pool. In the case of DON release, ^{15}N-labeled NH_4^+ or NO_3^- is added to a sample and the appearance of ^{15}N in the DON pool is monitored over time (Bronk and Glibert, 1991). To measure a DON flux, DON must be isolated prior to mass spectrometric analysis. There are three basic approaches used to isolate DON: (1) wet chemistry (e.g., Axler and Reuter, 1986; Bronk and Ward, 1999; Slawyk and Raimbault, 1995), (2) ion retardation (Bronk and Glibert, 1991; Nagao and Miyazaki, 1999), and (3) dialysis (Feuerstein *et al.*, 1997); all three approaches are discussed in more detail in Chapter 28 by McCarthy and Bronk (this volume).

There are two basic approaches to calculate rates of DON release. First, is to directly measure the passage of ^{15}N from the intracellular organic N (ON) pool to the extracellular DON pool (Bronk and Glibert, 1991, 1993) and is specified the intracellular pool (IP) DON release rate. Isolating the intracellular DON pool gives the added bonus of allowing the calculation of a transformation rate, which is a measure of the transformation of NH_4^+ to ON intracellularly (Bronk, 1999). Second, is to monitor the change in the ^{15}N atom % enrichment of the extracellular DON pool only and is the more commonly reported rate. The DON release rate is calculated as the difference between the gross N uptake rate (ρ_G) and the net N uptake rate (ρ). The second method of measuring DON is less labor intensive and requires fewer assumptions and so is more robust.

When measuring any release process one must be aware of three potential artifacts—filtration stress (e.g., Goldman and Dennett, 1985), incubation stress (e.g., Venrick *et al.*, 1977), and loss of DON during the isolation process (Bronk and Ward, 2000); see Chapter 28 by McCarthy and Bronk (this volume) for a more detailed discussion.

> ## 5. Rates of Nitrogen Regeneration in the Water Column

Here we review published rates of NH_4^+ regeneration and DON release in the water column. In general, these rates were measured in small volume incubations (<4 L) run for 1–24 h and could represent release due to many of the mechanisms discussed above for phytoplankton, bacteria, zooplankton, viruses and detritus. There have been two recent reviews of DON release rates in the field (Bronk, 2002 and Berman and Bronk, 2003) and so here we focus on the literature of the last ten years. There have been no similar reviews for NH_4^+ regeneration, however, and so we extend coverage back to the 1980s, when the isotope dilution technique to measure NH_4^+ regeneration was introduced (Glibert *et al.*, 1982).

5.1. NH_4^+ regeneration

Ammonium is the primary form of N released in the water column. Rates of NH_4^+ regeneration are often tightly coupled with uptake such that concentrations of NH_4^+ can be near the limit of detection even in systems that are running largely on NH_4^+ (e.g., McCarthy and Goldman, 1979). Though related, there is seldom a strong correlation between NH_4^+ uptake and NH_4^+ regeneration measured simultaneously because of the large number of processes that can result in NH_4^+ release (e.g., Bode and Dortch, 1996).

5.1.1. NH_4^+ regeneration versus DON release

Rates of NH_4^+ regeneration generally exceed DON release as observed in studies in Chesapeake Bay (Bronk *et al.*, 1998; Ward and Bronk, 2001), Monterey Bay (Bronk and Ward, 1999; Ward and Bronk, 2001), the Gulf of Lions (Diaz and Raimbault, 2000), shelf and oceanic waters off Northwest Spain (Varela *et al.*, 2003a), and the Southern California Bight (Bronk and Ward, 2005). In the water column, rates of NH_4^+ regeneration are often significantly correlated ($p < 0.001$) to rates of DON release as observed in Monterey Bay (Bronk and Ward, 1999; Ward and Bronk, 2001), the Southern California Bight (Bronk and Ward, 2005; Ward and Bronk, 2001), and in the oceanic and coastal waters off of Spain (Varela *et al.*, 2003a). To compare NH_4^+ regeneration to DON release, literature values were used to calculate a total DON release rate, defined as the sum of the rates of DON release due to both NH_4^+ uptake and NO_3^- uptake. Total DON release is plotted against the rate of NH_4^+ regeneration for studies in coastal and open ocean systems (Figs. 8.2 and 8.3, Table 8.7); comparable data for estuarine systems were not available due to a lack of DON release rates resulting from NO_3^- uptake. The slope of the linear regression indicates that across several studies, DON release was approximately 24% of the rate of NH_4^+ regeneration (Fig. 8.2 and Table 8.7). Clearly more data are needed at the higher regeneration rates to define the relationship.
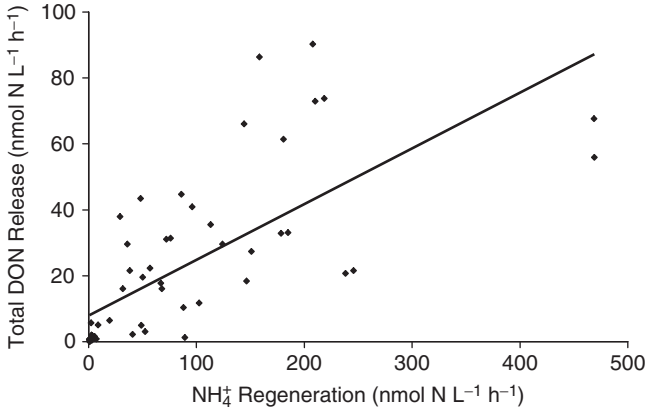
Figure 8.2 The relationship between total DON release, which is defined as the sum of DON released as a result of both NH_4^+ and NO_3^- uptake, and NH_4^+ regeneration in coastal and ocean systems. Model II regression parameters are reported in Table 8.7. The line for a hyperbolic function yields the following parameters: $y = 9.429Ln(x)$, $r^2 = 0.53$, $n = 56$. The graph includes data from Monterey Bay, CA (Bronk and Ward, 1999; Ward and Bronk, 2001), the Southern California Bight, CA (Bronk and Ward, 2005; Ward and Bronk, 2001), and the Gulf of Lions, Mediterranean (Diaz and Raimbault, 2000).
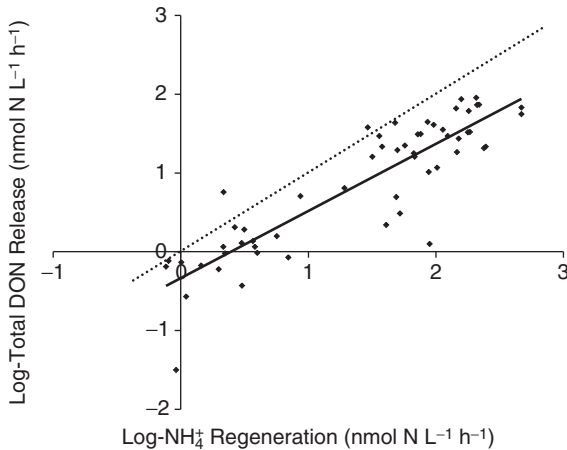
Figure 8.3 The log–log relationship between total DON release, which is defined as the sum of DON released as a result of NH_4^+ and NO_3^- uptake, and NH_4^+ regeneration in coastal and ocean systems. Model II regression parameters are reported in Table 8.7. The graph includes data from Monterey Bay, CA (Bronk and Ward, 1999; Ward and Bronk, 2001), the Southern California Bight, CA (Bronk and Ward, 2005; Ward and Bronk, 2001), and the Gulf of Lions, Mediterranean (Diaz and Raimbault, 2000).

Table 8.7 Regression parameters for Figs. 2–4

Rate 1	Rate 2	Depth	n	slope	Intercept	r^2	p
Total DON release	NH_4^+ regeneration	Euphotic zone	56^a	0.237	2.13	0.51	<0.001
Log-Total DON release	Log-NH_4^+ regeneration	Euphotic zone	56^a	0.852	−0.34	0.78	<0.001
Log-DON release (from NH_4^+)	Log-Gross NH_4^+ uptake	Euphotic zone	89^a	0.926	0.65	0.90	<0.001
Log-DON release (from NH_4^+)	Log-Gross NH_4^+ uptake	Euphotic zone	115^b	0.968	0.67	0.82	<0.001
Log-DON release (from NO_3^-)	Log-Gross NO_3^- uptake	Euphotic zone	98^a	0.807	0.51	0.79	<0.001
DON release (from NH_4^+)	Gross NH_4^+ uptake	Upper euphotic	48^a	0.132	2.27	0.71	<0.001
DON release (from NH_4^+)	Gross NH_4^+ uptake	Lower euphotic	41^a	0.861	−2.37	0.92	<0.001
DON release (from NO_3^-)	Gross NO_3^- uptake	Upper euphotic	55^a	0.572	−1.79	0.42	<0.001
DON release (from NO_3^-)	Gross NO_3^- uptake	Lower euphotic	43^a	0.929	−1.33	0.96	<0.001

[a] Rates from coastal and oceanic systems.
[b] Rates from estuarine, coastal and oceanic systems.

5.1.2. Rates of NH_4^+ regeneration in the field

A literature survey of NH_4^+ regeneration rates in oceanic, coastal, and estuarine systems is presented in Table 8.8. Only those rates measured on whole water or the smaller size fraction (i.e., <202 or 370 μm) were used in the calculation of means. In a survey of literature values, the lowest rates were measured in oceanic systems (mean 0.036 μmol N L^{-1} h^{-1}). Within a given study, however, this trend was not always observed. For example, along an ocean to coastal continuum off the coast of Spain, rates of NH_4^+ regeneration tended to be higher in oceanic, relative to coastal, waters (Varela *et al.*, 2003a). Comparisons between systems suggest that rates are highest in coastal waters (0.138 μmol N L^{-1} h^{-1}; note that the very high rates measured in coastal Louisiana (Dortch *et al.*, 1992) were excluded from this average), which is approximately twice that of the mean rate in estuarine waters (0.079 μmol N L^{-1} h^{-1}). Within estuarine systems NH_4^+ regeneration can vary widely along the salinity gradient (e.g., Cotner and Gardner, 1993), but not always. For example, rates of NH_4^+ regeneration measured along a salinity gradient from the Mississippi River to the mid-shelf region of the Gulf of Mexico did not vary with salinity in one study (Bode and Dortch, 1996), while in an earlier study in the same region, NH_4^+ regeneration rates were highest in higher salinity waters (Dortch *et al.*, 1992). Many parameters can affect NH_4^+ regeneration, which could also vary along a salinity gradient, such as phytoplankton standing stock, temperature, and plankton community structure. As a result it is difficult to tease apart the role of salinity itself.

5.1.3. Variables that affect NH_4^+ regeneration rates

A large number of variables can affect NH_4^+ regeneration. As a result it is there are few robust trends across as illustrated below. The size structure of the phytoplankton community can be an important determinant of N regeneration. Larger cells tend to occur in more turbulent areas with higher new N inputs, such as upwelling zones. Smaller cells, in contrast, are more common in stratified environments where primary production runs largely on regenerated N, mainly NH_4^+ (Legendre and Rassoulzadegan, 1995). During periods of low input of new N, grazing by micro and macrozooplankton affects primary production through N regeneration, which can enhance primary production (Gasol and Morán, 1999) as well as consume phytoplankton biomass. As discussed above, large zooplankton can add another level of complexity because they graze on the protozoa, which can be the primary regenerators of N thereby decreasing total N regeneration rates (Caron, 1991).

Due to the close association with grazing, N regeneration is likely to have a diel periodicity associated with the rates. For example, in the Southern California Bight in April, NH_4^+ regeneration rates were higher at night than in the day (Bronk and Ward, 2005). This trend was also observed in three out of four size fractionation experiments in April and five out of six experiments in October in the Southern California Bight (Ward and Bronk, 2001). Day/night differences also likely impact how NH_4^+ regeneration may vary with depth. While some studies observe the highest rates of regeneration near the surface (e.g., Probyn, 1987 in an upwelling zone, Diaz and Raimbault, 2000) other studies show higher release deeper in the water column (e.g., Bode *et al.*, 2002). When NH_4^+ regeneration is measured in the

Table 8.8 Rates of ammonium (NH_4^+) regeneration in the water column measured using ^{15}N-isotope dilution (^{15}N ID) with NH_4^+ isolated using distillation (Distill), solid phase extration (SPE), solvent extraction (SE), or diffusion (Diff)

Location	Date/Culture	NH_4^+ regeneration rate $\mu mol\,N\,l^{-1}h^{-1}$	NH_4^+ regen: Net NH_4^+ uptake (%)	Size Fraction (μm)	Method	Reference
Oceanic						
Atlantic—off Spain—oceanic	Apr 1997–Oct 1999[a]	0.033 ± 0.033		Whole water	^{15}N ID-Diff	Varela *et al.* (2003a)
Atlantic—off Spain—shelf break	Apr 1997–Oct 1999[a]	0.012 ± 0.006		Whole water	^{15}N ID-Diff	Varela *et al.* (2003a)
Bedford Basin, Nove Scotia	Feb–May 1980	0.041 ± 0.08	35.6	Whole water	^{15}N ID-Distill	La Roche (1983)
Bedford Basin, Nove Scotia	June–Aug. 1980	0.066 ± 0.12	3.6	Whole water	^{15}N ID-Distill	La Roche (1983)
Bellingshausen Sea (Zone 2)	Dec 1995 and Jan 1996	0.041 ± 0.012	109.8	Whole water	^{15}N ID	Bode *et al.* (2002)
Bransfield Strait (Zone 3)	Dec 1995 and Jan 1996	0.014 ± 0.005	196.2	Whole water	^{15}N ID	Bode *et al.* (2002)
Gerlache Strait (Zone 1)	Dec 1995 and Jan 1996	0.023 ± 0.016	118.7	Whole water	^{15}N ID	Bode *et al.* (2002)
Gulf Stream	May–June 1982	NDL to 0.012		<5	^{15}N ID-Distill	Glibert *et al.* (1988)
Gulf Stream	May–June 1982	NDL		<153	^{15}N ID-Distill	Glibert *et al.* (1988)
Offshore Hawaii	Aug–Sept 1982	~0.009–0.096		Whole Water	^{15}N ID-Distill	Harrison and Harris (1986)
Sargasso Sea	June–July 1979	0.044 ± 0.039		<10	^{15}N ID-Distill	Glibert (1982)
Sargasso Sea	June–July 1979	0.006 ± 0.007		10–35	^{15}N ID-Distill	Glibert (1982)

(*Continued*)

Table 8.8 Rates of ammonium (NH_4^+) regeneration in the water column measured using ^{15}N-isotope dilution (^{15}N ID) with NH_4^+ isolated using distillation (Distill), solid phase extration (SPE), solvent extraction (SE), or diffusion (Diff) (*continued*)

Location	Date/Culture	NH_4^+ regeneration rate $\mu mol\, N\, l^{-1} h^{-1}$	NH_4^+ regen: Net NH_4^+ uptake (%)	Size Fraction (μm)	Method	Reference
Sargasso Sea	June–July 1979	0.017 ± 0.029		35–130	^{15}N ID-Distill	Glibert (1982)
Sargasso Sea	May–June 1982	0.001–0.003		<5	^{15}N ID-Distill	Glibert et al. (1988)
Sargasso Sea	May–June 1982	0.002–0.003		<153	^{15}N ID-Distill	Glibert et al. (1988)
Scotia Sea, Antarctica	Feb–Mar 1981	0.165 ± 0.120	54.4 ± 36.6	<370	^{15}N ID	Koike et al. (1986)
Scotia-Weddell Confluence	Nov and Dec 1988	0.006 ± 0.006		Whole water	^{15}N ID-Diff	Goeyens et al. (1991)
Southern California Bight—offshore	Oct 1992	0.023 ± 0.003		Whole water	^{15}N ID-Distill	Bronk and Ward (2005)
Southern California Bight—offshore	Oct 1992 and Apr 1994	0.061 ± 0.040		<10	^{15}N ID-Distill	Ward and Bronk (2001)
Southern California Bight—offshore	Oct 1992 and Apr 1994	0.061 ± 0.056		<202	^{15}N ID-Distill	Ward and Bronk (2001)
Subarctic Pacific	June 1987	0.008 ± 0.008[b]		Whole water	^{15}N ID-SPE	Wheeler et al. (1989)
Subarctic Pacific	May	0.007 ± 0.0007		Whole water	^{15}N ID-SPE	Wheeler and Kokkinakis (1990)
	Mean	*0.036 ± 0.202*	*whole water*			

Coastal						
Atlantic—off Spain—mid-shelf	Apr 1997–Oct 1999[a]	0.022 ± 0.033			Whole water	[15]N ID-Diff	Varela *et al.* (2003a)
Atlantic—off Spain—coastal	Apr 1997–Oct 1999[a]	0.040 ± 0.016		Whole water	[15]N ID-Diff	Varela *et al.* (2003a)
Mediteranean— Gulf of Lions[c]	Mar 1997	0.004 ± 0.002	560	Whole water	[15]N ID-Diff	Diaz and Raimbault (2000)
Mediteranean— Gulf of Lions[c]	Apr 1997	0.004 ± 0.002	356	Whole water	[15]N ID-Diff	Diaz and Raimbault (2000)
Mediteranean— Gulf of Lions[c]	May 1997	0.003 ± 0.001	273	Whole water	[15]N ID-Diff	Diaz and Raimbault (2000)
Bedford Basin, Nova Scotia	Mar–Aug 1980	0–0.425		Whole water	[15]N ID-Distill	La Roche (1983)
Coastal Oregon— non-upwelling	July 1990–Aug 1991	0.370 ± 0.777		Whole water	[15]N ID-SPE	Dickson and Wheeler (1995)
Coastal Oregon— upwelling	July 1990–Aug 1991	0.838 ± 0.792		Whole water	[15]N ID-SPE	Dickson and Wheeler (1995)
Davies Reef, Australia	Aug 1984	0.001–0.011		Whole water	[15]N ID-SE	Hopkinson *et al.* (1987)
Gray's Reef, GA	July 1985	0.063 ± 0.023	<208	[15]N ID-SE	Hopkinson *et al.* (1991)
Gulf of Mexico	Oct 1990	0.330 ± 0.348	1303 ± 2028	<200	[15]N ID-Distill	Bode and Dortch (1996)
Gulf of Mexico	July 1991	0.370 ± 0.260	23.8 ± 12.8	<200	[15]N ID-Distill	Bode and Dortch (1996)
Gulf of Mexico— mid-shelf	Feb–Sept 1991 (monthly)	0.196 ± 0.181	77.6 ± 72.3	<200	[15]N ID-Distill	Bode and Dortch (1996)

(*Continued*)

Table 8.8 Rates of ammonium (NH_4^+) regeneration in the water column measured using ^{15}N-isotope dilution (^{15}N ID) with NH_4^+ isolated using distillation (Distill), solid phase extration (SPE), solvent extraction (SE), or diffusion (Diff) (*continued*)

Location	Date/Culture	NH_4^+ regeneration rate $\mu mol\, N\, l^{-1} h^{-1}$	NH_4^+ regen: Net NH_4^+ uptake (%)	Size Fraction (μm)	Method	Reference
Kaneohe Bay	Aug–Sept 1982	~0.020–0.11		Whole water	^{15}N ID–Distill	Harrison and Harris (1986)
Louisianna continental shelf	Spring 1991	0.070	14.9	Whole water	^{15}N ID	Dortch *et al.* (1992)
Louisianna continental shelf	Fall 1990	1.700	944	Whole water	^{15}N ID	Dortch *et al.* (1992)
Louisianna continental shelf	~Fall 1990, Spring 1991	0.03–4.701		Whole water	^{15}N ID	Dortch *et al.* (1992)
Mid–Atlantic Bight	Summer 1980	0.008–0.029				Harrison *et al.* (1983)
Mississippi River plume	Feb 1991	0–0.054		Whole water	^{15}N ID	Benner *et al.* (1992)
Mississippi River plume	Feb 1991	0.037 ± 0.007		Whole water	^{15}N ID	Cotner and Gardner (1993)
Mississippi River plume	Sept 1991	0.076 ± 0.030		Whole water	^{15}N ID	Cotner and Gardner (1993)
Mississippi River plume	Sept 1991	0.021 ± 0.024		<1	^{15}N ID	Cotner and Gardner (1993)
Mississippi River plume	May 1992	0–0.180		Whole water	^{15}N ID	Gardner *et al.* (1997)
Mississippi River plume	July 1993	~0.06–0.750		Whole water	^{15}N ID	Gardner *et al.* (1997)
Mississippi River plume	July 2001	0.021–0.441		Whole water	^{15}N ID–SPE	Wawrik *et al.* (2004)
	Mar and Sept 1993	0.209 ± 0.219		<10	^{15}N ID–Distill	Ward and Bronk (2001)

Monterey Bay, CA	Mar and Sept 1993	0.152 ± 0.070		<202	[15]N ID-Distill	Ward and Bronk (2001)
Northern Benguela System	Apr 1988	0.026 ± 0.016		<200	[15]N ID-Distill	Probyn et al. (1990)
South Atlantic, offshore Brazil	Mar and Dec 1994	0.750 ± 0.310	94	<1	CC + [15]N uptake[d]	Metzler et al. (2000)
South Atlantic, nearshore Brazil	Mar and Dec 1994	0.874 ± 0.559	97 ± 9.1	<1	CC + [15]N uptake[d]	Metzler et al. (2000)
Southeastern US continental shelf	April 1985	0.224 ± 0.041	201.8 ± 60.5	Whole water	[15]N ID-Diff	Hanson and Robertson (1988)
Southern Benguela System	Dec 1983–July 1985[e]	BD to 0.033		<1	[15]N ID-Diff	Probyn (1987)
Southern Benguela System	Dec 1983–July 1985[e]	0.0003–0.120		<15	[15]N ID-Diff	Probyn (1987)
Southern Benguela System	Dec 1983–July 1985[e]	0.0003–0.125		<200	[15]N ID-Diff	Probyn (1987)
Southern Benguela System	Dec 1983–July 1985[e]	0.0001–0.106		<3	[15]N ID-Diff	Probyn (1987)
Southern California Bight— nearshore	Oct 1992 and Apr 1994	0.388 ± 0.351		<10	[15]N ID-Distill	Ward and Bronk (2001)
Southern California Bight— nearshore	Oct 1992 and Apr 1994	0.442 ± 0.325		<202	[15]N ID-Distill	Ward and Bronk (2001)

(*Continued*)

Table 8.8 Rates of ammonium (NH_4^+) regeneration in the water column measured using ^{15}N-isotope dilution (^{15}N ID) with NH_4^+ isolated using distillation (Distill), solid phase extration (SPE), solvent extraction (SE), or diffusion (Diff) (*continued*)

Location	Date/Culture	NH_4^+ regeneration rate $\mu mol\,N\,l^{-1}\,h^{-1}$	NH_4^+ regen: Net NH_4^+ uptake (%)	Size Fraction (μm)	Method	Reference
Southern California Bight	Oct 1992	0.028 ± 0.003		Whole Water	^{15}N ID–Distill	Bronk and Ward (2005)
Vineyard Sound, MA	Oct 1979–Aug 1980	0–0.270		<10	^{15}N ID–Distill	Glibert (1982)
Vineyard Sound, MA	Oct 1979–Aug 1980	0–0.190		<202	^{15}N ID–Distill	Glibert (1982)
Vineyard Sound, MA	Oct 1979–Aug 1980	0–0.310		<35	^{15}N ID–Distill	Glibert (1982)
Western English Channel	Jan–Dec 1988	0–0.028		Whole Water	^{15}N ID–Diff	Le Corre *et al.* (1996)
Western English Channel	Jan–Dec 1988	0–0.006		<1	^{15}N ID–Diff	Le Corre *et al.* (1996)
Western English Channel	Jan–Dec 1988	0–0.015		1–15	^{15}N ID–Diff	Le Corre *et al.* (1996)
Western English Channel	Jan–Dec 1988	0–0.012		15–200	^{15}N ID–Diff	Le Corre *et al.* (1996)
Western English Channel	Jan–Dec 1988	0.0006–0.027		Whole water	^{15}N ID–Diff	Maguer *et al.* (1999)
Western English Channel	Jan–Dec 1988	NDL to 0.006		<1	^{15}N ID–Diff	Maguer *et al.* (1999)
Western English Channel	Jan–Dec 1988	0.0002–0.010		1–15	^{15}N ID–Diff	Maguer *et al.* (1999)
Western English Channel	Jan–Dec 1988	0.0001–0.014		15–200	^{15}N ID–Diff	Maguer *et al.* (1999)
	Mean	*0.138^f ± 1.252*	*Whole water*			

Estuarine

Chesapeake Bay	May–June 1980	0.062 ± 0.055	<1	^{15}N ID-Distill	Glibert (1982)
Chesapeake Bay	May–June 1980	0.225 ± 0.035	1–10	^{15}N ID-Distill	Glibert (1982)
Chesapeake Bay	May–June 1980	0.750	10–35	^{15}N ID-Distill	Glibert (1982)
Chesapeake Bay	May–June 1980	0.075 ± 0.041	35–102	^{15}N ID-Distill	Glibert (1982)
Chesapeake Bay	Feb 1985	0.010 ± 0.010	<10	^{15}N ID-Distill	Glibert *et al.* (1992)
Chesapeake Bay	Feb 1985	0.020 ± 0.030	Whole water	^{15}N ID-Distill	Glibert *et al.* (1992)
Chesapeake Bay	Apr 1986	0.140 ± 0.240	<10	^{15}N ID-Distill	Glibert *et al.* (1992)
Chesapeake Bay	Apr 1986	0.110 ± 0.130	<202	^{15}N ID-Distill	Glibert *et al.* (1992)
Chesapeake Bay	June 1985	0.120 ± 0.110	<10	^{15}N ID-Distill	Glibert *et al.* (1992)
Chesapeake Bay	June 1985	0.150 ± 0.090	<202	^{15}N ID-Distill	Glibert *et al.* (1992)
Chesapeake Bay	Aug 1985	0.130 ± 0.110	<10	^{15}N ID-Distill	Glibert *et al.* (1992)
Chesapeake Bay	Aug 1985	0.220 ± 0.310	<202	^{15}N ID-Distill	Glibert *et al.* (1992)
Chesapeake Bay	Feb 1985	0–0.09	Whole water	^{15}N ID-Distill	Glibert *et al.* (1991)
Chesapeake Bay	Apr 1986	0–0.45	<202	^{15}N ID-Distill	Glibert *et al.* (1991)
Chesapeake Bay	June 1985	0–0.32	<202	^{15}N ID-Distill	Glibert *et al.* (1991)

(*Continued*)

Table 8.8 Rates of ammonium (NH_4^+) regeneration in the water column measured using ^{15}N-isotope dilution (^{15}N ID) with NH_4^+ isolated using distillation (Distill), solid phase extration (SPE), solvent extraction (SE), or diffusion (Diff) (*continued*)

Location	Date/Culture	NH_4^+ regeneration rate $\mu mol\,N\,l^{-1}h^{-1}$	NH_4^+ regen: Net NH_4^+ uptake (%)	Size Fraction (μm)	Method	Reference
Chesapeake Bay	Aug 1985	0–1.38		<202	^{15}N ID-Distill	Glibert *et al.* (1991)
Delaware River (tidal portion)	July–Sept 1983	0.165 ± 0.338	210.4 ± 155	Whole water	^{15}N ID	Lipschultz *et al.* (1986)
Fourleague Bay, LA	Mar 1991	0.27 ± 0.04	27.1 ± 23.4	<200	^{15}N ID-Distill	Bode and Dortch (1996)
Oslofjord, Norway	May 1988	0.03–0.14				
Oslofjord, Norway	June–Sept 1981	0.028 ± 0.021				
	Mean	*0.079 ± 0.030*	*Whole water*			

[a] Rates were meausured in April 1997, August and September 1998, and October 1999.
[b] Rates were averaged; when a rate was below the level of detection (0.4 nmol L^{-1} h^{-1}) a zero was included in the calculation.
[c] Rates were measured using 24 h incubations and converted to per hour.
[d] Rates were measured based on concentration changes then the rate of NH_4^+ uptake was added.
[e] Rates were measured in December 1983, October 1984, and March and July 1985.
[f] Extremmely high values from Dortch *et al.* 1992 were excluded from mean. If they were included the mean would be 0.357.
Data are presented as mean ± standard deviation. When data from a given site were reported with several averages and standard deviations, the data were pooled for an overall site average and a new estimate of the standard deviation was calculated using propogation of error. Rates are confined to those measured *in situ* in papers published since 1980. NDL = near detection limits. Many rates were estimated from figures.

light and the dark, in parallel, rates are often higher in the light incubation (e.g., Gardner *et al.*, 1997; Hanson and Robertson, 1988) although, again, this trend is not always observed (e.g., Lipschultz *et al.*, 1986).

Rates of NH_4^+ regeneration can also vary seasonally with higher rates in the spring and summer and lower rates in the fall and winter (e.g., Maguer *et al.*, 1999). In a seasonal study in Chesapeake Bay, however, the highest rates of NH_4^+ regeneration were measured in the fall, relative to the spring and summer, although the rates were highly variable ranging from extremely high (>1.2 µmol N L^{-1} h^{-1}) to unmeasurable (Bronk *et al.*, 1998).

5.2. DON release

The number of studies that have quantified DON release is finally increasing to the point that general trends are emerging, particularly with respect to DON release resulting from NH_4^+ uptake. Here we focus on DON release and the percent of uptake released across systems, release resulting from NH_4^+ versus NO_3^- uptake, and how DON release varies with respect to N uptake, water depth, and diel and seasonal cycles.

5.2.1. Rates of DON release in the field

A literature survey indicates that rates of DON are highly variable but that the average rate of DON release (resulting from NH_4^+ uptake) appears to be lowest in oceanic systems (0.006 µmol N L^{-1} h^{-1}), higher in coastal systems (0.044), and higher still in the one estuary for which there are measurements, Chesapeake Bay (0.074, Table 8.9). This same trend was observed in a transect north and south through the central Atlantic, where rates of DON release, resulting from NH_4^+ uptake, were higher in shelf waters relative to oceanic waters farther offshore (Varela *et al.*, 2006). In contrast, another study along a continuum of stations from coastal to oceanic found that rates of DON release tended to be higher at offshore stations relative to stations nearer shore (Varela *et al.*, 2003a).

5.2.2. DON release versus nitrogen uptake

DON release rates can be compared to two types of uptake rates—gross and net. The rate of gross N uptake is the total amount of N taken up by cells regardless of whether its ultimate fate is PON production or DON production (Bronk *et al.*, 1994). The net N uptake rate is the rate traditionally measured with ^{15}N tracer techniques that only includes PON production (Bronk *et al.*, 1994). As a percentage of gross N uptake, termed percent extracellular release (PER) in many studies, DON release appears similar but slightly higher in coastal (39 to 48%) versus oceanic environments (29–45%; Table 8.9). A similar trend was observed in a north-south transect through the central Atlantic where a larger percentage of gross NH_4^+ uptake was released as DON in shelf relative to oceanic waters (Varela *et al.*, 2006; a gross uptake rate was calculated as the sum of the published NH_4^+ uptake and DON release rate). Within the oceanic sites, however, a higher percentage of NH_4^+ uptake was released as DON at sites classified as oligotrophic (14%), defined as areas

Table 8.9 Rates of dissolved organic nitrogen (DON) release published and ratios of DON release to gross nitrogen (N) uptake, expressed as a percent, in the field. Rates were determined using ^{15}N tracer techniques (^{15}N) or changes in concentrations (CC)

Location	Date/Culture	Substrate	DON release rate $\mu mol\,N\,L^{-1}\,h^{-1}$	DON release: Gross N Uptake (%)	Method	Reference
Oceanic						
Atlantic—off Spain—oceanic	Apr 1997–Oct 1999[a]	NH_4^+	0.0146 ± 0.0226		^{15}N-WC	Varela *et al.* (2003a)
Atlantic—off Spain—shelf break	Apr 1997–Oct 1999[a]	NH_4^+	0.0022 ± 0.0023		^{15}N-WC	Varela *et al.* (2003a)
Atlantic Ocean— productive (chl $>0.25\,\mu g\,L^{-1}$)	Aug 1998–Oct 2000	NH_4^+	0.0014 ± 0.0003	11.3	^{15}N-WC	Varela *et al.* (2006)
Atlantic Ocean— oligotrophic (chl $<0.25\,\mu g\,L^{-1}$)	Aug 1998–Oct 2000	NH_4^+	0.0089 ± 0.0026	14.4	^{15}N-WC	Varela *et al.* (2006)
Caribbean Sea	Nov 1988	NH_4^+	0.0098 ± 0.0030	27.8 ± 8.0	^{15}N-IRC	Bronk *et al.* (1994)
Equatorial and oligotrophic Pacific	Nov 1994	$NH_4^+\ NO_3^-$	NP	15.3 ± 13.4	^{15}N-WC	Slawyk *et al.* (1998)
Equatorial and oligotrophic Pacific	Nov 1994	$NH_4^+\ NO_3^-$	NP	20–100	^{15}N-WC	Raimbault *et al.* (1999)
North Atlantic Drift Providence	Sept–Oct 2000	NH_4^+	0.0007 ± 0.0002^b	20.7 ± 11.0^c	^{15}N-WC	Varela *et al.* (2005)
North Atlantic Subtropical Gyre Providence	Sept–Oct 2000	NH_4^+	0.0002 ± 0.0000^b	24.7 ± 4.8^c	^{15}N-WC	Varela *et al.* (2005)
East Canary Coastal Providence	Sept–Oct 2000	NH_4^+	0.0002 ± 0.0001^b	15.8 ± 7.4^c	^{15}N-WC	Varela *et al.* (2005)

Eastern Tropical Atlantic Providence	Sept–Oct 2000	NH_4^+	0.0002 ± 0.0000^b	17.4 ± 4.1^c	^{15}N–WC	Varela *et al.* (2005)
South Atlantic Subtropical Gyre Providence	Sept–Oct 2000	NH_4^+	0.0003 ± 0.0000^b	22.7 ± 5.4^c	^{15}N–WC	Varela *et al.* (2005)
Brazil Current Coastal Providence	Sept–Oct 2000	NH_4^+	0.0001 ± 0.0000^b	14.2 ± 3.7^c	^{15}N–WC	Varela *et al.* (2005)
Oligotrophic North Pacific	Sept–Oct 1992	NH_4^+	$0.0016–0.0031^d$	$19.7–71.6^d$	^{15}N–WC	Slawyk and Raimbault (1995)
Southern California Bight	Oct 1992	NH_4^+	$0.0001–0.0385$	$0.5–89.0$	^{15}N–WC	Bronk and Ward (2005)
Southern California Bight	Apr 1994	NH_4^+	$0.0008–0.0088$	$6.4–63.2$	^{15}N–WC	Bronk and Ward (2005)
Southern California Bight	Oct 1992 and Apr 1994	NH_4^+	$0.0006–0.031^e$	$13.2–89.0$	^{15}N–WC	Ward and Bronk (2001)
Southern California Bight	Oct 1992 and Apr 1994	NH_4^+	$0.0007–0.019^f$	$14.4–79.7$	^{15}N–WC	Ward and Bronk (2001)
		Mean	*0.006 ± 0.023*	*29.2 ± 22.4*		
North Atlantic Drift Providence	Sept–Oct 2000	NO_3^-	0.0007 ± 0.0003^b	51.3 ± 30.1^c	^{15}N–WC	Varela *et al.* (2005)
North Atlantic Subtropical Gyre Providence	Sept–Oct 2000	NO_3^-	0.0003 ± 0.0001^b	60.6 ± 18.7^c	^{15}N–WC	Varela *et al.* (2005)
East Canary Coastal Providence	Sept–Oct 2000	NO_3^-	0.0108 ± 0.0075^b	31.3 ± 24.9^c	^{15}N–WC	Varela *et al.* (2005)

Table 8.9 Rates of dissolved organic nitrogen (DON) release published and ratios of DON release to gross nitrogen (N) uptake, expressed as a percent, in the field. Rates were determined using ^{15}N tracer techniques (^{15}N) or changes in concentrations (CC) (*continued*)

Location	Date/Culture	Substrate	DON release rate μmol N L^{-1}h^{-1}	DON release: Gross N Uptake (%)	Method	Reference
Eastern Tropical Atlantic Providence	Sept–Oct 2000	NO$_3^-$	0.0043 ± 0.0016[b]	47.6 ± 23.6[c]	^{15}N-WC	Varela *et al.* (2005)
South Atlantic Subtropical Gyre Providence	Sept–Oct 2000	NO$_3^-$	0.0006 ± 0.0003[b]	39.5 ± 25.4[c]	^{15}N-WC	Varela *et al.* (2005)
Brazil Current Coastal Providence	Sept–Oct 2000	NO$_3^-$	0.0005 ± 0.0003[b]	31.1 ± 21.1[c]	^{15}N-WC	Varela *et al.* (2005)
Oligotrophic North Pacific	Sept–Oct 1992	NO$_3^-$	0.0002–0.0005[d]	25.8–98.5[d]	^{15}N-WC	Slawyk and Raimbault (1995)
Ross Sea, Antarctica	Nov–Dec 1994	NO$_3^-$	0.066[g]	19.0	^{15}N-IRC	Hu and Smith (1998)
Ross Sea, Antarctica	Dec–Jan 1995 and 1996	NO$_3^-$	0.027[g]	8.0	^{15}N-IRC	Hu and Smith (1998)
Southern California Bight	Oct 1992 and Apr 1994	NO$_3^-$	0.0011–0.072[e]	22.2–96.0	^{15}N-WC	Ward and Bronk (2001)
Southern California Bight	Oct 1992 and Apr 1994	NO$_3^-$	0.0011–0.386[f]	22.0–99.0	^{15}N-WC	Ward and Bronk (2001)
Southern California Bight	Oct 1992	NO$_3^-$	0.0011–0.0292	26.3–98.5	^{15}N-WC	Bronk and Ward (2005)
Southern California Bight	Apr 1994	NO$_3^-$	0.0003–0.0342	3.0–92.7	^{15}N-WC	Bronk and Ward (2005)
		Mean	*0.029 ± 0.008*	*44.7 ± 59.4*		

Coastal						
Akkeshi Bay, Japan	Mar–Nov 1998	NH_4^+	$0.0060–0.015^h$		^{15}N-WC	Hasegawa *et al.* (2000b)
Atlantic—off Spain—mid-shelf	Apr 1997–Oct 1999[a]	NH_4^+	0.0061 ± 0.0095		^{15}N-WC	Varela *et al.* (2003a)
Atlantic—off Spain—coastal	Apr 1997–Oct 1999[a]	NH_4^+	0.0181 ± 0.0266		^{15}N-WC	Varela *et al.* (2003a)
Atlantic Shelf—high productivity region	Aug 1998–Oct 2000	NH_4^+	0.0129 ± 0.0023	18.2	^{15}N-WC	Varela *et al.* (2006)
Atlantic Shelf—low productivity region	Aug 1998–Oct 2000	NH_4^+	0.0105 ± 0.0031	23.6	^{15}N-WC	Varela *et al.* (2006)
Gulf of Lions (Mediterranean Sea)	March–May 1997	NH_4^+	$\sim0–0.0015$	26.0	^{15}N-WC	Diaz and Raimbault (2000)
Monterey Bay, CA	Mar 1993	NH_4^+	0.0619 ± 0.0472	16.2 ± 12.0	^{15}N-WC	Bronk and Ward (1999)
Monterey Bay, CA	Sept 1993	NH_4^+	0.0208 ± 0.0210	64.7 ± 22.1	^{15}N-WC	Bronk and Ward (1999)
Monterey Bay, CA	Mar and Sept 1993	NH_4^+	$0.0035–0.263^e$	21.1–94.0	^{15}N-WC	Ward and Bronk (2001)
Monterey Bay, CA	Mar and Sept 1993	NH_4^+	$0.0195–0.334^f$	14.1–97.7	^{15}N-WC	Ward and Bronk (2001)
Northwest Spain	Oct 1998–Sept 1999	NH_4^+	$0.0044–0.064$	6.5–62.7	^{15}N-WC	Bode *et al.* (2004b)
Southern California Bight	Oct 1992	NH_4^+	0.0124 ± 0.0032	20.5 ± 1.6	^{15}N-IRC	Bronk *et al.* (1994)
Southern California Bight	Oct 1992	NH_4^+	$0.0164–0.0315$	18.5–71.7	^{15}N-WC	Bronk and Ward (2005)

Table 8.9 Rates of dissolved organic nitrogen (DON) release published and ratios of DON release to gross nitrogen (N) uptake, expressed as a percent, in the field. Rates were determined using ^{15}N tracer techniques (^{15}N) or changes in concentrations (CC) (*continued*)

Location	Date/Culture	Substrate	DON release rate μmol N L^{-1}h^{-1}	DON release: Gross N Uptake (%)	Method	Reference
Southern California Bight (inshore)	Oct 1992	NH$_4^+$	0.0169–0.360	29.6–97.2	^{15}N-WC	Ward and Bronk (2001)
		Mean	*0.044 ± 0.059*	*38.7 ± 25.2*		
Gulf of Lions (Mediterranean Sea)	Mar–May 1997	NO$_3^-$	~0–0.0006	24.0	^{15}N-WC	Diaz and Raimbault (2000)
Monterey Bay, CA	Mar 1993	NO$_3^-$	0.0014 ± 0.0007	22.2 ± 33.6	^{15}N-WC	Bronk and Ward (1999)
Monterey Bay, CA	Sept 1993	NO$_3^-$	0.0122 ± 0.0022	85.7 ± 14.3	^{15}N-WC	Bronk and Ward (1999)
Monterey Bay, CA	Mar and Sept 1993	NO$_3^-$	0.0007–0.027^e	9.3–95.0	^{15}N-WC	Ward and Bronk 2001
Monterey Bay, CA	Mar and Sept 1993	NO$_3^-$	0.0014–0.021^f	4.1–92.0	^{15}N-WC	Ward and Bronk (2001)
Southern California Bight	Oct 1992	NO$_3^-$	0.0034 ± 0.0002	34.3 ± 3.6	^{15}N-IRC	Bronk *et al.* (1994)
Southern California Bight	Oct 1992	NO$_3^-$	0.0003–0.0014	5.0–35.6	^{15}N-WC	Bronk and Ward (2005)
Southern California Bight (inshore)	Oct 1992	NO$_3^-$	0.0050–0.027	53.0–95.4	^{15}N-WC	Ward and Bronk (2001)
		Mean	*0.007 ± 0.002*	*48.1 ± 15.2*		
Estuarine						
Chesapeake Bay, north	Apr 1989	NH$_4^+$	0.052	34.0	^{15}N-IRC	Bronk *et al.* (1994)
Chesapeake Bay, south	Apr 1989	NH$_4^+$	0.037 ± 0.032	26.3 ± 12.6	^{15}N-IRC	Bronk *et al.* (1994)

Chesapeake Bay, mesohaline	May 1988	NH_4^+	0.072 ± 0.059	27.8 ± 18.3^i	^{15}N–IRC	Bronk *et al.* (1998)
Chesapeake Bay, mesohaline	Aug 1988	NH_4^+	0.055 ± 0.047	14.2 ± 8.3^i	^{15}N–IRC	Bronk *et al.* (1998)
Chesapeake Bay, mesohaline	Oct 1988	NH_4^+	0.033 ± 0.039	28.4 ± 25.4^i	^{15}N–IRC	Bronk *et al.* (1998)
Choptank River, MD	Aug 1990	NH_4^+	0.193 ± 0.118	22.1 ± 15.1^i	^{15}N–IRC	Bronk and Glibert (1993)
		Mean	*0.074 ± 0.149*	*25.5 ± 37.9*		
Chesapeake Bay, north	Apr 1989	NO_3^-	0.061	11.0	^{15}N–IRC	Bronk *et al.* (1994)

[a] Rates were measured in April 1997, August and September 1998, and October 1999.
[b] Rates were calculated by taking the mean euphotic zone integrate rate and dividing by the mean integration depth.
[c] Percentage was recalculated using gross nitrogen uptake rates.
[d] Percentate was calculated using raw data from manuscript and equations in Slawyk *et al.* (1998).
[e] <10 μm size fraction.
[f] < 202 μm size fraction.
[g] Rate was calculated from uptake and percent release data.
[h] Release was mediated by grazers.
[i] Gross uptake rates were calculated from original data.
Tracer methods include ion retardation columns (IRC) and wet chemical (WC) isolation. Data are presented as mean ± standard deviation. When ranges are presented, the mean % release was calculated using the median value in the range. When data from a given site were reported with several averages and standard deviations, the data were pooled for an overall site average and a new estimate of the standard deviation was calculated using propogation of error. NP = not presented. Many rates were estimated from figures.

with <0.25 µg Chl L^{-1}) relative to those classified as productive (11%; Varela *et al.*, 2006). Similarly, within the shelf sites, the percent of NH_4^+ released as DON was higher at the low productivity sites (23%) relative to high productivity sites (18%; Varela *et al.*, 2006).

Using ^{15}N tracer techniques, rates of DON release are often measured as a result of either NH_4^+ or NO_3^- incorporation. One trend emerging from field data is that a higher percentage of gross NO_3^- uptake is released as DON relative to incubations where NH_4^+ is the substrate (Table 8.9). In oceanic systems 45% of NO_3^- uptake was released as DON while only 29% of NH_4^+ was released, although the errors are large (Table 8.9). The value for DON release from NH_4^+ agrees well with the 27% cited in a review table in Varela *et al.* (2006). In coastal systems 48% of NO_3^- uptake was released as DON relative to only 39% of NH_4^+, but again the errors are large (Table 8.9). Note that the 39% is almost twice that of the 20% cited in the review table of Varela *et al.* (2006). The reason for the discrepancy is the inclusion of estuarine data in their coastal mean. They also included three very low averages from a study in Akkeshi Bay where grazers were purposely excluded (Hasagawa *et al.*, 2000b) that were not included in Table 8.9 because they did not represent *in situ* rates.

Within individual studies this trend of higher percent extracellular release when NO_3^- is used is even more apparent. At six providences in the central Atlantic, the mean percentage of NO_3^- uptake released as DON was 1.7–2.7 times greater than the mean percentage of NH_4^+ uptake released as DON (Varela *et al.*, 2005 and Table 8.9). A higher percentage of NO_3^- uptake was also released as DON, relative to NH_4^+ uptake, in Monterey Bay (Bronk and Ward, 1999) and the Southern California Bight (Bronk and Ward, 2005). This trend is not universal, however, and the reverse was seen in the Gulf of Lions (Diaz and Raimbault, 2000) and in a culture study with *Scenedesmus quadricauda* and *Microcystis novacekii* (Nagao and Miyazaki, 2002). Although published DON release rates are most commonly the result of NH_4^+ uptake, followed by NO_3^- uptake, theoretically DON release could result from uptake of any N substrate—inorganic or organic. Varela *et al.* (2005) used an isotope dilution approach to measure DON release due to urea uptake. Percent release when urea was used tended to be similar to the percent release observed in parallel NH_4^+ incubations (Varela *et al.*, 2005). Most importantly, the fact that multiple substrates can contribute to DON release means that DON release rates measured as a result of just one or two substrates (i.e., NH_4^+ and NO_3^-) likely underestimates the true rate of DON release *in situ*.

Rates of DON release usually correlate well with N uptake. For example, rates of DON release (from NH_4^+) were linearly related to rates of NH_4^+ uptake in the waters off of Spain (Bode *et al.*, 2004b). In a similar fashion, net and gross uptake was positively correlated (linearly) to each other in the Gulf of Lions for both NH_4^+ ($r^2 = 0.93$) and NO_3^- ($r^2 = 0.95$) uptake (Diaz and Raimbault, 2000); the difference between net and gross nitrogen uptake is DON release. This relationship, however, is a function of the methods used to measure DON release. Using the ^{15}N approaches published to date (e.g., Bronk and Glibert, 1991, Slawyk and Raimbault, 1995) the cell must first take up the ^{15}N label thereby making a
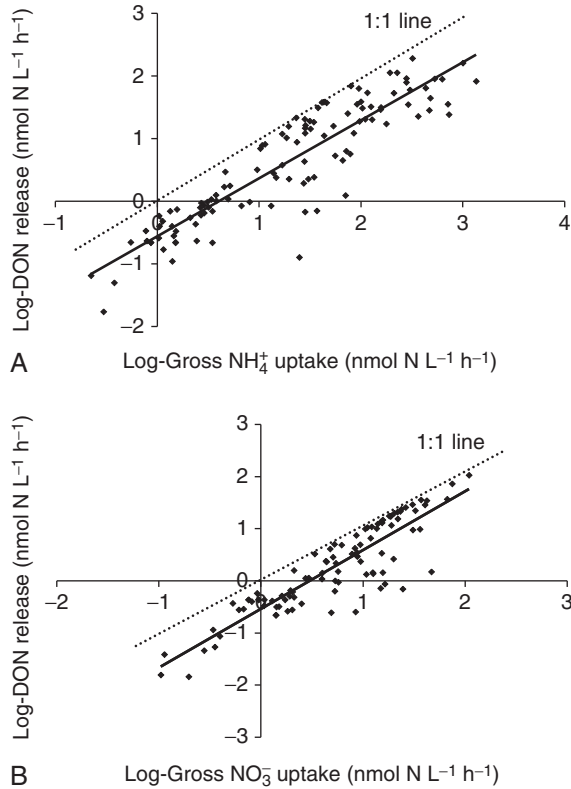
Figure 8.4 The log-log relationships between DON release and gross nitrogen uptake for (A) DON release as a result of NH_4^+ uptake and gross NH_4^+ uptake and (B) DON release as a result of NO_3^- uptake and gross NO_3^- uptake. Model II regression parameters are reported in Table 8.7. Graphs include data from Monterey Bay, CA (Bronk and Ward, 1999; Ward and Bronk, 2001), the Southern California Bight, CA (Bronk and Ward, 2005; Ward and Bronk, 2001), the Gulf of Lions, Mediterranean (Diaz and Raimbault, 2000), and six sites in the Central Atlantic (Varela *et al.*, 2005). Graph A also includes data from Chesapeake Bay (Bronk *et al.*, 1998).

release rate measurement dependent on prior N utilization. When data from a number of different studies were combined, log-transformed DON release rates from NH_4^+ and NO_3^- were positively correlated with their respective log-transformed gross uptake rates at the $p < 0.001$ level (Fig. 8.4 and Table 8.7). Varela *et al.* (2006) included a log-transform plot of DON release, from NH_4^+ uptake, and net NH_4^+ uptake for 180 incubations performed in ocean and shelf waters in the Atlantic (data not included in Fig. 8.3). The equation of their regression was log DON release = 0.84 log net NH_4^+ uptake-0.56, $r^2 = 0.76$, $p < 0.001$. Note that their plot used net uptake rates, as opposed to the gross uptake rates used in Fig. 8.4.

5.2.3. Variables that affect DON release rates

Rates of DON release also vary with depth in the water column. DON release rates were generally higher in surface waters and lower at depth in the Gulf of Lions, from both NH_4^+ and NO_3^- uptake (Diaz and Raimbault, 2000), and in the Central Atlantic Ocean when NH_4^+ was the substrate (Varela *et al.*, 2005). In contrast, the reverse was true for DON release resulting from both NH_4^+ and NO_3^- uptake in the Southern California Bight (Bronk and Ward, 2005). When expressed as a percentage of uptake, however, DON release is generally higher deeper in the euphotic zone indicating that deeper in the water column a smaller percentage of the N taken up is incorporated into cells and therefore sinking particles (Bronk and Ward, 1999). This was also observed in rates of DON release measured when NO_3^- was the substrate in the Central Atlantic Ocean (Varela *et al.*, 2005) and as a result of NH_4^+ uptake in the waters off of Spain, where greater than 50% of the NH_4^+ taken up was released as DON deep in the euphotic zone, as compared to surface waters where the percent extracellular release was generally less than 20% (Bode *et al.*, 2004b). This trend was also seen in the cross system comparison conducted for this review. In four coastal and open ocean studies, for which complete data sets were available, there was a higher percentage of gross NH_4^+ and NO_3^- uptake released as DON at the base of the euphotic zone, relative to near surface waters. In near surface waters, the slopes of the regression lines indicate that 13% and 57% of the NH_4^+ and NO_3^- taken up, respectively, was released as DON (Table 8.7). Deeper in the euphotic zone, the percentages increase to 86% and 93% for NH_4^+ and NO_3^- uptake respectively (Table 8.7). Similarly, high rates of urea release are also observed at the base of the euphotic zone (Cho and Azam, 1995).

The question is why would cells lose or release so much of the N that was recently taken up. Bronk and Ward (1999) suggested possible mechanisms that could contribute to higher measured DON release. Higher grazing pressure deeper in the euphotic zone could increase release rates. Similarly, greater rates of viral–induced release or cell death could also enhance release rates. Lower rates of incorporation of recently release DON at the base of the euphotic zone could also lead to higher measured release rates. Bacterial production tends to be lower deeper in the water column (e.g., Carlson and Ducklow, 1996) and concentrations of inorganic N are higher such that reincorporation of recently release DON would not be as likely. Most troublesome is the possibility of a photoprotective futile cycle (Lomas and Glibert, 1999). Cells in a NO_3^-–rich environment, as is common at the base of the euphotic zone in or near the nitracline, may rapidly take up NO_3^- and release reduced compounds as a way of protecting their photosystems when exposed to high light levels. The high light exposure could have occurred when the incubations were being assembled on deck. If this mechanism was operating, high rates measured at the base of the euphotic zone could be an artifact of the experimental protocol. A similar circumstance could occur in nature during deep mixing events, however, when cells in low light, high NO_3^- waters could be mixed up into higher light environments.

The question of a diel periodicity in rates of DON release is still an open one. In the Southern California Bight, rates of DON release from both NH_4^+ and NO_3^- uptake were generally higher at night deeper in the water column, but higher during

the day nearer the surface (Bronk and Ward, 2005). In cultures of both *Scenedesmus quadricauda* and *Microcystis novacekii*, DON release increased in the light relative to the dark (Nagao and Miyazaki, 2002). In Chesapeake Bay, DON release rates in surface waters were highest at dawn and dusk, than during the day, although the difference was not statistically significant (Bronk *et al.*, 1998). Based purely on physiology, phytoplankton do not synthesize the organic C component of DON in the dark, which should lead to lower rates of direct DON release from cells. As a result, when higher rates of DON release are observed in the field at night, it is likely more a function of trophic dynamics, such as zooplankton sloppy feeding or excretion, than direct release by cells.

The fate of N uptake also appears to change seasonally. In Monterey Bay, the primary fate of N uptake is particle production in March but DON production in September, suggesting that the DON pool acts as an intermediate between DIN assimilation and the net formation of particles for export (Bronk and Ward, 1999). In a seasonal study of DON release off the coast of Spain, the percentage of NH_4^+ uptake released as DON was highest during the winter and fall, when rates of primary production were relatively low, and lower during the higher productivity periods during seasonal upwelling (Bode *et al.*, 2004b). Measured rates of DON release are also affected by simultaneous uptake of DON. As a result, this pattern of lower percent DON release during upwelling could be enhanced to some extent by relatively low rates of bacterial DOM consumption during the upwelling period (Varela *et al.*, 2003b). In another study, upwelling appeared to largely control the seasonal and spatial variability in regeneration rates (Varela *et al.*, 2003a). Rates of NH_4^+ regeneration and DON release, integrated throughout the euphotic zone, were at a minimum during upwelling conditions in the summer and at a maximum in early fall (Varela *et al.*, 2003a). With respect to seasonal, temporal, and spatial differences in NH_4^+ and DON release rates, the only statistically significant source of variability was a seasonal component to NH_4^+ regeneration (Varela *et al.*, 2003a). This suggests that in general these rates are governed by biological processes on relatively small spatial and temporal scales.

5.3. Individual organic compounds

In addition to the bulk DON pool, rates of release have also been measured for a suite of individual compounds including urea, DFAA, DCAA and proteins, and nucleic acids. In many respects urea behaves similarly to NH_4^+ though it can be much more variable (e.g., Bronk *et al.*, 1998). Rates of uptake and release of urea can be tightly coupled, as observed in the Bering Sea (Hansell and Goering, 1989). They also tend to be lower than NH_4^+ regeneration, though in one study in Chesapeake Bay urea release rates were high enough to supply over 100% of the phytoplankton N requirement (Bronk *et al.*, 1998). A review of urea regeneration rates in Chesapeake Bay found that mean Bay-wide surface urea regeneration rates are highest but most variable during the fall (Lomas *et al.*, 2002). In the Southern California Bight, urea release in the bacterial size fraction was very high at the base of the euphotic zone (Cho and Azam, 1995). These data suggest that urea is an important intermediate between sinking particles and release of NH_4^+ mediated by bacteria.

Elevated concentrations of dissolved amino acids are often measured during and after phytoplankton blooms (e.g., Børsheim et al., 1999). The copepod *Eucalanus pileatus* was shown to release bursts of DFAA when fed (Gardner and Paffenhöfer, 1982). In another study DFAA release was found to be higher in the presence of copepods (Roman et al., 1988) and bactivory can also result in DFAA release (Ferrier-Pagès et al., 1998b). A number of culture studies have investigated DFAA release. In general, DFAA often accumulate in the media with the highest concentrations present during stationary phase (Poulet and Marin-Jezequel, 1983) while diatoms showed the highest rates of DFAA release during exponential growth (Myklestad et al., 1989). The types of amino acids released from the diatom *Chaetoceros affinis* also changed during exponential growth relative to stationary growth (Myklestad et al., 1989). Marsot et al. (1991) observed an accumulation of glycine, threonine, and serine in cultures of *Phaeodactylum tricornutum*. Sea ice can be a large reservoir for DFAA due largely to their production by ice algae. Concentrations of DFAA in ice were as high as 1.83 µM, which represented 21% of the total DOC in the sea ice (Amon et al., 2001).

Meon and Kirchman (2001) measured DCAA production and degradation rates during phytoplankton blooms in two mesocosms—one with nutrient enrichment and one without. Concentrations of DCAA increase in the enriched mesocosm but not in the unenriched. They concluded that degradation processes, not production, were more important in governing the composition of the DCAA pool. In another study, *Paraphysomonas imperforata* grazing on bacteria released DCAA (Nagata and Kirchman, 1991).

Nucleic acids are another ON pool that has received attention recently. In the North Pacific Subtropical gyre 11–35% of total enzymatically hydrolyzable dissolved DNA (ehD-DNA) production was due to lysis of bacteria (Brum, 2005) indicating that other processes contribute significantly to the ehD-DNA pool; likely candidates were bacterial exudation, bacterial autolysis, and bactivory. Rates of D-DNA production from viruses were lowest at the surface (5 m, 0.019–0.042 ng ml^{-1} h^{-1}), fairly constant within the upper 45 m, and highest at the deepest station sampled (75 m, 0.062–0.120 ng ml^{-1} h^{-1}, Brum, 2005).

5.4. Linking DON release with DOC flux

Release of DON and DOC in short-term incubations is ultimately dependent on primary production and so a positive relationship between DON release and primary production and DOC release could be expected. Similarly, bacteria are often considered the primary users of DON and so an inverse relationship between measured DON release and bacterial production is also likely. To date few studies have investigated these relationships directly, although studies linking DON with DOC release are becoming more common.

A study off northwest Spain measured DON release and primary production in parallel (Bode et al., 2004b). A model II regression of log-transformed data indicated only a weak positive relationship between rates of DON release and primary production (slope = 0.55, r^2 = 0.14, p = 0.023, n = 36) or chlorophyll a concentrations (slope = 1.01, r^2 = 0.13, p = 0.028, n = 36). Log-transformed DON release rates (from both NH_4^+ uptake, NO_3^- uptake and both combined) were plotted

against log-transformed chlorophyll *a* concentrations for the studies used in Fig. 8.4 but no significant relationships were observed.

Similar to DON release, release of DOC as a fraction of particulate and dissolved C production is highly variable in many systems (reviewed in Carlson, 2002), with rates of DOC release generally being higher at the surface (e.g., Bode *et al.*, 2004b). A seasonal study in Chesapeake Bay showed that the ratio of DOC:DON release (from NH_4^+ uptake only) was quiet low in May, August, and October, ranging from 3.4 to 4.5. These low ratios suggest release consisting primarily of N-rich compounds such as DFAA. In a study off northwest Spain, the slope of a linear regression between rates of DOC and DON release (from NH_4^+ uptake only) was 7.4 ($r^2 = 0.867$, $p < 0.001$) again suggesting the release of relatively N-rich compounds (Bode *et al.*, 2004b). At any given depth, however, the DOC:DON release ratio varied widely from ∼5 to 23 at the 100% light depth to ∼1 to 5 at the 1% light depth (Bode *et al.*, 2004b). In another study off the coast of Spain, which experiences seasonal upwelling, the ratio of DOC:DON release (from NH_4^+ uptake only) always exceeded 30 indicating the release of C-rich compounds such as mono-or polysaccharides. High DOC:DON release ratios could also be the result of an underestimation of DON release rates because only release as a result of NH_4^+ uptake was measured. DOC:DON release ratios could also increase due to a rapid reincorporation of the released DON during the course of the incubation; if DON was reincorporated it would not be included in the measured DON release rates. Several studies suggest that DOC release, as a percentage of primary production (i.e., PER), is higher in oligotrophic, relative to more productive, systems (Morán *et al.*, 2002; Nagata, 2000). In a transect through the central Atlantic, a similar trend was observed where the PER of DON release, resulting from NH_4^+ uptake, was higher in oligotrophic waters relative to shelf waters (Varela *et al.*, 2006).

Similar to the situation with primary production, relatively few studies have measured DON release and bacterial production simultaneously. In a transect through the central Atlantic, DON release accounted for <15% of the variability observed in bacterial production rates. In this study, rates of DON release were in excess of the bacterial N requirements in oceanic waters but not in shelf waters. The calculations assumed that only 20% of the recently released DON was labile. While it is true that a large fraction of the DON pool could be composed of refractory compounds (reviewed in Chapter 3 by Aluwihare and Meador, this volume and Bronk, 2002) the DON released in short-term incubations would likely be composed predominantly of labile biomolecules such as amino acids, proteins, nucleic acids, and enzymes. For example, in a study with *Trichodesmium* virtually all of the recently released DON (Glibert and Bronk, 1994) was in the form of amino acids, based on amino acid release measurements performed in parallel (Capone *et al.*, 1994).

6. RECOMMENDATIONS FOR FUTURE RESEARCH

We are only beginning to understand the who, what, and where of regeneration—both in the water column and sediment. With respect to the who—studies are needed that determine the magnitude of release from different trophic levels. In the

water column we need new approaches that will allow researchers to separately quantify release due to direct release by phytoplankton from release mediated by viruses and the various pathways whereby grazers affect release (excretion, sloppy feeding, fecal pellet dissolution). Along these lines, only a handful of studies have examined changes in plankton community trophic structure and resultant changes in N regeneration. While experimentally challenging, the use of mesocosms and other approaches have proved useful in several studies. With respect to the what—more studies are needed to characterize the organic pools released. Though there has been substantial effort directed at characterizing HMW DON, we know relatively little about the range of organic N compounds produced during the different regeneration processes. Studies that address the stoichiometry of release are also needed as we work to define how the different elemental cycles of C, N, and P interact. In particular more studies are needed that measure inorganic and organic release simultaneously. With respect to the where—it is no surprise, given sampling constraints, that rates of N regeneration are best known for coastal surface waters of coastal and near-shore sediments. Little is known of the relative role of different trophic levels in N regeneration in mesopelagic waters, and DON flux rates from sediments, particularly deep sea sediments, are still few and far between. Quantifying sediment DON fluxes is particularly important for accurate N budgets in coastal and estuarine systems because they help constrain processes that are much more difficult to quantify like denitrification.

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Land-Based Nitrogen Sources and Their Delivery to Coastal Systems

Sybil P. Seitzinger *and* John A. Harrison

Contents

1. Introduction

Inputs to terrestrial systems of newly fixed N_2 (the conversion of relatively inert N_2 gas to more bioavailable N forms) have more than doubled as a result of human activities including synthetic N fertilizer production, cultivation of legume crops, and combustion of fossil fuels (Galloway *et al.*, 2004; Vitousek and Matson, 1993). A portion of this chemically combined N is transported to coastal waters where it can lead to changes in coastal ecosystem function (e.g. Chapter 18 by Boynton and Kemp, this volume; Deegan *et al.*, 2002; Glibert *et al.*, 2005a; Nixon, 1995; Rabalais *et al.*, 2002; Valiela *et al.*, 1997; Carpenter *et al.*, 1998). Changes in phytoplankton species composition,
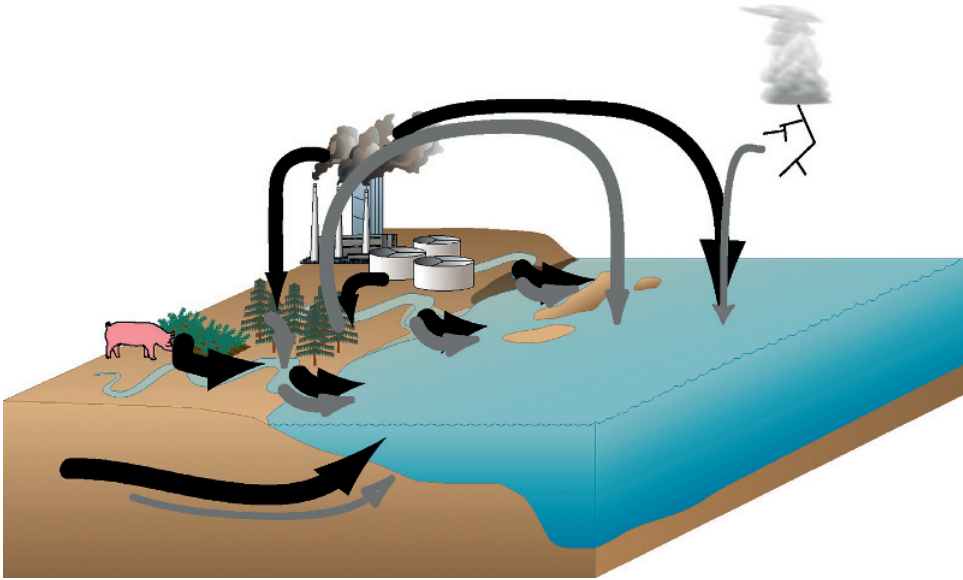
Figure 9.1 Land-based sources of N to coastal and marine waters from natural (grey arrows) and anthropogenic (black arrows) sources. Sources of N that contribute to river N export are represented including non-point inputs from atmospheric deposition from natural sources (soil emissions and lightning) and fossil fuel combustion, non-agricultural and agricultural biological N_2-fixation, and synthetic fertilizer and livestock manure; point sources include wastewater treatment facilities. Some portion of watershed N inputs can enter coastal regions directly with submarine groundwater discharge. Atmospheric deposition directly to the surface of coastal and oceanic waters also is a transport pathway of land-based N. Figure created using the IAN symbol library (http://ian.umces.edu).

alteration and loss of seagrass habitats, and increases in carbon fixation, extent and duration of anoxic and hypoxic water, harmful algal blooms, and coral reef degradation are some of the effects associated with excess N inputs to coastal systems (e.g., Anderson et al., 2002; Carpenter et al., 1998; Diaz, 2001; Nixon, 1995; NRC, 2000; Rabalais, 2002) as reviewed in Chapter 11 by Paerl and Piehler (this volume).

Rivers are the major pathway for delivery of land-based N sources from watersheds to coastal systems (Fig. 9.1). In addition, submarine groundwater discharge and direct atmospheric deposition can be important pathways for delivery of land-based N sources. This chapter focuses on export of N by rivers to the coastal zone (i.e., estuaries, bays, continental shelves) and includes investigations of: (1) spatial patterns of river N export, by N form; (2) N sources (natural and anthropogenic) and other factors controlling the form and magnitude of N transport by rivers from watersheds; (3) temporal patterns in river export of N (e.g., inter-decadal, inter-annual, seasonal, events); and (4) effects of human modification of discharge (dams, consumptive water use) on N export. We also include brief discussions of coastal N loading via submarine groundwater discharge and direct atmospheric N deposition to coastal waters. Site specific case studies as well as regional and global perspectives are presented.

We specifically address land-based inputs of N to the coastal zone in this chapter. Biological N_2-fixation occurring within coastal and open ocean regions can contribute substantial additional inputs of N at some times and locations (Chapter 4 by Carpenter

and Capone, this volume; Chapter 22 by Hopkinson and Giblin, this volume). Of particular note is N from oceanic N_2-fixation which is transported onto continental shelves from advection of open ocean waters and can considerably exceed land-based N sources to continental shelves (e.g., Fennel et $al.$, 2006; Seitzinger and Giblin, 1996).

2. Spatial Patterns in Amount and Form of River Nitrogen Export

Biologically available N transported by rivers to coastal systems occurs in several forms. These forms include dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON), and particulate nitrogen (PN). DIN is made up of three major components: nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+). DON is operationally defined as organic N that can pass through a filter with a minimum pore size of 0.2–1 µm. PN is the N that is retained by the filter. DON and PN each are composed of complex mixtures of specific N-based compounds, most of which are chemically uncharacterized at the molecular level, although considerable advances are being made (Chapter 3 by Aluwihare and Meador, this volume; Benner, 2002; Bronk 2002; Chapter 8 by Bronk and McCarthy, this volume; Koch et $al.$, 2005; Seitzinger et $al.$, 2005a).

From a nitrogen mass balance perspective, understanding total N export from watersheds to coastal systems may be sufficient. However, information about the various forms of N transported by rivers is important in order to understand factors controlling N export and effects of N inputs on coastal systems. This is because factors controlling export of N from watersheds are not the same for all N forms, as discussed below. Furthermore, different forms of N have different ecosystem effects. For example, all of the DIN pool is generally considered to be bioavailable, while only a portion of river transported DON is readily available for uptake by micro-organisms, including bacteria and some phytoplankton (Bronk, 2002; Seitzinger et $al.$, 2002a). In particular DON is implicated in the formation of some coastal harmful algal blooms (Berg et $al.$, 1997; Glibert et $al.$, 2005a,b; Granéli et $al.$, 1999; Paerl, 1988; 2003). Particulate and dissolved species can have very different impacts on receiving ecosystems. Of course, it is also not sufficient to understand N alone; other bioactive elements (e.g., phosphorus, silica, carbon, etc.) must also be considered in order to fully understand effects of nutrient inputs to coastal systems (Conley et $al.$, 1993; Turner et $al.$, 1998). In this chapter we focus on only N, while recognizing the importance of other elements. Recent global perspectives on river export of P and C can be found in Harrison et $al.$ (2005a,b), Mackenzie et $al.$ (1998), Smith et $al.$ (2003), and Van Drecht et $al.$ (2005).

2.1. Approaches

During the last several decades there have been many studies quantifying and characterizing N export by individual rivers to the coastal zone through direct measurements of N concentrations and water discharge (e.g. Boynton et $al.$, 1995;

Eyre and Pont, 2003; Jordan *et al.*, 2003; Nixon *et al.*, 1995; Wollast, 1983; Table A1). In addition, a number of relatively complex, deterministic models of river nitrogen flux have been developed and applied to individual rivers (e.g., GWLF, Haith and Shoemaker, 1987; Lee *et al.*, 1999; HSPF, Bicknell *et al.*, 1997; Filoso *et al.*, 2004; SWAT, Srinivasan *et al.*, 1993). Several of these, as well as other models, have been described recently in Alexander *et al.* (2002).

However, syntheses of results from individual studies have been rarer. One of the first global syntheses of measurements of river N export, by N form, was by Meybeck (1982). Since then, several databases have been created documenting measured N export from rivers for specific regions (e.g., Alexander *et al.*, 1996—U.S.; Lewis *et al.*, 1999—South America; Holmes *et al.*, 2000—Arctic; UNEP/MAP, 2003—Mediterranean; EEA, 1998—Europe; Table A1) and globally (GEMS-Water Triennial Reports; LOICZ; Meybeck and Ragu, 1995; Peierls *et al.*, 1991; Smith *et al.* 2003). The creation of these databases has highlighted the large variation among rivers, both in terms of N yield (kg N km^{-2} watershed yr^{-1}) and N load (kg N watershed^{-1} yr^{-1}; Table A1), and made it possible to develop a more refined understanding of patterns of N export at local, regional and global scales. For example, regional syntheses of measured N fluxes have characterized rates of N export in the Northeast US (Boyer *et al.*, 2002), Eastern and Gulf US (Castro *et al.*, 2001), and in relatively unimpacted rivers in the Americas (Lewis *et al.*, 1999). These studies have shown that fluxes of TN to the coastal zone can vary substantially on a regional scale and that TN export correlates reasonably well with N inputs to watersheds (Sources contributing to N export are discussed in detail in Section 3).

Development of databases for a wide range of rivers also has made it possible to develop models that are widely applicable without tuning to individual basins. This is particularly important because in many basins around the world there is inadequate observational data on surface water quantity and quality. In some regions, such as the US, the number of river locations monitored (e.g., by the US Geological Survey) for water quality and discharge has decreased dramatically in the past decades; in some other world regions open access to data is still very limited. Progress continues to be made in the development of remote-sensing-based measurements for rivers and streams to obtain continuous data on water discharge (Brakenridge *et al.*, 2005) and automated sensors capable of measuring concentrations of dissolved and particulate constituents (e.g. Johnson and Coletti, 2002). However, until these techniques are substantially improved and widely implemented, we must rely on models to estimate N export from watersheds which have few or no available measurements of nutrient export.

Significant progress has been made in the development and application of spatially explicit regional and global models of N export by rivers to the coastal zone as a function of watershed N inputs and biogeophysical properties (e.g., Alexander *et al.*, 2000; Beusen *et al.*, 2005; Boyer *et al.*, 2006; Caraco and Cole, 1999; Green *et al.*, 2004; Harrison *et al.*, 2005b; Howarth *et al.*, 1996; Jordan and Weller, 1996; Seitzinger and Kroeze, 1998; Smith *et al.*, 2003; Van Drecht *et al.*, 2003). One of the first models to explain N export by a wide range of rivers globally related human population to nitrate export (Peierls *et al.*, 1991). That model was further developed with additional parameters, including nonpoint sources related to agricultural fertilizer use and atmospheric N deposition, point source sewage inputs, and hydrological parameterization (Caraco and Cole, 1999). Global databases of input parameters were then applied which resulted

in the first spatially explicit, global view of DIN export as a function of N inputs to watersheds (Kroeze and Seitzinger, 1998; Seitzinger and Kroeze, 1998; Seitzinger *et al.*, 2002b). A model describing TN export by rivers as a function of atmospheric deposition and net anthropogenic N inputs (NANI model) was developed using data from rivers in the North Atlantic Basin (Howarth *et al.*, 1996). A modified version of the NANI model has now been applied to global databases (Boyer *et al.*, 2006). The SPARROW model (Spatially Referenced Regression on Watershed attributes; Smith *et al.*, 1997) relates TN export by rivers with spatial data on watershed N sources, landscape characteristics, and stream properties. The SPARROW model has been applied to a range of rivers in the US and New Zealand (e.g., Alexander *et al.*, 2000, 2001; McBride *et al.*, 2000). A non-spatial, global model has examined changes in the biogeochemical cycles N, P, C, and S over decadal to century time scales (Mackenzie *et al.*, 1998).

A system of spatially explicit, global nutrient export models has recently been developed by the Global **N**utrient **E**xport from **W**atersheds (Global NEWS) working group (Seitzinger *et al.*, 2005b). The Global NEWS system of models includes models that predict annual average export of DIN, DON, and PN for individual river basins globally (P and C forms are also included). These sub-models are referred to as NEWS-DIN, NEWS-DON and NEWS-PN, respectively. The NEWS-DIN model predicts DIN yield (kg N km^{-2} yr^{-1}) as a function of water runoff, inorganic N fertilizer application, manure N excretion, sewage N inputs, atmospheric N deposition, and biological N_2-fixation (described in detail in Dumont *et al.*, 2005). NEWS-DIN also includes N retention and loss terms such as N retention in river networks, N retention in dammed reservoirs, N loss via consumptive water use, and N removed from a watershed via harvesting and grazing. The NEWS-DON model predicts DON yield (kg N km^{-2} yr^{-1}) as a function of water runoff, inorganic N fertilizer application, manure N excretion, sewage N inputs, and biological N_2-fixation (described in detail in Harrison *et al.*, 2005b). It contains many of the same retention and loss pathways as NEWS-DIN. NEWS-PN is somewhat different in that it predicts PN yield (kg N km^{-2} yr^{-1}) as a function of land-use, precipitation intensity, landscape rugosity, and lithology (described further in Beusen *et al.*, 2005).

2.2. Patterns

According to global model predictions, total N export by rivers at the global scale is approximately 40–65 Tg N yr^{-1} under 1990s conditions (Boyer *et al.*, 2006; Galloway *et al.*, 2004; Green *et al.*, 2004; Seitzinger *et al.*, 2005b; Van Drecht *et al.*, 2003). It is remarkable how small the range of estimates is, given the uncertainties in modeling approaches, global input data bases, and availability and quality of measurements of river TN export. Though this consistency is encouraging, some of it may result from the fact that there is considerable overlap between TN-export datasets used to calibrate each of these models, and some overlap in the input data sets used to apply the models (Van Drecht *et al.*, 2005).

Relatively few large rivers account for a large proportion of the N exported to coastal zones globally for all N forms, based on NEWS model predictions. For example, the 25 rivers with the greatest water discharge globally (<0.5% of the rivers with a watershed >2500 km^2) account for approximately half of the exported DIN and DON, and about a quarter of the exported PN, globally (Table 9.1). Some of

Table 9.1 The largest 25 rivers (in order of decreasing annual discharge; Meade 1996 Milliman and Meade 1983), river watershed areas, river discharge estimates, and NEWS-model-estimated DIN, DON, PN, and TN (Total exports from the 25 largest rivers and the percent of total global export for which these rivers account are also shown, $Tg = 10^{12}$ g)

River name	Receiving ocean	Watershed surface area 10^6(km^2)	Q (km^2 yr^{-1})	DIN (Tg N basin^{-1} yr^{-1})	DON (Tg N basin^{-1} yr^{-1})	PN (Tg N basin^{-1} yr^{-1})	TN (Tg N basin^{-1} yr^{-1})
Amazon	Atlantic Ocean	5.88893	6300	3.43	1.89	2.75	8.07
Zaire	Atlantic Ocean	3.73374	1250	0.82	0.68	0.82	2.32
Orinoco	Atlantic Ocean	1.03931	1200	0.50	0.48	0.15	1.13
Ganges	Indian Ocean	1.62681	970	2.17	0.32	1.29	3.78
Chang Jiang	Pacific Ocean	1.79304	900	1.05	0.16	0.14	1.35
Yenisei	Arctic Ocean	2.62072	630	0.07	0.08	0.11	0.26
Mississippi	Atlantic Ocean	3.20312	530	0.57	0.08	0.17	0.82
Lena	Arctic Ocean	2.50029	510	0.04	0.05	0.11	0.20
Parana	Atlantic Ocean	2.71483	470	0.26	0.19	0.16	0.61
Mekong	Pacific Ocean	0.757852	470	0.35	0.14	0.34	0.83
St. Lawrence	Atlantic Ocean	1.05277	450	0.15	0.14	0.03	0.32
Irrawaddy	Indian Ocean	0.40554	430	0.21	0.13	0.37	0.72
Ob	Arctic Ocean	3.10111	400	0.13	0.07	0.13	0.33
Amur	Pacific Ocean	1.79413	325	0.25	0.05	0.08	0.38
Mackenzie	Arctic Ocean	1.71057	310	0.03	0.03	0.07	0.13
Zhujiang	Pacific Ocean	0.408123	300	0.32	0.05	0.07	0.45
Salween	Indian Ocean	0.272866	300	0.06	0.03	0.09	0.18
Columbia	Pacific Ocean	0.731473	250	0.06	0.04	0.02	0.12
Indus	Indian Ocean	1.14155	240	0.19	0.02	0.02	0.23

Table 9.1 (*continued*)

River name	Receiving ocean	Watershed surface area $10^6(km^2)$	Q $(km^2\ yr^{-1})$	DIN (Tg N $basin^{-1}$ yr^{-1})	DON (Tg N $basin^{-1}$ yr^{-1})	PN (Tg N $basin^{-1}$ yr^{-1})	TN (Tg N $basin^{-1}$ yr^{-1})
Magdalena	Atlantic Ocean	0.251789	240	0.21	0.07	0.13	0.40
Zambezi	Indian Ocean	1.36377	220	0.14	0.09	0.06	0.29
Danube	Black Sea	0.787291	210	0.18	0.06	0.06	0.30
Yukon	Pacific Ocean	0.895236	195	0.02	0.03	0.05	0.10
Niger	Atlantic Ocean	2.23885	190	0.16	0.12	0.13	0.41
Purari	Pacific Ocean	0.0337825	150	0.02	0.01	0.03	0.07
Total		42.96	17440	11.38	5.02	7.41	23.81
Total as Percent of Global Total		38	47	48	46	27	38

the highest rates of TN export (>100 Gg N yr^{-1}) by individual rivers occur in Europe and southern and eastern Asia, although high export is also predicted for a number of rivers in North America, Africa, and South America (Fig. 9.2). The highest rates of TN export are often, although not always (e.g., Amazon), estimated to occur in large rivers with substantial anthropogenic N inputs.

If we distinguish N export by form, at the global scale, DIN and PN export are approximately equal in magnitude ($\sim$25 Tg yr^{-1} and 30 Tg yr^{-1}, respectively), with
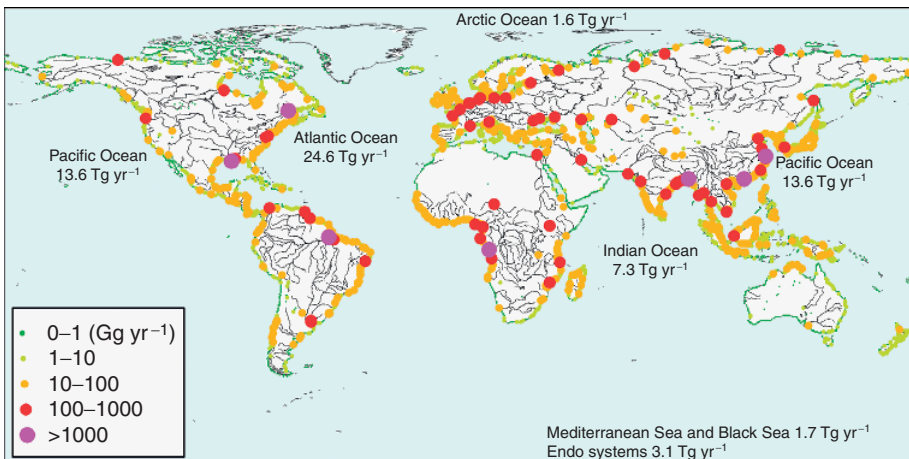
Figure 9.2 Modeled river TN export to coastal areas from Van Drecht *et al.* (2003). Gg = 10^9 g and Tg = 10^{12} g.
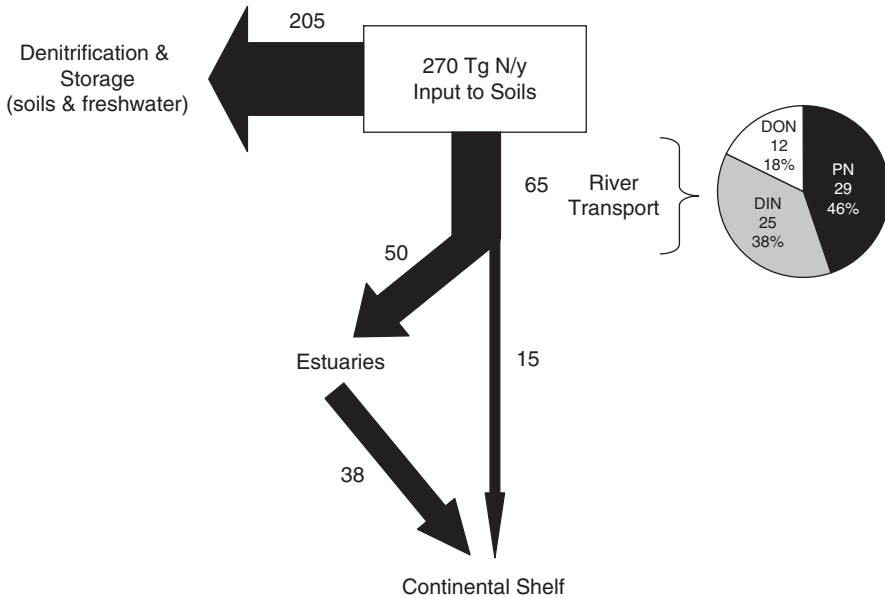
Figure 9.3 Globally, approximately 270 Tg of newly fixed N enters terrestrial soils each year from biological N_2-fixation (natural and agricultural), lightning, combustion of fossil fuels (leading to increased atmospheric N deposition), and synthetic fertilizer use as of the mid-1990s (Galloway *et al.* 2004). According to NEWS-model-predictions, 65 Tg N yr^{-1} of the N entering soils is exported by rivers to coastal systems as DIN, DON, and PN (Beusen *et al.*, 2005; Dumont *et al.*, 2005; Harrison *et al.*, 2005b). We estimate that ∼15 Tg of the river N is discharged by large rivers directly to the continental shelves. The other 50 Tg of river N enters estuaries, of which ∼38 Tg is then exported to continental shelves. Units: Tg N yr^{-1} (Tg = 10^{12} g).

each accounting for approximately 40% of the global TN export, according to NEWS-model-predictions (Fig. 9.3). In contrast, global DON export is substantially less (∼12 Tg yr^{-1}), and accounts for approximately 20% of TN export. (The C exported in the dissolved organic and particulate matter amounts to 170 and 197 Tg C yr^{-1}, respectively, according to NEWS model predictions—Harrison *et al.*, 2005b; Beusen *et al.*, 2005). There is, however, substantial spatial variability in both the magnitude and form of N exported to the coastal zone (Table A1; Fig. 9.4).

There are a number of hot-spots and cold-spots for N yield (N export per km^2 watershed) that are consistent across all N forms. For example, regions with high runoff such as northern South America, tropical West Africa, and Indonesia have high predicted yields for DIN, DON, and PN (Fig. 9.4) (Seitzinger *et al.*, 2005b). Conversely, arid regions such as the southwestern US, Northern Africa, and Australia all have low predicted yields for all N forms.

Substantial differences in the magnitude of export of the N forms are observed in many regions. For example, regions with high rates of human activity such as the NE US, Western Europe, and China all have high predicted rates of DIN export, but relatively lower predicted rates of DON and PN export (Fig. 9.4). South Asia and Southeast Asia have high predicted rates of DIN and PN export, but generally lower
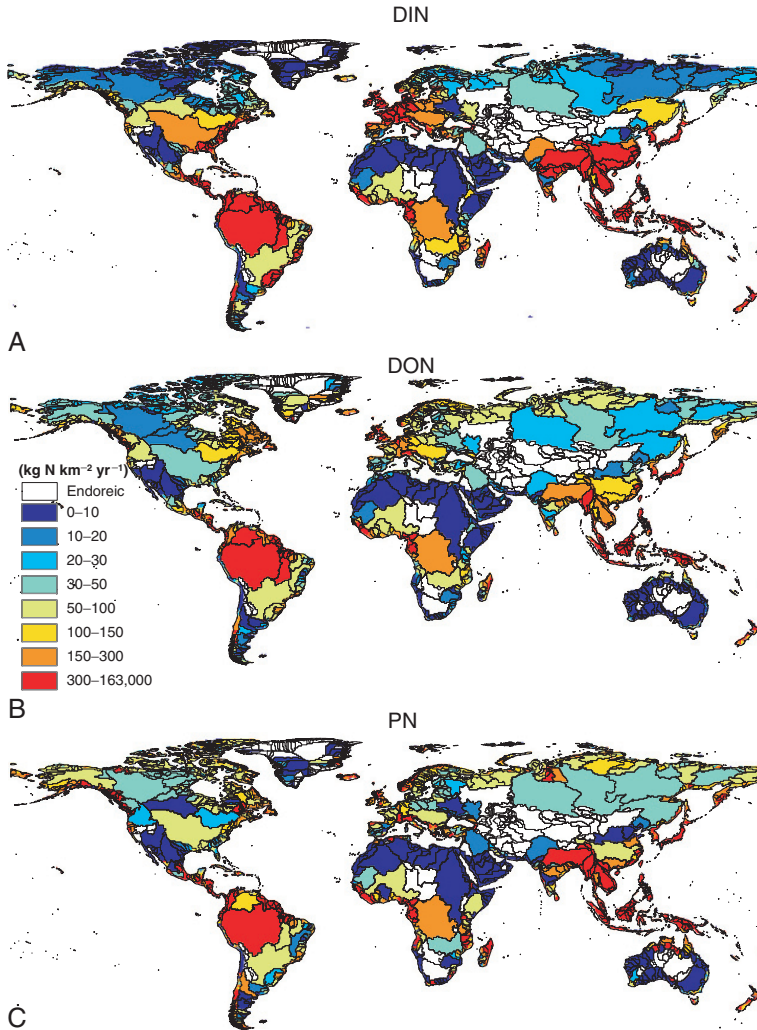
Figure 9.4 NEWS–model–predicted (A) DIN, (B) DON, and (C) PN yield (kg N km^{-2} yr^{-1}) for 0.5 × 0.5 degree (latitude × longitude) or larger river basins globally for mid-1990's conditions. Model output replotted from Beusen *et al.* (2005), Dumont *et al.* (2005), and Harrison *et al.* (2005b).

predicted rates of DON export. High predicted DIN yields in these regions are likely due to the high rates of human activity in these regions and the high predicted PN yield is likely due to the mountainous nature of the region. Basins where DON constitutes the largest pool of exported N are widely distributed, with a slight tendency to occur more frequently in northern boreal basins (Figs. 9.4 and 9.5).

When we aggregate predicted N export by latitude, several interesting patterns emerge. DIN constitutes the largest single form of N exported to coastal zones in north temperate regions, but PN constitutes the largest fraction of exported N from the rest of the world's latitudinal regions (Fig. 9.5; Table 9.2). The greatest fraction
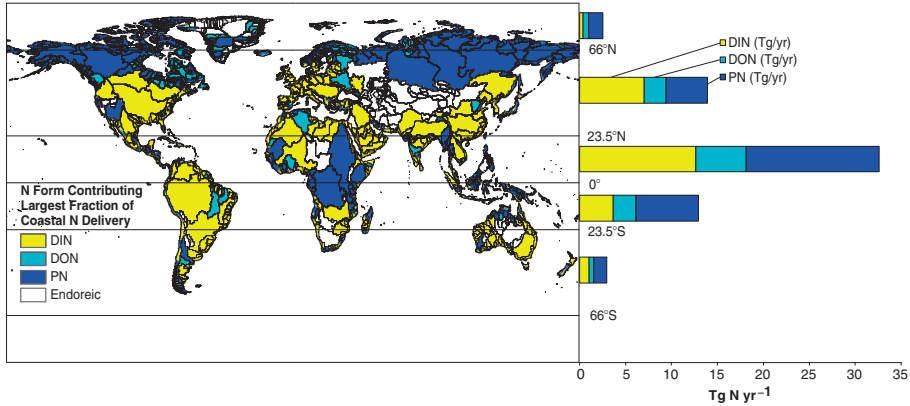
Figure 9.5 DIN, DON, and PN export to the coastal zone by latitude (mid-1990s). Dominant forms are shown by basin in map, and magnitudes of coastal TN export are represented by size of bars to right of map. Global map has been slightly distorted to make room for bar graph to right. Model predictions taken from Beusen *et al.* (2005), Dumont *et al.* (2005), and Harrison *et al.* (2005b).

Table 9.2 DIN, PN, DON, total N (TN) export (Tg N yr^{-1}) by rivers, and exoreic land surface area (10^6 km^2) for tropical (0°–23.5°), temperate (23.5°–66°), and polar (66°–90°) latitude bands and global totals, based on Global NEWS model predictions for mid-1990s (for spatially explicit representation of data, see Figs. 6.4 and 6.5)

Latitude band	DIN (Tg N yr^{-1})	DON (Tg N yr^{-1})	PN (Tg N yr^{-1})	Total N (Tg yr^{-1})	Surface area (10^6 km^2)
Arctic (66°N–90°N)	0.4	0.6	1.6	2.5	17.0
North Temperate (23.5°N–66°N)	7.0	2.8	4.5	13.9	43.8
North Tropical (0°–23.5°N)	12.6	5.7	14.5	32.6	27.6
South Tropical (0°–23.5°S)	3.7	2.5	6.8	12.9	15.0
South Temperate (23.5°S–66°S)	1.0	0.6	1.4	2.9	10.8
Totals	24.8	12.1	28.6	64.9	114.1

of exported N occurring as DON is in arctic latitudes, where approximately a quarter of the exported N is DON. DON constitutes a somewhat smaller proportion of N exported from other latitudinal bands (17–20%). Although most of the exoreic land surface area is located in the north temperate latitude band, the greatest rates of N export for all N forms occur in the north tropical latitude band (Table 9.2; Figs. 9.4 and 9.5). This is due largely to the high rates of water runoff in tropical

regions, though in many regions high levels of human activity also play an important role (Seitzinger *et al.*, 2005b). The above discussion of the differences in the spatial patterns of river export of the various N forms demonstrates the need to explicitly include parameterization of the different N forms in watershed N export models.

Where does the river N enter the coastal zone? Some rivers discharge into estuaries while others (primarily large rivers) discharge directly to continental shelves (e.g., Mississippi, Zaire). Of the river N entering estuaries, a portion is permanently removed within the estuary by denitrification or burial, with the remainder exported to continental shelves. How much of river transported N enters estuaries and how much reaches continental shelves? Using NEWS model predictions of global river TN export (DIN + DON + PN = 65 Tg TN yr^{-1}) and arguments presented in Seitzinger *et al.* (2006), we estimate the following distribution. Rivers discharge 50 Tg N yr^{-1} to estuaries and 15 Tg N yr^{-1} (from large rivers) directly to continental shelves (Fig. 9.3). Estuaries export 38 Tg N yr^{-1} of their river N to continental shelves, bringing total inputs of river N to continental shelves to 53 Tg N yr^{-1}. Therefore, estuaries (directly) and continental shelves (directly plus indirectly) receive about equal amounts of river N. Most of the river transported N does not enter the open ocean, but is denitrified in estuarine and continental shelf sediments (reviewed in Seitzinger *et al.* 2006).

3. Sources of Nitrogen and Factors Controlling Nitrogen Export

Both natural and anthropogenic factors are important in controlling among-watershed differences in DIN, DON, and PN export. Natural N sources include N$_2$-fixed by lightning and biological N$_2$-fixation (Fig. 9.1). Prior to recent anthropogenic increases in N fixation, the amount of naturally fixed N washed into surface waters was likely controlled by a mixture of biological N$_2$-fixation rates and hydrology, with high rates of runoff transferring a relatively large proportion of the naturally fixed N into surface drainage waters. The tight coupling between runoff and N export in relatively unimpacted tropical and temperate rivers supports this conceptual model (Lewis *et al.*, 1999). Major anthropogenic sources of fixed (bioavailable) N inputs to watersheds include inputs of newly fixed N from agricultural sources (inorganic fertilizers and biological N$_2$-fixation by crops) and atmospheric N deposition, as well as recycled N from animal manure, human sewage, and industrial sources.

3.1. Approaches

Nitrogen isotope studies and watershed modeling are two approaches that have been used to identify the contribution of different watershed sources of N to river N export. For example, differences in the δ^{15}N signature of biogenic (plants, soils) and lithogenic (rock) PN has been used to infer that biogenic sources accounted for only ~10% of the total PN exported from the mainstem of the Lanyang-His River in Taiwan (Kao and Liu, 2000). The δ^{15}N signature of nitrate has been used to indicate differences in N

sources in watersheds (e.g., Billen *et al.*, 2005; Böhlke *et al.* 1997; Mayer *et al.*, 2002). For example, increases over time in the δ^{15}N signature of nitrate in groundwater beneath agricultural land were used to relate increases in groundwater nitrate concentration to a change in the sources of N for crops from biological N_2-fixation plus mineralization to primarily synthetic fertilizer (Böhlke 2002). δ^{15}N signatures were used to demonstrate a strong link between N in estuarine organisms and land-based anthropogenic N sources in several shallow coastal systems in the Northeast US (Martinetto *et al.* 2006; McClelland and Valiela, 1998; see also Montoya, this volume).

Another approach to estimating the relative importance of N sources within a watershed entails the use of models. Models have been used to estimate the relative importance of various land-based N sources at basin, region and global scales. Numerous models have been developed for use in individual watersheds (e.g., Alexander *et al.*, 2000; Billen *et al.*, 2001; Donner *et al.*, 2004; Lee *et al.*, 1999; Valiela *et al.*, 2000). Though these models are useful for the watersheds to which they have been tuned, it is problematic to apply these models to address regional and global scale questions as the input data required for these models are often unattainable at regional and global scales. Furthermore, they often require coefficients to be tuned to specific system types through sampling programs that would be resource-prohibitive at the regional or global scale.

Regional scale modeling analyses have been done for 15 watersheds in the NE US (Boyer *et al.*, 2002), 31 watersheds in the eastern and Gulf coasts of the US (Castro *et al.*, 2001), and for the coterminous US divided into 15 regions (Jordan and Weller 1996). These analyses were limited to predicting export of TN only or nitrate only, not individual N forms, and examined the magnitude of various N sources to those watersheds but did not explicitly estimate the contribution of each source to the river export. Due to different rates of retention of N sources in watersheds, relative inputs of different N sources are not equal to their relative contributions to N export. However, insights from these studies are still quite useful. In addition to these regional studies, the recent development of global nutrient export models (such as the NEWS and some of the other models described in section 2) has made it possible to estimate the spatial distribution of the contributions of various land-based N sources to coastal N export globally.

3.2. Patterns

In their regional modeling analysis, Boyer *et al.* (2002) found that TN sources to watersheds varied substantially throughout the NE US. However, averaged across all the 15 study watersheds, atmospheric N deposition constituted the greatest N input (31% of total N inputs); net inputs of food and feed constituted the second largest source of N to these watersheds (25%), followed by agricultural N_2-fixation (24%), fertilizer use (15%), and forest N_2-fixation (5%) (Boyer *et al.*, 2002; Driscoll *et al.*, 2003). Castro *et al.* (2001) found similar patterns for TN sources to NE watersheds, but also found that sources of TN to watersheds differed substantially by region, with N fertilizer being substantially more important in mid-Atlantic, Southeastern, and Gulf Coast watersheds than in Northeast US watersheds.

At the global scale, it is possible to use NEWS models to make several predictions regarding watershed N sources driving the regional patterns of N export. First, human activity is estimated to be responsible for approximately 60% of the global export of DIN to coastal zones and has significantly increased DON export in many regions as well (Dumont *et al.*, 2005; Harrison *et al.*, 2005b) (Fig. 9.6; Tables 9.3 and 9.4). Total anthropogenic point and non-point sources of DON in rivers are estimated to be over three times greater in the tropics (1.85 Tg N yr^{-1}) than temperate latitudes (0.51 Tg N yr^{-1}) and account for a larger proportion of total DON (Table 9.4). Despite the massive scale of human intervention in the N cycle that has altered the input and transport of N by rivers (and the atmosphere) to coastal zones, natural sources still dominate the export (i.e., are the single largest
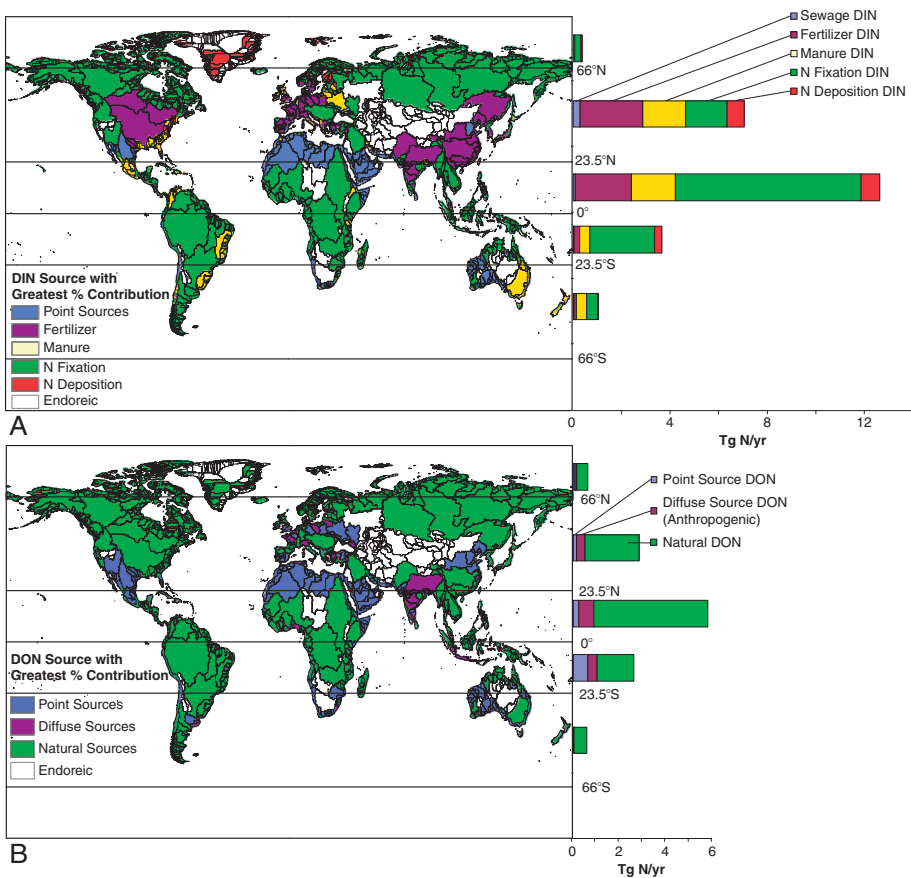
Figure 9.6 Dominant sources of N to the coastal zone by 0.5 × 0.5 degree or larger basin and relative contributions of sources of exported DIN and DON by latitude band as predicted by NEWS-DIN (A) and NEWS-DON (B). Global maps have been compressed horizontally to fit both map and bar graph in same figure. For actual values of predicted DIN and DON, see Tables 9.3 and 9.4. Model predictions have been reprocessed from Dumont *et al.* (2005) and Harrison *et al.* (2005b).

Table 9.3 DIN sources: contribution of N sources in watersheds to the global DIN export by rivers by latitude bands and global total, as predicted by Global NEWS models for mid-1990s (Units: Tg N yr^{-1})

Latitude band	Sewage DIN (Tg N yr^{-1})	Fertilizer DIN (Tg N yr^{-1})	Manure DIN (Tg N yr^{-1})	N$_2$-fixation DIN (Tg N yr^{-1})	N Dep DIN (Tg N yr^{-1})
Northern Polar (66°N–90°N)	0.01	0.01	0.04	0.25	0.05
Northern Temperate (23.5°N–66°N)	0.28	2.59	1.76	1.72	0.70
Northern Tropical (0°–23.5°N)	0.09	2.31	1.82	7.65	0.78
Southern Tropical (0°–23.5°S)	0.03	0.24	0.43	2.67	0.29
Southern Temperate (23.5°S–66°S)	0.03	0.11	0.41	0.45	0.05
Totals	0.44	5.26	4.46	12.74	1.87

Table 9.4 DON sources: contribution of N sources in watersheds to the global DON export by rivers by latitude bands and global total, as predicted by Global NEWS models, for mid-1990's (Units: Tg N yr^{-1})

Latitude band	Point source DON (Tg N yr^{-1})	Diffuse source DON (Tg N yr^{-1})	Natural DON (Tg N yr^{-1})
Northern Polar (66°N–90°N)	0.07	0.07	0.46
Northern Temperate (23.5°N–66°N)	0.13	0.35	2.28
Northern Tropical (0°–23.5°N)	0.23	0.63	4.79
Southern Tropical (0°–23.5°S)	0.61	0.38	1.55
Southern Temperate (23.5° S–66°S)	0.02	0.01	0.54
Totals	1.06	1.44	9.62

source) of DIN, DON, and PN from many watersheds in Arctic regions and in much of South America and Africa (Fig. 9.6). In the case of DON, natural sources still account for approximately 80% of export globally (Harrison *et al.*, 2005b).

Fertilizer is estimated to be the single most important source of exported DIN in north temperate latitudes, contributing 37% of the exported DIN, according to

NEWS-DIN (Fig. 9.6; Table 9.3). N_2-fixation was estimated to be the largest contributor in tropical and Arctic regions, responsible for over half of the exported DIN in each region. N_2-fixation is estimated to contribute the smallest proportion of exported N in the industrialized north temperate range (~24%). Manure was estimated to contribute a relatively small fraction of exported DIN (11–25%) in most latitude bands, but was estimated to contribute a substantial proportion (39%) of the exported DIN in the south temperate range. N deposition was most important in the Arctic, contributing an estimated 14% of the exported DIN, and least important in the south temperate range (5%). When examined at the level of large latitudinal regions, sewage point sources constituted a fairly small fraction of exported DIN and DON (<5% and <10%, respectively). However, in individual basins, sewage point sources can dominate DIN and DON export (Fig. 9.6). Most of the point-source-dominated basins are arid, and therefore have very small predicted rates of diffuse source (natural or anthropogenic) N input (Fig. 9.6).

It is important to note that often only a relatively small percentage of the total N inputs to the landscape are exported by rivers to the coast. Data from a variety of coastal watersheds throughout the world, indicate that, on average, approximately 25% of total net nitrogen inputs from anthropogenic plus natural sources is exported by rivers as TN to the coast (Boyer et al., 2006; Fig. 9.3). Similarly, estimates for watersheds throughout the North Atlantic Basin suggest that about 25% of anthropogenic N inputs are exported by rivers (Howarth et al., 1996). There can be considerable variation among basins in the percentage of N inputs that are exported by rivers. For example, the percentage of N loaded onto basins from point and non-point sources (both anthropogenic and natural) that is exported as DIN ranged from 0.0001% to 43%, based on NEWS-DIN model predictions (Dumont et al., 2005). Rivers which exported the largest fraction of their watershed N inputs had high water runoff (>1 m yr^{-1}); rivers exporting the smallest fractions had extensive damming or very low annual precipitation and runoff. According to NEWS-DIN, in exoreic dry areas of Africa, Asia, Australia, and North America where runoff is less than 0.1 m yr^{-1} (30% of the exoreic world), only 5% (on average) of N applied to watersheds is exported to coastal waters as DIN. Export from these arid systems is predicted to be low because there is very little runoff to transport soluble nitrogen (nitrate) from the soil and unsaturated zone to streams.

A number of caveats must be considered in the discussion of large–scale patterns of N sources. There is considerable variation among watersheds in a region in the relative importance of various N sources. Global models primarily focus on relatively large basins (25,000 km^2). These large watersheds account for ~72% of global water discharge and 79% of exoreic land area and thus likely a large proportion of N export. However, small coastal watersheds drain most of the coastline globally. For example, out of 6152 watersheds identified globally in a simulated topological network (STN-30p), 93% of them are entirely contained in coastal grid cells (0.5° latitude × 0.5° longitude) that are immediately adjacent to an ocean (Vörösmarty et al., 2000). Therefore, while small coastal watersheds may not appear to contribute much coastal N at regional and global scales, they are very important in determining rates and patterns of N delivery to many of the world's estuaries, and thus important in affecting coastal ecosystems and water quality where much of the world's population lives.

4. Temporal Patterns in River Export of Nitrogen

Temporal variability of river N export spans a range of scales from geological (e.g., glacial, inter-glacial) to hourly. On human time scales, long-term trends occur over decades associated with changes in anthropogenic N inputs to watersheds (Meybeck, 2003), inter-annual variability can be associated with differences in weather patterns, and intra-annual variability occurs on seasonal, diurnal and event (e.g. storm) scales. Variability on all of these scales can affect the response of coastal ecosystems to N inputs (Deegan *et al.*, 2002; Grenz *et al.*, 2000; Chapter 11 by Paerl and Piehler, this volume).

4.1. Long-term trends

Long-term (multi-decadal) records of N export from watersheds are rare and generally limited to nitrate (see also Boynton and Kemp, this volume). In general, human-impacted rivers demonstrate higher NO_3^- loads than non-impacted rivers, and the NO_3^- loads increase over time as anthropogenic N inputs to those watersheds increase. For example, an approximately 30-year record for the Yangtze River (Changjiang River), China, demonstrates a more than ten-fold increase in NO_3^- export as human population and agricultural activity have increased in the watershed (Yan *et al.*, 2003) (Fig. 9.7). Several-fold increases in NO_3^- export have also been observed in the Mississippi (Goolsby and Battaglin, 2001), Seine (Billen *et al.*, 2001), Scheldt (Billen *et al.*, 2005), and Susquehanna (Hagy *et al.*, 2004) basins associated with changing anthropogenic watershed activities (Fig. 9.7). In contrast, as expected, relatively stable NO_3^- export is observed in basins with relatively low and stable levels of human activity, such as in the Yukon and S. Dvina Rivers (Alexander *et al.*, 1996; Holmes *et al.*, 2000) (Fig. 9.7).

At the global scale, TN river export to coastal systems is estimated to have approximately doubled between 1860 and 1990, due to anthropogenic activities on land (Galloway *et al.*, 2004). Over the next 50 years the human population is predicted to increase markedly in certain world regions, notably Southern and Eastern Asia, South America, and Africa (United Nations, 1996). Growing food to feed the expanding world population will require increased use of nitrogen and phosphorus fertilizers (Alcamo *et al.*, 1994; Bouwman, 1997; Bouwman *et al.*, 1995). Increased industrialization, with the associated combustion of fossil fuels and NO_x production, is predicted to increase atmospheric deposition of N (Dentener *et al.*, 2006; IPCC, 2001). Thus, unless substantial technological innovations and management changes are implemented, increasing food production and industrialization will undoubtedly lead to increased export of N to coastal ecosystems (Galloway *et al.* 2004), with resulting water quality degradation. For example, inorganic N export to coastal systems is predicted to increase 3-fold by the year 2050 (relative to 1990) from Africa and South America (Fig. 9.8) (Kroeze and Seitzinger, 1998; Seitzinger *et al.*, 2002b). Substantial increases are predicted for Europe (primarily from eastern Europe) and N. America. Alarmingly large absolute increases are predicted for eastern and southern Asia; almost half of the total global increased N export is predicted for those regions alone.
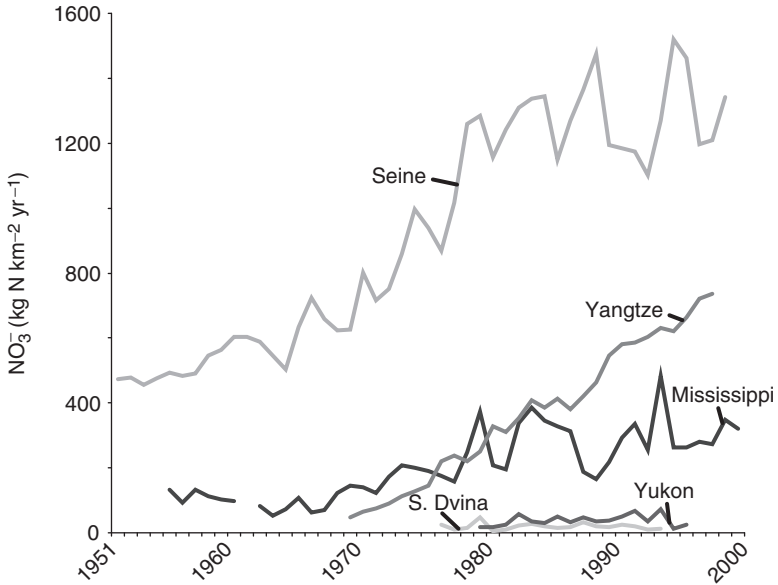
Figure 9.7 NO_3^- yield (kg N km^{-2} yr^{-1}) trends for three human-impacted rivers (Seine, Mississippi, and Yangtze), and two relatively non-impacted rivers (Severnaya Dvina and Yukon). (Alexander *et al.*, 1996; Billen *et al.*, 2001; Goolsby and Battaglin, 2001; Holmes *et al.*, 2000; Yan *et al.*, 2003).
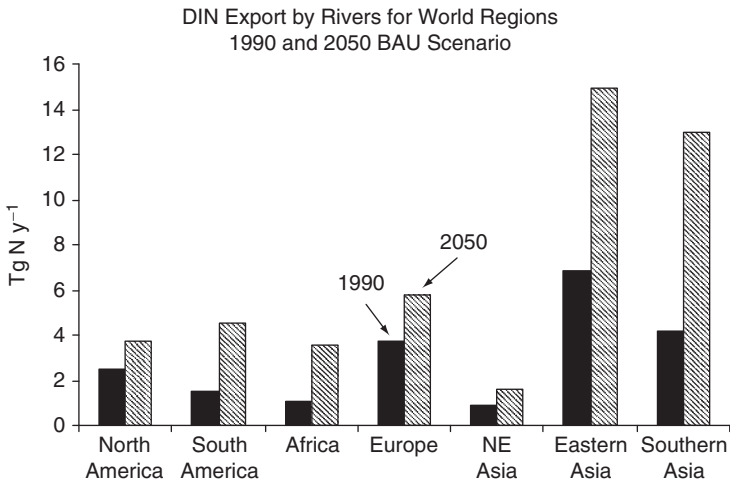
Figure 9.8 Predicted increases in DIN export to coastal systems between the years 1990 and 2050 under a business–as–usual (BAU) scenario. Modified from Kroeze and Seitzinger (1998).

Increases in river N export over time often correlate relatively well with increases in N inputs to watersheds. However, the response of river N export to *decreases* in watershed N inputs is not necessarily as rapid or well understood. In at least one case, the Patuxent River (MD, USA), point source reductions in N resulted in rapid reductions in N export (Boynton *et al.*, 1995; Jordan *et al.*, 2003). However, N export is generally dominated by non-point sources, not point sources (Carpenter *et al.*, 1998; Dumont *et al.*, 2005; Seitzinger *et al.*, 2005b; others), so substantially reducing N export often requires a reduction in non-point N inputs. Reductions of non-point nutrient inputs have been attempted in a number of systems, but response of river N export to these reductions is often much slower and less predictable than responses to reductions in point sources (Stålnacke *et al.*, 1999).

The complex response of N export to nonpoint reductions is well illustrated by Eastern European rivers. Despite a drastic reduction in fertilizer use and animal production as a result of the economic decline in the former Soviet Union and in Eastern Europe in the early 1990's, DIN concentrations showed statistically significant downward trends in only 4 of 12 sites studied in Latvian Rivers (study period 1987–1998) (Stålnacke *et al.*, 2003). No decreases were reported in nitrogen export from major Eastern European rivers such as the Vistula and Oder Rivers in Poland (1989–1995) (Stålnacke, 2005) or the Danube River (at Reni; 1995–2000) (Behrendt *et al.*, 2005a). Only moderate decreases in TN or nitrate export by the Elbe River to the North Sea were measured (1985–2000) (Hussian *et al.*, 2004). A number of factors have been proposed to explain the weak response of river N export to decreases in diffuse sources of N in the watersheds, including the long-water transit times of water in soils and in aquifers and continued mineralization of the large pools of organic N which have accumulated over time in soils. The above examples demonstrate the uncertainty in our knowledge of the response of river N export to decreases in N inputs to watersheds after prolonged periods of high inputs and the need for future research.

4.2. Interannual variability

In addition to long-term trends, there is also substantial inter-annual variation in N export (Fig. 9.7). There are several potential causes for this inter-annual variability, including: year-to-year variation in climate, variation in N inputs, changes in land-use, or changes in the relative timing of N application and runoff events. For example, inter-annual variation in climate is a major factor affecting river water discharge, and discharge is an important variable controlling non-point N (natural or anthropogenic) export. This is observed in the long-term pattern (1945–2001) of nitrate export by the Susquehanna River to Chesapeake Bay which was strongly affected by inter-annual and inter-decadal differences in river discharge (Hagy *et al.*, 2004). Similarly, in the Odra River inter-annaual variation (1960–2000) in DIN load and discharge showed similar patterns (Behrendt *et al.*, 2005b). It is not clear whether the positive relationship between discharge and N export is simply due to increased hydrologic connectivity between potential N sources and flowing surface waters under wetter conditions or whether higher discharges also reduce N removal by denitrification in the river and on the landscape. The overall effect of water

discharge on DIN, DON, and PN export is indicated by the importance of water discharge as an explanatory variable in models that predict N export (TN as well as each of the N forms) across a wide range of rivers/watersheds globally (Caraco and Cole, 1999; Dumont et al., 2005; Harrison et al., 2005b; Smith et al., 1997; Smith et al., 2003). This is consistent with analysis of data from 16 major watersheds in the Northeastern US in which watersheds with higher discharge exported a greater fraction of the net anthropogenic N inputs (Howarth et al. 2006). Future changes in water discharge in various world regions are predicted in response to changing climate (due to natural and anthropogenic effects). Already, increases in water discharge in the 6 largest Eurasian Arctic rivers which appear to be related to global warming have been documented (7% from 1936 to 1999) (McClelland et al., 2004; Peterson et al., 2002); the effect of such changes in river flow on N export in these rivers is under investigation. A preliminary estimate of the effect on TN export of increases in precipitation and discharge due to future climate change indicated that TN export by the Susquehanna River to Chesapeake Bay might increase by 3–17% by 2030 and by 16–65% by 2095 (Howarth et al., 2006).

It is possible to examine the relationship between climate and N export by de-trending long-term data and evaluating the strength of the relationship between de-trended N export and water discharge via regression analysis. When this analysis is done, we find that discharge exerts a relatively strong effect on NO_3^- export in some rivers, explaining approximately half of the variability in NO_3^- export from the Mississippi and Yukon River basins. This is similar to what others have found for the Mississippi using other approaches (Goolsby and Battaglin, 2001). However, curiously, in other rivers (e.g. the Yangtze, Seine, and Severnaya Dvina), there is little or no detectable relationship between water discharge and NO_3^- export through time, even when changes in N inputs are accounted for.

4.3. Seasonal variability

The response of coastal ecosystems to river nutrient inputs depends, in part, on the seasonal pattern of the N delivery, including seasonal changes in the form of N delivered (e.g., DIN, DON, and PN). Most studies of river nutrient export only report annual rates and often only report TN export. Where seasonal patterns of N export by individual river basins have been reported, a number of patterns have been observed, and the different forms of N can exhibit different patterns of export (e.g., Alexander et al., 1996; Eyre and Pont, 2003; Lesack et al., 1984) (Figs. 9.9 and 9.10). Seasonal patterns are observed in both N concentration and in N export (Fig. 9.10).

One might expect that river nutrient export from point sources would show a constant export rate, with concentration varying inversely with discharge, while rivers dominated by diffuse N sources would show an export rate more directly related to discharge with less variation in concentration with discharge (Billen et al., 2005). However, to our knowledge this dynamic has not yet been demonstrated across a broad range of rivers, or with consideration of the N form. Seasonal patterns of river nutrient export may be complicated by seasonal variations in biological retention (plant uptake) or removal (e.g. denitrification) within soils or within the
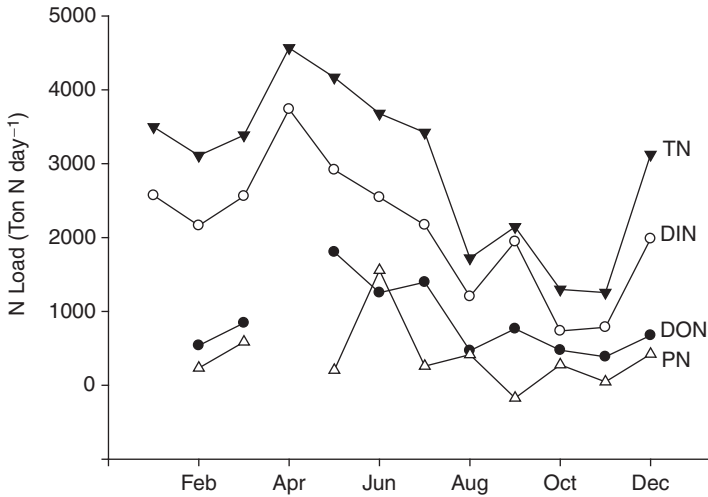
Figure 9.9 Mean annual N load for the Mississippi River at Belle Chase, LA (1979–1981), by N form, including total nitrogen (closed triangles), dissolved inorganic nitrogen (open circles), dissolved organic nitrogen (closed circles), and particulate nitrogen (open triangles) (Alexander *et al.*, 1996). Units: metric Ton N day^{-1}.

river network. Extremes of river discharge with season, such as occur in monsoonal regions, would be expected to greatly affect river N export. The natural seasonal cycle of N export can also be markedly dampened by dams such as demonstrated in California's Mokelumne River, as compared to a similar, but undammed neighboring river (Ahearn *et al.*, 2005). In this case, dams shifted the pulse of nitrate export from winter to summer, and distributed the winter pulse of TN evenly throughout the year.

Despite a general lack of synthetic understanding regarding seasonal dynamics of N export from rivers, some initial work has been done at the global scale to estimate seasonal patterns of TN export by world rivers. Green *et al.* (2004) simply distributed annual river TN export for individual rivers based on the pattern of monthly water discharge (Fig. 9.11). This approach suggests that Arctic rivers exhibit the largest TN export during the summer (June–August). Many Northern Hemisphere temperate latitude rivers are predicted to have highest TN export during winter (December–February) or spring (March–May), according to this approach. A comparison of the seasonal pattern of model predicted TN export for the Mississippi River (Fig. 9.11) with measured export (Fig. 9.9) suggests that, at least for this river, discharge alone can capture the major patterns in seasonal variation of TN export. Additional analyses we have conducted for the Sacramento, San Joaquin, and Yukon Rivers are consistent with the above, suggesting that, while the absolute magnitude of N export is highly dependent upon watershed N inputs, the overall seasonal patterns in TN export can be relatively well predicted from the seasonal pattern in river water discharge. However, the pattern in other rivers is not as straight forward and will require more comprehensive analyses.
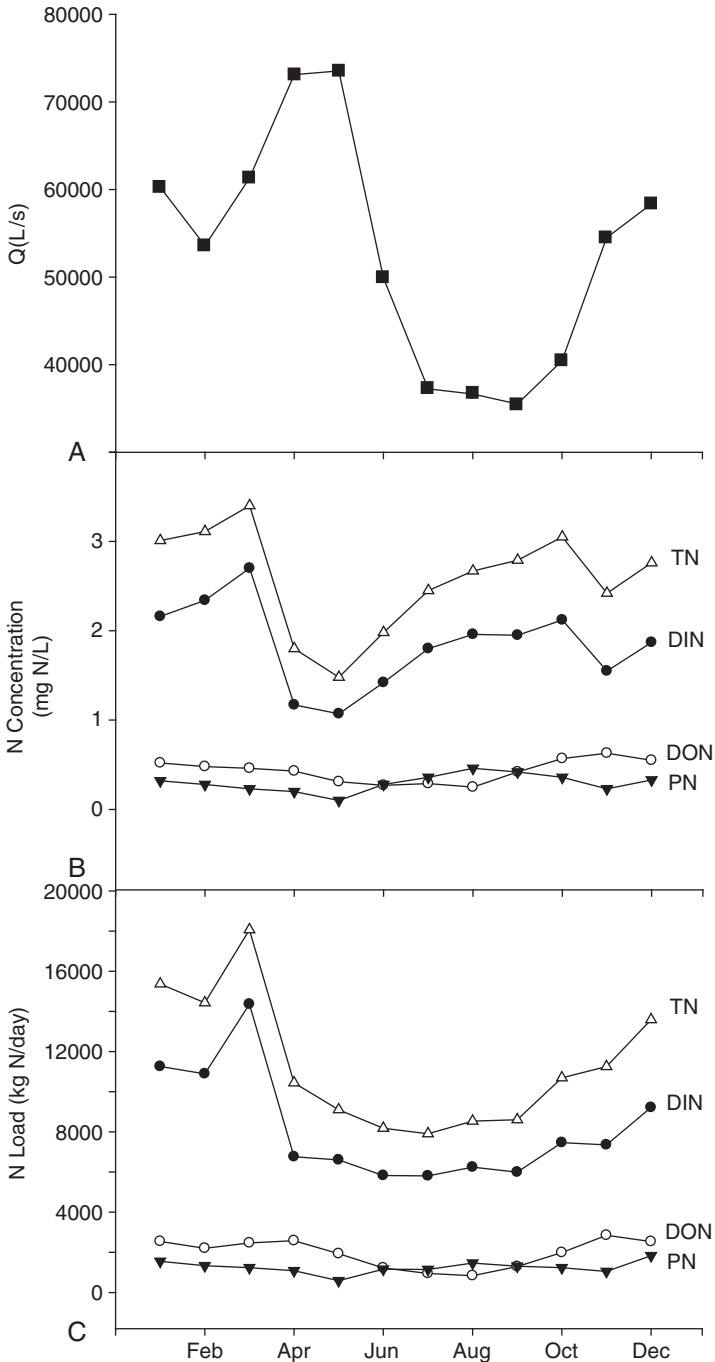
Figure 9.10 Seasonal discharge and N dynamics in the San Joaquin River (California, USA). Monthly average discharge (A), DIN (closed circles), DON (open circles), TN (open triangles), and PN (closed, inverted triangles) concentrations (B), and loads (C) are shown. Data from 2001–2002 (Kratzer *et al.*, 2004).
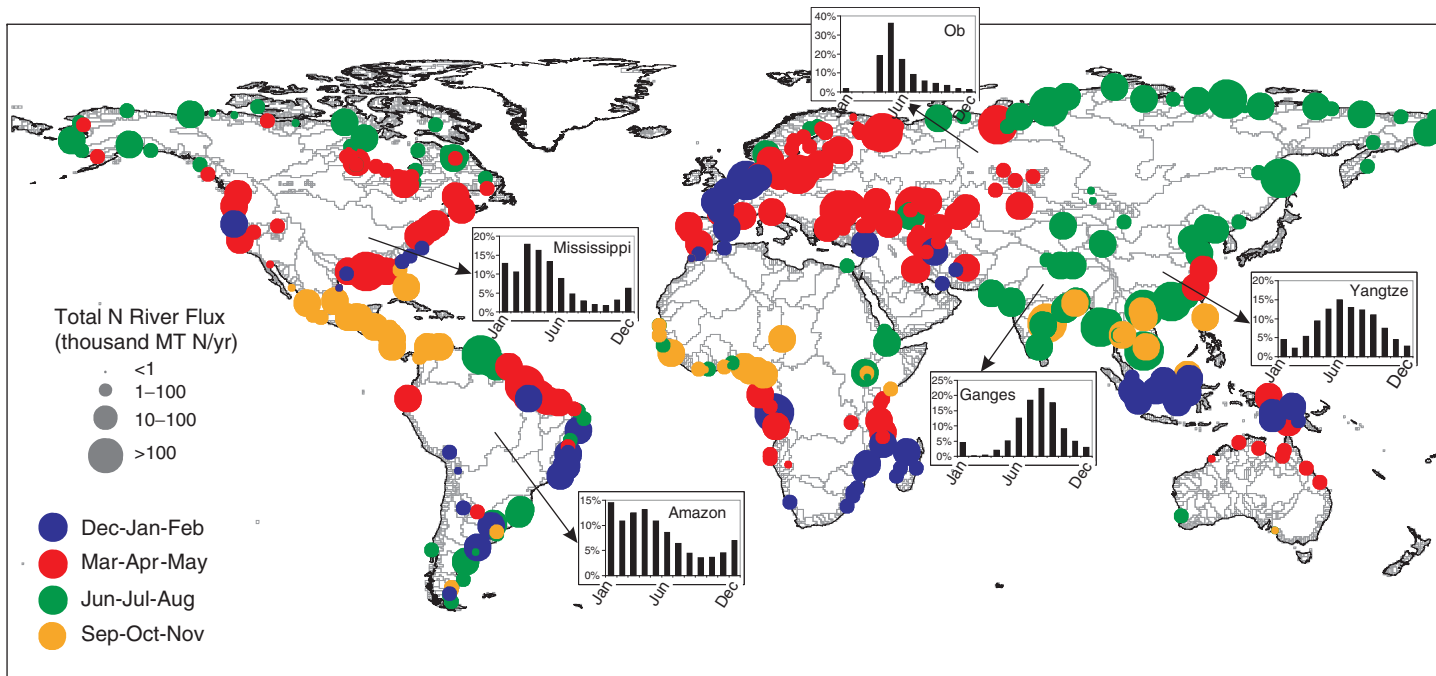
Figure 9.11 Global seasonal patterns of N export as predicted using hydrology alone. Hydrographs of five large, representative rivers are shown. Reprinted with permission from Green *et al.* (2004).

4.4. Diel variability

Shorter term variations in nutrient export due, for example, to diel variations in N processing, can also be significant and may affect coastal ecosystem processes. A study in the highly productive, subtropical Yaqui Valley region in Mexico suggests that night-time anoxia can shut down nitrification and coupled nitrification–denitrification, thereby substantially changing the amount and form of exported N on a time-scale of hours (Harrison *et al.*, 2005c). Similarly, a study of three rivers in the Midwest US showed a decrease in denitrification during the night (Laursen and Seitzinger 2004). The San Joaquin River, a highly productive river in California, also exhibits pronounced diel fluctuations in N concentrations and dynamics (Dahlgren, personal communication). In the case of the Mexican stream, the authors estimated that failing to measure night-time N fluxes would lead to a 17–38% underestimate of N export during their 24-h study period (Harrison *et al.*, 2005c). However, understanding the impact of diel variation in N cycling on longer term (seasonal and annual) patterns and magnitudes of N flux will require additional work. Also, the effects of diel variation on downstream ecosystems have yet to be explored.

4.5. High intensity, low frequency events

Tropical storms, typhoons, or hurricanes, depending on their strength and geographical context, often deliver torrential rains. Increased river flow during such high intensity, low frequency rainfall events can transport a major portion of annual river N load to coastal systems. Historically, there has been a paucity of data on such events due to their irregular and extreme nature and the generally manual nature of water sample collection for nutrient analyses. However, with the use of *in situ* automatic water samplers and nutrient analysis systems, data from such events can now more readily be captured.

Large increases in river nutrient export associated with hurricanes have been documented in rivers in the Chesapeake Bay watershed and in the Neuse River watershed (North Carolina, USA) (Chesapeake Research Consortium, 1976; Paerl *et al.*, 2005). In years with hurricanes that travel inland and deposit large amounts of rain (e.g., hurricanes Fran 1996 and Floyd 1999), the annual DIN load to Pamlico Sound from the Neuse River can be twice as large as during non–hurricane years (Paerl *et al.*, 2005 Chapter 11 by Paerl and Piehler, this volume). DON load is also elevated (Paerl, pers comm.). In the Lanyang-His in Taiwan, a small subtropical river with a forested watershed, as much as 90% of the annual load of suspended matter can be transported during a single typhoon, although water discharge is often <10% of the annual total runoff (Kao and Liu, 1996, 2000).

In arid regions the majority of water and nutrients in un–dammed rivers can be exported during a few major storms. In a study of 7 subtropical, Australian rivers, approximately 75% of the annual TN load was delivered in 20% of the time (two 10-day flood periods during 1996; Eyre and Pont, 2003). This contrasts with a number of temperate systems in which it takes 23–52% of the time to deliver 75% of the annual TN load (Eyre and Pont, 2003). Different N forms also exhibited different patterns of export in this study of Australian rivers, with DON typically accounting

for a relatively constant proportion (60–80%) of the total N load in each non–flood month and PN increasing in importance during flood months (Eyre and Pont, 2003).

5. EFFECTS ON NITROGEN EXPORT OF LONG-TERM HUMAN MODIFICATION OF DISCHARGE

Human modification of water discharge associated with dams and consumptive water use can greatly decrease river N export. Both dissolved and particulate N forms are affected. Dams trap particulates and also can increase water residence time, which can increase the proportion of N inputs that are denitrified or buried. Consumptive water use (e.g. irrigation) transfers N out of the river system to terrestrial soils where it can be assimilated by crops, fixed by soils, or denitrified, and therefore not transported downstream. Consumptive water use also can influence PN export by decreasing water flow, which increases suspended sediment trapping within the river system.

Over 45,000 dams at least 15 m high are in operation today around the world (Vörösmarty et al., 2003; World Commission on Dams, 2000). There are many additional smaller dams. For example, there are more than 79,000 registered dams in the US alone (National Inventory of Dams), and an estimated >500,000 globally (Downing et al., 2006). While soil erosion due to anthropogenic activities has increased sediment transport by global rivers by over 2 billion metric tons yr, the amount of sediment reaching the world's coast has decreased by about 1.4 billion metric tons, due to retention in reservoirs (Syvitski et al., 2005). Globally, trapping of suspended sediments behind dams is estimated to have decreased the modern sediment transport to coastal systems by about 26% (Syvitski et al., 2005; Vörösmarty et al., 2003). PN data are often not available for pre and post dam conditions. However, there is a reasonably strong, non-linear relationship between total suspended sediment (TSS) and PC, PN, or PP based on data from a wide range of world rivers, so that PN and PC can be estimated based on TSS export (Beusen et al., 2005; Ittekkot and Zhang, 1989). Globally, approximately 4–7 Tg yr^{-1} of PN are estimated to be buried with sediments in large reservoirs alone (about 600), which is equivalent to a reduction of 14–23% of the current global export of PN (Beusen et al., 2005; Seitzinger et al., 2002b). Substantial additional PN is undoubtendly trapped in the tens to hundreds of thousands of small reservoirs around the world (Smith et al., 2001).

Dams can increase water residence time in a river, thereby increasing denitrification and decreasing N export to coastal systems (Dumont et al., 2005; Seitzinger et al., 2002c). Globally, denitrification is estimated to remove about 31 Tg N yr^{-1} in lakes and reservoirs, with about 11 Tg N yr^{-1} in large lakes and reservoirs and 20 Tg N yr^{-1} in small lakes (Seitzinger et al., 2006). These estimates are associated with considerable uncertainty, but (given that global N export by rivers is about 60 Tg yr^{-1}; Bouwman et al., 2005; Boyer et al., 2006; Seitzinger et al., 2005b) suggest that human modification of rivers may significantly impact river N export.

The degree to which dams increase N removal by denitrification in a given river depends on a number of factors, such as placement of dams within the watershed, reservoir water residence time, reservoir depth, and the proportion of the river N that passes through reservoirs. Analysis of 16 rivers in the Northeastern US suggested that while any one reservoir could remove a substantial portion of the N input to that reservoir, at the scale of the whole river, the total amount of N removed in all dams within a watershed decreased the export of N to the coastal zone by less than 10% (Seitzinger *et al.*, 2002c). In general, a relatively small proportion of the N inputs to those rivers passed through reservoirs. However, reservoirs with long water residence times, which intercept a large proportion of a basin's water and N load, can have a marked effect on export of N to coastal systems. The Nile provides a dramatic example. Nitrate export is estimated to have decreased by more than a factor of ten due to closure of the high dam at Aswan in 1965 (Nixon, 2003). Denitrification in reservoirs is predicted to also have a marked effect on N export in a number of other rivers heavily influenced by dams such as the Colorado, Rio Grande, Orange, and Huang He (Dumont *et al.*, 2005). Interestingly, N export by the Nile is estimated to now exceed the pre–dam export due to increased fertilizer use, changes in agricultural draining, increasing human population, and marked extensions of urban water and wastewater systems, and the Nile delta fishery now surpasses that immediately preceding the dam (Nixon, 2003).

6. Groundwater

Submarine groundwater discharge (SGD) is a potentially important but as yet poorly quantified source of nutrients to coastal systems (Burnett *et al.* 2003; Slomp and Van Cappellen, 2004). The discharge of groundwater into the sea has been known since Roman times, when offshore springs were used as a source of freshwater (reviewed by Burnett *et al.*, 2003). However, recognition of the potential importance of SGD as a source of nitrogen to coastal systems has been relatively recent (e.g., Burnett *et al.*, 2003; Capone and Bautista 1985; D'Elia *et al.*, 1981; Johannes, 1980; Slomp and Van Cappellen, 2004; Valiela and D'Elia, 1990; Valiela *et al.*, 2002). Coastal systems in which SGD is an important source of N tend to be those characterized by shallow, permeable (sand, limestone) coastal aquifers with high rates of groundwater recharge (Slomp and Van Cappellen, 2004).

Nitrogen concentrations in submarine groundwater are influenced by many of the same natural and anthropogenic watershed characteristics that control river water concentrations. A model that predicts total dissolved N loads in groundwater to shallow estuaries for watersheds underlain by unconsolidated sands, has been developed and successfully applied to a number of systems on Cape Cod, MA (USA) (Valiela *et al.*, 2000, 2002). Inputs to the model include N in wastewater (via septic systems), fertilizer use, and atmospheric deposition. Quantifying N inputs to coastal waters, however, is complicated by high spatial and temporal variability. In addition to geologically determined preferential flow-paths, large changes in nitrogen concentrations can occur during groundwater movement through the aquifer and

coastal sediments. In particular, groundwater nitrate can be removed by denitrification, and diagensis of organic matter in the coastal sediments can add nitrogen or change the composition of nitrogen species in the groundwater (Capone and Slater, 1990; Nowicki *et al.*, 1999; Valiela *et al.*, 1990).

Despite difficulties, there are a number of studies at both local and regional scales in which SGD of nitrogen (generally only nitrate measurements) has been quantified within the context of whole system N budgets. SGD has been shown to be an important N source in some coral reefs areas (e.g., Corbett *et al.* 1999; D'Elia *et al.* 1981; Umezawa *et al.* 2002), shallow coastal lagoons and bays (Capone and Bautista 1985; Capone and Slater, 1990; Gobler and Boneillo, 2003; Moser, 1997; Valiela and D'Elia, 1990; Valiela *et al.*, 1990; 2002), and salt marsh dominated systems (Krest *et al.*, 2000). In a number of systems, N inputs from SGD equal or exceed river inputs (Table 9.5).

At regional scales, there are also examples in which SGD of nitrogen equals or exceeds river fluxes. For example, DIN fluxes in groundwater discharges through salt marshes along the South Carolina coast are estimated to be equivalent to input from major rivers in this region ($\sim 60 \times 10^6$ mol yr^{-1}) (Krest *et al.*, 2000) (Table 9.5).

Regional and global scale budgets of SGD and nutrients are very difficult to formulate with confidence. However, most estimates of SGD at the global scale range from about 6–10% of surface water inputs (reviewed by Burnett *et al.*, 2003), with large variations in time and space. If we assume that the water budget is a rough proxy for dissolved N inputs, then SGD could transport approximately 4 Tg N yr^{-1} to coastal systems (10% of global river DIN plus DON transport). While at the global scale, SGD may be a relatively small source of N to coastal systems, it is becoming

Table 9.5 Comparison of estimated N fluxes through submarine groundwater discharge (SGD) relative to river fluxes (modified from Slomp and Van Cappellen, 2004; sites with relatively large SGD inputs relative to river inputs were specifically selected for this table)

Location	SGD N/ river N	Reference
Coastal bays, New England	2:1–32:1	Valiela *et al.* (1990)
Perth Region, West Australia	3:1–5:1	Johannes (1980) and Johannes and Hearn (1985)
Kahana Bay, Hawaii	2:1	Garrison *et al.* (2003)
Florida Bay	$\sim$1: $\sim$1	Corbett *et al.* 1999
Turkey Pt., St. Joseph Bay, Florida	1:1	Rutkowksi *et al.* (1999)
S. Carolina, salt marsh input	1:1	Krest *et al.* (2000)
S. Carolina, incl. offshore input	3.6:1	Moore *et al.* (2002)
Tomales Bay, CA, summer	7:1	Oberdorfer *et al.* (1990)
Tomales Bay, CA, winter	1:3.4	Oberdorfer *et al.* (1990)
Kashima Sea, Japan	1:420	Uchiyama *et al.* (2000)
Yeoja Bay, Korea	14:1	Hwang *et al.* (2005)
Barnegat Bay, NJ	1:7	Moser (1997)

increasingly clear that at local and regional scales it can be a very important term in some cases (Table 9.5). There is a need for greater understanding of SGD as a coastal N source and for the formulation and application of spatially explicit models of groundwater N inputs.

7. ATMOSPHERIC DEPOSITION DIRECTLY TO COASTAL WATERS

Atmospheric N deposition can be an important source of N to coastal and open ocean ecosystems. The potential importance of N in atmospheric deposition has been recognized for over twenty years (e.g., Correll and Ford, 1982; Duce, 1986; Paerl, 1985). Recognition of the importance of atmospheric deposition as a source of N to coastal waters increased rapidly following the analysis by Fisher and Oppenheimer (1991) for a number of coastal systems, including Chesapeake Bay. Atmospheric deposition to watersheds contributes to diffuse N loads in rivers as discussed previously in this chapter. In this section we are specifically referring to N deposited (wet and dry) directly to the surface of coastal and open ocean waters.

N in rainwater includes both DIN (nitrate, nitrite, ammonium) and DON. Gas phase N species in the atmosphere contributing to dry deposition include ammonia, nitric acid and organic N compounds. Particulate N can also be associated with dust and organic debris, and deposited either as dry deposition or with rainfall. Globally, anthropogenic sources account for over 70% of NOx and 65% of ammonium deposition (Galloway et al., 2004). Sources of organic N compounds are not well known although a significant fraction (up to 80%) is available to plankton communities as a nitrogen source (Peierls and Paerl, 1997; Seitzinger and Sanders, 1999; Timperley et al., 1985).

In some estuaries and coastal embayments, atmospheric deposition directly to the water surface can account for a substantial fraction of N input (as much as 40% of the N inputs from river plus atmospheric inputs; Table 9.6). However, the relative importance of atmospheric deposition as an N source varies considerably among coastal systems and depends on a number of factors, including the nature of watershed N sources and the relative sizes of the contributing watershed and receiving estuary (Valigura et al., 2001).

In continental shelf regions, there is also a wide range in the relative magnitude of atmospheric N deposition. For example, in North Atlantic shelf regions, direct atmospheric deposition can account for from as little as 5% to more than half of the land-based N input (rivers plus direct atmospheric deposition; Table 9.6). The proportion of land-based N inputs that are from atmospheric deposition depends not only on the magnitude of river N export, but also the area of shelf over which atmospheric N is deposited (Nixon et al., 1996; Seitzinger and Giblin, 1996). At the global scale, atmospheric N deposition to continental shelves ($\sim$8 Tg N yr^{-1}) is estimated to account for about 17% of total N inputs from land-based sources (Table 9.6). This is based on estimated N inputs to global continental shelves from rivers that includes river N exported from estuaries and N delivered from large rivers that discharge directly to shelves (Fig. 9.3 and Seitzinger et al., 2006). These atmospheric deposition estimates do

Table 9.6 Proportion (as a percent) of N inputs to estuaries, bays and continental shelves from atmospheric deposition *directly to the water surface* compared to river plus atmospheric inputs

Receiving waters	Percent DIRECT atmospheric N to water surface (%)	Reference
Estuaries and bays		
Narragansett Bay, RI[a,b]	5	Nixon 1995
Barnegat Bay, NJ[b,c]	40	Moser 1997
Chesapeake Bay[a,d]	8	Castro *et al.* 2001
Casco Bay, ME[a,d]	22	Castro *et al.* 2001
Buzzards Bay[a,d]	19	Castro *et al.* 2001
St. Catherine-Sapelo, GA[a,d]	37	Castro *et al.* 2001
Tampa Bay, FL[a,d]	4	Castro *et al.* 2001
Neuse River Estuary, NC[b,c]	5	Whitall and Paerl 2001
Continental shelves		
Global shelves[a,e]	17	Seitzinger *et al.* 2006; This chapter
North Atlantic Shelf[a,e]		
Western 45°–66°N	29–50	Nixon *et al.* (1996);
20°–45°	17–21	Prospero *et al.* (1996)
0°–20°	5 (incl. Amazon)	
Eastern 45°–66°N	40–55	
20°–45°	17–29	
0°–20°	3–5	
Open Ocean	17–51	This chapter
Global Coastal and Open Ocean	15–42	This chapter

[a] Wet and dry deposition.
[b] Inorganic and organic N.
[c] Wet deposition only.
[d] Nitrate only.
[e] Inorganic only.
For open ocean regions atmospheric deposition is calculated as a percent of biological N_2-fixation plus atmospheric deposition, assuming river N inputs are removed within coastal and shelf sediments. For continental shelves the range includes uncertainties in river N inputs; for open ocean estimates, the range is that calculated with and without including DON in rainwater. See Fig. 6.6 for the contribution of atmospheric deposition in watersheds to river N export.

not include DON, as we know of no measurements for DON in rainwater collected directly from continental shelf locations. However, we estimate that DON may comprise anywhere from 20% to 80% of the total dissolved N in rainwater (Cornell *et al.*, 1995, 2001, 2003; Mace *et al.* 2003; Valigura *et al.*, 2001).

Open ocean regions receive significant amounts of land-based N from atmospheric deposition ($\sim$25 Tg N yr^{-1} Dentener pers. comm.). This estimate of atmospheric deposition does not include dissolved organic nitrogen (DON), which may account for from 20% to as much as 80% of the total dissolved N in rainwater in remote marine locations (Cornell *et al.*, 1995, 2001, 2003; Mace *et al.* 2003). Keeping in mind that this estimate of DON is uncertain because of the

limited number of measurements, including DON could increase current estimates of N deposition to oceanic regions by a factor of 1.25–5 or to 31–125 Tg N yr^{-1}, increasing it to the range of estimated oceanic biological N_2-fixation (57–152 Tg yr^{-1}; Mahaffey *et al.* 2005; Chapter 4 by Carpenter and Capone, this volume).

8. Summary and Future Directions

In the past several decades, considerable progress has been achieved toward a comprehensive understanding of land–based N inputs to coastal waters (estuaries, bays and continental shelves). Local studies of patterns and controls of N export have been carried out and synthesized. These syntheses have, in turn, been used to develop spatially explicit regional and global models that can be used to estimate the patterns and magnitudes of N input to coastal ecosystems, as well as the N sources in watersheds and other factors controlling N export. We now have reasonable, though still quite uncertain, estimates of land–based N inputs to coastal waters globally via a number of different pathways, including rivers ($\sim$60 Tg N yr^{-1}), submarine groundwater discharge (roughly 4 Tg N yr^{-1}) and direct atmospheric deposition ($\sim$8 Tg N yr^{-1}). A better understanding of the magnitude of transport of different forms of N is also developing. Globally, DIN and PN each account for approximately 40% of river transported N is in the form of DIN and PN, with DON comprising approximately 20%.

There is substantial variability in the magnitude of N inputs to coastal regions, as well as the contribution of different sources driving that export, that is starting to be addressed by spatially explicit models. There are now many watersheds throughout the world with high yields (i.e., kg N exported by rivers to the coastal zone km^{-2} watershed yr^{-1}). Some of the highest TN yields occur in watersheds in Europe and southern and eastern Asia. These are watersheds with high anthropogenic N inputs and N export from these systems is dominated by DIN, with fertilizer being the single largest N source of that DIN. Anthropogenic factors, however, are not always responsible for high rates of N yield from watersheds, and high yields are not always dominated by DIN. Watersheds with high water runoff, such as the Amazon and Congo, are estimated to have high yields of all N forms (DIN, DON, and PN). In much of Oceania, a combination of high water runoff and anthropogenic activity lead to high yields of all N forms. Throughout most of the world, non-point sources (natural and/or anthropogenic) dominate N export, regardless of N form. This includes fertilizer which is estimated to be the single most important source of exported DIN in many north temperate latitude watersheds; manure is estimated to contribute a substantial portion of the exported DIN in a number of watersheds scattered throughout temperate and tropical latitudes. Except for some watersheds in the Eastern United States, atmospheric deposition does not appear to be the dominant N source driving DIN export. Despite the massive scale of human intervention in the N cycle which has affected transport of N by rivers, natural sources (N_2-fixation) are still the single largest contributor of DIN, DON, and PN from many watersheds in the Arctic, South America, and

Africa. PN export may be one of the largest uncertainties in river N export. Its potential importance is suggested by current estimates that PN accounts for about 40% of global river TN export, and that it constitutes the largest fraction of exported N from all regions, except the north temperate zone, when information is aggregated at the larger scale of major latitudinal zones (tropical, temperate, and arctic regions). Within a region, all the patterns noted above can vary among individual watersheds. Differences in the spatial patterns of river N export among N forms, in combination with differences in coastal ecosystem effects of these different N forms, highlight the need to explicitly include parameterization of the different N forms in watershed N export models.

Often only a relatively small proportion of the total N inputs to a watershed are exported by rivers to the coast, although the range is considerable (e.g., <1% to >40%). Much of the N is denitrified within soils and freshwater ecosystems. Hydrology has a large impact on the proportion of N exported by rivers with the largest proportion exported from watersheds with high water runoff (>1 m yr^{-1}) and lowest from basins with extensive damming or very low annual precipitation and runoff.

There is still much important work that needs to be done to understand patterns and controls of different N forms delivered to coastal waters. Below we highlight a few of these needs. At present, there is considerably more information on DIN inputs than other forms of N. The ability to predict seasonal or event driven changes in export, by N form, is largely limited to intensive studies in individual watersheds, as at present most regional and global N export models are only able to predict mean annual river export from large river basins. A comprehensive synthesis of seasonal patterns of river nutrient export that includes different N forms, as well as the factors contributing to those seasonal patterns, is needed. Such an analysis should include rivers across a wide range of geographic, hydrological, geological, and human disturbance regimes. These studies should also include small coastal watersheds which drain a large portion of the land immediately adjacent to the coastline globally, but which have often received less attention than larger watersheds. There is also a need for better integration of terrestrial biogeochemistry and ecosystem models with river nutrient export, including multiple element interactions. All the above are particularly pressing needs in tropical and subtropical systems, which are simultaneously the most poorly understood and subject to the greatest anticipated increases in N loading. While there has been relative success relating increases in N inputs to watersheds to increases in N export by rivers, there is considerable uncertainty in our knowledge of the response of river N export to decreasing N inputs to watersheds after prolonged periods of high inputs. Given the suggested large magnitude of N entering marine systems from atmospheric DON deposition, research is needed to obtain better quantitative information DON deposition and to determine whether the source of that DON is from land-based processes in which case it is an input of new N to the oceans, or whether it is from volatilization of DON from the oceans, in which case it would not represent new N, but rather recycled N.

APPENDIX

Table A1 DIN, DON, and PN yield (kg N km^{-2} yr^{-1}) and load (Ton N basin^{-1} yr^{-1}) from a range of river basins for which data are available

River	Continent	Basin area (km^2)	DIN yield (kg N km^{-2} yr^{-1})	DIN load (Ton N basin^{-1} yr^{-1})	Source	DON yield (kg N km^{-2} yr^{-1})	DON load (Ton N basin^{-1}yr^{-1})	Source	PN yield (kg N km^{-2} yr^{-1})	PN load (Ton N basin^{-1} yr^{-1})	Source
Alabama	N. America	56894				241.9	13762.7	2			
Altamaha	N. America	41450	113.2	4692.1	2						
Amazon	S. America	5833000	172.5	1006192.5	1	172.8	1007942.4	1	180.6	1053639.0	1
Amur	Asia	1748000	79.7	139315.6	1						
Anabar	Asia	98550	11.7	1153.0	1	42.5	4188.4	1			
Appalachicola	N. America	54660	235.1	12850.6	2	157.5	8609.0	2			
Apure	S. America	170000	53	9010	5	80	13600	5	154	26180	5
Balsas	N. America	122600	73.1	8962.1	1						
Brazos	N. America	124600	56	6977.6	2	52.8	6578.9	2	1824.6	227340.1	1
Bug	Europe	68980	28.3	1952.1	4						
Caroni	S. America	95000	207	19665	5	235	22325	5	75	7125	5
Caura	S. America	47500	238	11305	5	400	19000	5	364	17290	5
Chang Jiang	Asia	1788000	327.5	585570.0	1						
Churchill (Hudson Bay)	N. America	302400	9.5	2872.8	1						
Colorado (CA)	N. America	638951				5.6	3578.1	2			
Colorado (Texas)	N. America	120800	24.2	2923.4	2	16.6	2005.3	2			
Columbia	N. America	729300	74.1	54041.1	1	0.0					
Colville	N. America	53535				750.2	40162.0	2			
Connecticut	N. America	25019				162.5	4065.6	2			
Copper	N. America	66990	325.2	21785.1	2	431.2	28886.1	2			
Dalalven	Europe	29820	56.7	1690.8	1						
Danube	Europe	817000				150.0	122550.0	1			
Daugava	Europe	83160	151.3	12582.1	3						
Don	Europe	421600	19.1	8052.6	1						

Table A1 DIN, DON, and PN yield (kg N km^{-2} yr^{-1}) and load (Ton N basin^{-1} yr^{-1}) from a range of river basins for which data are available (*continued*)

River	Continent	Basin area (km^2)	DIN yield (kg N km^{-2} yr^{-1})	DIN load (Ton N basin^{-1} yr^{-1})	Source	DON yield (kg N km^{-2} yr^{-1})	DON load (Ton N basin^{-1}yr^{-1})	Source	PN yield (kg N km^{-2} yr^{-1})	PN load (Ton N basin^{-1} yr^{-1})	Source
Elbe	Europe	148000	795.4	117719.2	1						
Gambia	Africa	42000							13.6	569.8	1
Ganges	Asia	1050000				28.2	29610.0	1	569.5	598018.9	1
Glama	Europe	47310	191.8	9074.1	1						
Guaviare	S. America	150000	124	18600	5			5			5
Huang He	Asia	890500	120.5	107305.3	1				1711.4	1524018.7	1
Hudson	N. America	43070	381.1	16414.0	1						
Indigirka	Asia	362000				59.5	21539.0	1			
Indus	Asia	1139000	136.9	155929.1	1						
Japura	S. America	300000	204	61200	5	184	55200	5	244	73200	5
Jurua	S. America	220000	182	40040	5	121	26620	5	144	31680	5
Kamchatka	Asia	50370	88.8	4472.9	1						
Khatanga	Asia	364000				94.3	34325.2	1			
Klamath	N. America	32080	71	2277.7	2	151.9	4873.0	2			
Kobuk	N. America	24657				303.6	7485.9	2			
Kolyma	Asia	663200	18	11937.6	1	70.0	46424.0	1			
Kuban	Europe	63630	330.9	21055.2	1						
Kuskowin	N. America	115400	136.9	15798.3	2	503.2	58069.3	2			
Lena	Asia	2433000	21.1	51336.3	1	96.6	235027.8	1			
MacKenzie	N. America	1787000				17.0	30379.0	1	46.3	82790.4	1
Madeira	S. America	1300000	130	169000	5	130	169000	5	203	263900	5
Meta	S. America	110000	214	23540	5			5			5
Mezen	Europe	75430	24.8	1870.7	1						
Mississippi	N. America	3191000	255.6	815619.6	1	139.4	444825.4	2	428.5	1367356.9	1
Murray	Australia	1028000	1.1	1130.8	1						
Narva	Europe	58010	73.3	4252.1	3						
Negro	S. America	620000	67	41540	5	248	153760	5	2	1240	5

Nemanus	Europe	96630	138.4	13373.6	1						
Neva	Europe	283500	74.1	21007.4	1						
Niger	Africa	1200000				16.9	20280.0	1			
Nile	Africa	2870000						1	0.1	389.4	1
Nueces	N. America	39956				22.5	899.0	2			
Nushagak	N. America	35300	105.3	3717.1	2	466.4	16463.9	2			
Ob	Asia	3015000	98	295470.0	1						
Odra	Europe	119400	389.8	46542.1	1						
Olenek	Asia	219000				65.6	14366.4	1			
Orange	Africa	1000000				1.5	1500.0	1			
Orinoco	S. America	1100000				164.8	181280.0	1	300.1	330103.4	1
Paraguay River	S. America	363500		0	5			5	16	5816	5
Paraiba do Sul	S. America	62760	185.2	11623.2	1						
Parana	S. America	2654000	43.9	116510.6	1	16.0	42464.0	1	39.7	105485.1	1
Pechora	Europe	313100	64.7	20257.6	1						
Pee Dee	N. America	27640	219.3	6061.5	2	114.4	3162.0	2			
Penzhina	Asia	85540	25.5	2181.3	1				1500.3	128331.6	1
Po	Europe	70000				224.0	15680.0	1			
Potomac	N. America	38300	395.7	15155.3	1	294.8	11290.8	2			
Rhine	Europe	164500	2200.4	361965.8	1						
Rio Coatzacoalcos	N. America	29497				565.5	16680.6	4			
Rio Grande (US)	N. America	801900	0.6	481.1	1	5.7	4570.8	2			
Roanoake	N. America	22458				79.2	1778.7	2			
Rufiji	Africa	186100	275.9	51345.0	1						
Sabine	N. America	18982				265.2	5034.0	2			
Sacramento	N. America	58690	38.1	2236.1	1	180.4	10587.7	2			
Saint John	N. America	52850	59.8	3160.4	1	264.0	13952.4	2			
Sakarya	Asia	56830	155	8808.7	1						
San Joaquin	N. America	35058				48.4	1696.8	2			
Savannah	N. America	25511				118.8	3030.7	2			
Sebou	Africa	38492				63.0	2425.0	4			
Seine	Europe	73190	1364.9	99897.0	1	100.0	7319.0	1			

(*Continued*)

Table A1 DIN, DON, and PN yield (kg N km^{-2} yr^{-1}) and load (Ton N basin^{-1} yr^{-1}) from a range of river basins for which data are available (*continued*)

River	Continent	Basin area (km^2)	DIN yield (kg N km^{-2} yr^{-1})	DIN load (Ton N basin^{-1} yr^{-1})	Source	DON yield (kg N km^{-2} yr^{-1})	DON load (Ton N basin^{-1}yr^{-1})	Source	PN yield (kg N km^{-2} yr^{-1})	PN load (Ton N basin^{-1} yr^{-1})	Source
Solimoes at Ica	S. America	1200000	240	288000	5	136	163200	5	390	468000	5
St. Lawrence	N. America	1020000				9.9	10098.0	1			
Stikine	N. America	51170	233	11922.6	2	1159.2	59316.3	2			
Susitna	N. America	50246				744.0	37383.0	2			
Susquehanna	N. America	71860	493.2	35441.4	1	414.7	29800.3	2			
Tejo	Europe	73090	122.8	8975.5	1						
Tornionjoki	Europe	34510	9	310.6	1						
Trinity	N. America	47380	92.2	4368.4	2	114.4	5420.3	2			
Trombetas	S. America	130000	53	6890	5			5			5
Usumacinta	N. America	67890	562.2	38167.8	1						
Waikato	Oceania	13700							268.5	3679.1	1
Weser	Europe	45470	1204.7	54777.7	1						
Wisla	Europe	180000	371.8	66924.0	1						
Yana	Asia	224200	25.9	5806.8	1	46.2	10358.0	1			
Yenisei	Asia	2569000	43.1	110723.9	1						
Yukon	N. America	852700	30.6	26092.6	1	205.2	174974.0	2			
Zaire	Africa	3698000				57.6	213004.8	1			
Zhujiang	Asia	407100	523.3	213035.4	1						

1 = Meybeck and Ragu [1995], 2 = USGS [Alexander *et al.*, 1996], 3 = European Environmental Agency [*EEA*, 1998], 4 = GEMS–Water Triennial Reports, 5 = Lewis *et al.*, 1999.

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Phototransformations of Dissolved Organic Nitrogen

Waldemar Grzybowski *and* Lars Tranvik

Contents

1. Introduction

Within the last decade it has become obvious that photochemical reactions play an important role in the transformations of organic matter. Most studies in this respect have focused on organic carbon. However, photochemical reactions are not restricted to carbon, but also affect other elements including nitrogen (see below), sulfur (e.g. Brugger *et al.*, 1998; Dierdre *et al.*, 2003; Hatton, 2002), phosphorus (observations limited to humic-rich lake water—see Mopper and Kieber, 2002), and metals (see Zepp, 2003).

The interest in environmental photochemistry is additionally stimulated by the awareness of an increasing flux of harmful UVB radiation due to stratospheric ozone depletion (e.g. Crutzen, 1992; Smith *et al.*, 1992). As a consequence, there has been a sizable increase in the number of publications concerning interactions between sunlight and both biotic and abiotic components of aquatic environments (Fig. 10.1).

The data that have accumulated so far are summarized and discussed in several excellent reviews. Of those, information on the impact of sunlight on aquatic nitrogen compounds can be found in Bronk (2002), Kieber (2000), Mopper and Kieber (2002), Moran and Covert (2003), and Zepp (2003). A reader interested in mechanisms behind the reported phenomena is advised to turn to more chemistry-oriented works by Boule *et al.* (1999) and Mopper and Kieber (2000). The present chapter is a supplement and update of the above-mentioned studies, specifically focusing on phototransformations of dissolved organic nitrogen (DON).
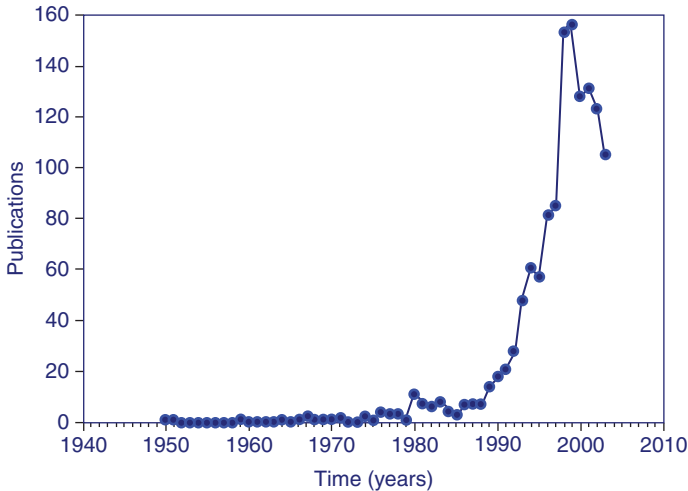
Figure 10.1 Development of publications on UV and the aquatic ecosystem between 1949 and 2003 (Web source: Ruben Sommaruga, http://homepage.uibk.ac.at/homepage/c719/c71986/).

2. PHOTOCHEMICAL PRODUCTION OF INORGANIC NITROGEN

Organic carbon and organic nitrogen differ in the environmental role of their final photoproducts. The ultimate product of photochemical oxidation of organic carbon is carbon dioxide and its dissolved forms. Although intermediate organic photoproducts are important substrates for heterotrophic bacteria (e.g. Bertilsson and Tranvik, 1998), the product of complete photomineralization of organic carbon is of little ecological importance. Inorganic carbon is highly abundant in marine waters, and its photochemical formation can hence cause only minor relative changes in concentration. Complete photochemical mineralization of organic nitrogen compounds, however, results in production of ammonium, the most easily assimilated nitrogen species, used by both auto- and heterotrophs.

Despite the conceptual simplicity of the phenomenon, the first proof of production of ammonium via photodegradation of natural dissolved organic matter was presented relatively recently, when Bushaw *et al.* (1996) showed that short-term irradiation of water with high concentrations of UV-absorbing natural organic matter resulted in a significant increase in ammonium concentration. Biotic mechanisms were ruled-out by these authors (and by their followers) by exclusion of bacteria by filtering through $0.2\ \mu m$ pore-size membrane filters.

Since most of the organic nitrogen is of limited bioavailability to autotrophs, photochemical transformation of recalcitrant DON into bioavailable forms implies an important (and unaccounted for) potential source of assimilable nitrogen. The study of Bushaw *et al.* (1996) was followed by several investigations containing

data on photoammonification in different aquatic environments. The results published so far, however, are not consistent (Table 10.1), and include positive results as well as decreased ammonium concentrations upon light exposure, and studies where no change in ammonium concentrations could be detected. The great potential significance of photoammonification, combined with the relatively low number of follow-up studies that were able to corroborate the initial results of Bushaw *et al.* (1996), suggest that there may be a "file drawer effect" (Bachau, 1997), i.e. an accumulation of conflicting results that have remained unpublished.

Since only absorbed light can initiate phototransformations, it may be expected that samples of different optical properties show different photoproduction rates. For comparative purposes, the rates are thus usually absorbance-normalized. However, the inconsistency when comparing results from different studies is not resolved by such normalization and the variability of normalized rates exceeds an order of magnitude (Table 10.1). An attempt to find a correlation between available bulk characteristics (DON, DOC, pH, absorbance) and irradiation effects proved unsuccessful (Grzybowski, 2003). Additional confusion is introduced by reports on lack of ammonium release and even its removal during irradiation, observed in apparently similar samples (Table 10.1).

The method descriptions in most of the published experiments are not detailed enough to allow us to elucidate whether differences in experimental setup are responsible for the observed variability. For example, data on radiation intensity at the surface of the irradiated water layer are not sufficient in the case of optically thick samples where a significant fraction of the light is absorbed within the upper layer of the sample. Accordingly, differences among samples that differ in the fraction of incident light that is absorbed may not be proportional to the photochemical reactivity of the organic matter.

The published results are commonly normalized against absorbance at 350 nm (UVA range). Since the total energy absorbed in the water column is usually higher in the UVA than in the UVB range, UVA normalization may be a reasonable approximation. However, it should be noted that UVB radiation is suggested to be most important for photoammonification (Buffam and McGlathery, 2003; Bushaw *et al.*, 1996). Data normalization limited to the UVA range may be misleading, since a highly energetic, singular UVB photon can not be directly substituted by numerous (but less energetic) UVA photons. So far, no comprehensive study has been presented on the relative importance of different wavelengths of radiation for photoammonification and other DON photoreactions.

Another factor responsible for the observed variability in "positive" results may be adsorption of ammonium and other nitrogen compounds onto humic substances (the main photoreactive fraction of DOM). Hence, detection of photoammonification may to some extent reflect photochemically induced desorption of ammonium from large organic molecules, rather than the mineralization of organic nitrogen. This phenomenon, however, would be limited to fresh and low salinity waters, since at higher salinities ammonium ions are replaced by cationic macroconstituents of seawater.

Table 10.1 Results of experiments on ammonium release due to photochemical transformations of dissolved organic matter

Irradiated sample	Irradiation conditions	Photoproduction rate (μM h^{-1})	DOC (μM)	DON (μM)	Absorption coefficient (m^{-1})	Absorbance normalized photoproduction rate (μM m h^{-1})	Reference
Boreal pond water, pH = 7.6	Artificial (Wavelength >290 nm), exposure time 18 h	0.15 ± 0.01	3000	55	a$_{350}$ ~43	0.0035	Bushaw *et al.* (1996)
	Wavelength >320 nm	0.11					
	Wavelength >360 nm	0.08					
Boreal pond inlet (fulvic acid), pH = 6.5	Artificial (wavelength >290 nm), exposure time 6.6 h	0.065	1133	24	a$_{350}$ ~20	0.0025	Bushaw *et al.* (1996)
Swamp water, pH = 3.9	Artificial (wavelength > 290 nm), exposure time 18 h	0.34	3840	86	a$_{350}$ ~106	0.032	Bushaw *et al.* (1996)
	Wavelength >320 nm	0.22					
	Wavelength >360 nm	0.059					
	Wavelength >425 nm	0.040					

Suwannee River water	Artificial (wavelength >320 nm), exposure time 36 h	0.36	not available	74	Not available		Bushaw et al. (1996)
Fluka humic acid solution	Artificial (wavelength >290 nm), exposure time 18 h	0.23	1942	51	a_{350} ~127	0.018	Bushaw et al. (1996)
Satilla River water	Artificial, exposure time 4 h	0.1	2100	40	a_{350} ~90	0.001	Gao and Zepp (1998)
Lake water	Artificial (UV range), exposure time 12 h	No photo-production	~880 and ~1000	~6 and ~15	Not available		Jørgensen et al. (1998)
Maracaibo Lake water, pH = 7.72	Sunlight	0.22	not available	Not available	Not available		Gardner et al. (1998)
Concentrate of Satilla River fulvic acids	Sunlight, exposure time 7 h	0.058	2200	40	a_{350} ~86	0.001 (corrected for internal shading)	Bushaw-Newton and Moran (1999)
Boreal rivers and groundwater, pH = 5.1–7.1	Artificial (UV range) exposure time 12 h	No photo-production	200–2192	14–47	a_{365} 0.1–34.7		Bertilsson et al. (1999)

Table 10.1 Results of experiments on ammonium release due to photochemical transformations of dissolved organic matter (*continued*)

Irradiated sample	Irradiation conditions	Photoproduction rate (μM h^{-1})	DOC (μM)	DON (μM)	Absorption coefficient (m^{-1})	Absorbance normalized photoproduction rate (μM m h^{-1})	Reference
Baltic Sea water, Gulf of Riga	Artificial (UV range) exposure time 12 h	58–82% concentration decrease	Not available	10–25	Not available		Jørgensen *et al.* (1999)
West Pearl River water, pH = 6	Artificial, exposure time 10 h	0.33	2491	24	a$_{350}$ 33	0.01	Wang *et al.* (2000)
Caribbean Sea water, Gulf of Paria	Sunlight, exposure time ~16 h	~0.04	Not available	~40	Not available		Morell and Corredor (2001)
Groundwater	Sunlight, exposure time up to 80 h	No photo-production	1100	80	Not available		Weigner and Setzinger (2001)
Satilla River water	Artificial, exposure time 8 h	0.018	1440	37	a$_{350}$ 43.8	0.00052 (corrected for internal shading)	Koopmans and Bronk (2002)
Groundwater	Sunlight, 8 h	Concentration decrease −0.04	2881	67	a$_{350}$ 56.8	Concentration decrease −0.002	Koopmans and Bronk (2002)
North Pacific water	Not available	0.005	Not available	Not available	Not available		Bronk (2002)
South Atlantic Bight water	Not available	0.035	Not available	Not available	Not available		Bronk (2002)

Baltic Sea water, Gulf of Gdansk	Artificial (UV range), exposure time 10 h	No photo-production	480–520	20–30	a_{350} 1.6–2.1		Grzybowski (2002)
Vistula River water	Artificial (UV range), exposure time 10 h	No photo-production	980–1050	40–50	a_{350} 7.1–8.2		Grzybowski (2002)
Coastal lagoon	Artificial (UV range), exposure time 36 h	No photo-production	141	8.2	a_{350} 1.1		Buffam and McGlathery (2003)
Ground water	Artificial (UV range), exposure time 36 h	Up to 0.032	612	32	a_{350} 10 (diluted)	Up to 0.0032	Buffam and McGlathery (2003)
Aged Baltic Sea water	Artificial, exposure time 44–132 h	0.007–0.077	Not available	15.8–19.8	a_{300} 5.0–9.9		Vähätalo and Zepp (2005)
Eastern Mediterranean water	Sunlight, exposure time $\leq$ 3 h	0.0007–0.0029	51–110	Not available	a_{300} 0.19–0.36	0.0026–0.0116	Kitidis et al. 2006
Baltic Sea water	Artificial (UV range) exposure time 48 h	0.035 0.045 (first 24 h) No photo-production	316 574 347	10.5 18.0 7.8	a_{350} 1.49 a_{350} 6.60 a_{350} 3.33	0.023 0.007 –	Stedmon et al. (2007)

Elemental nitrogen in humic compounds include structural nitrogen as well as different chemical moieties bound to the humic structure (Schnitzer, 1985). One may reasonably expect different photolability of these fractions, with the structural nitrogen showing bigger resistance to photorelease and the associated nitrogen sensitive to ionic strength and/or pH. In fact, increasing photoammonification rates with decreasing pH were reported by Wang *et al.* (2000), but this finding does not hold true for some of the data presented in Table 10.1.

The reverse process, i.e. decrease in ammonium during irradiation, was observed in some studies (Jørgensen *et al.*, 1999; Koopmans and Bronk, 2002). During long-term irradiation fluctuations in factors such as pH and temperature may affect the results. There may be a shift of the NH_4^+/NH_3 equilibrium towards ammonia (NH_3) if there is an increase in pH, and enchanced temperature during irradiation would favor volatilization of ammonia. Another reason for ''negative'' results may be irreversible binding of ammonium to other components of natural waters in a way that renders it undetectable, as has been reported for solutions of humic substances (e.g. Thorn and Mikita, 1992). It is conceivable that irreversible binding takes place also during irradiation, simultaneously with ammonium production from photodegraded DOM. The net formation of free, analytically available ammonium depends on the relative importance of binding as opposed to new formation of ammonium. The significance of ammonium binding (and its apparent removal) should be higher in samples of high initial ammonium concentration. This can actually be implied from observations in irradiated ground water samples (Fig. 10.2).
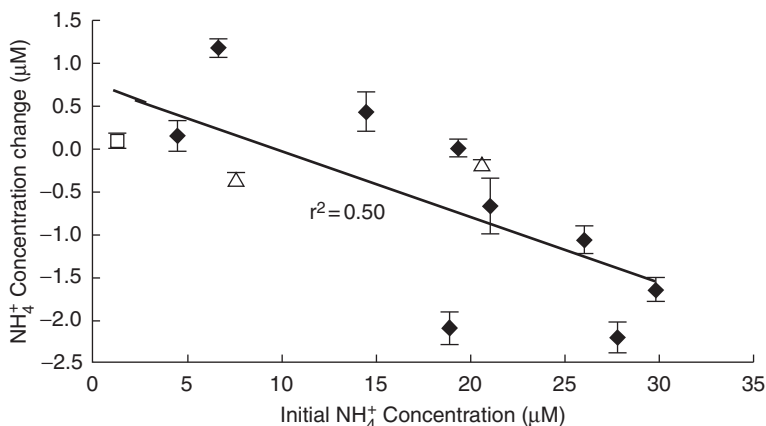
Figure 10.2 Change in ammonium (NH_4^+) concentration after an exposure of unmanipulated (◆), NH_4^+ removed (□), and DON concentrated (Δ) groundwater samples to natural or artificial sunlight (xenon arc lamp, 1000 μE m^{-2} s^{-1}), plotted against the initial NH_4^+ concentration. Means ± SD shown, $n = 3$ (Koopmans and Bronk, 2002).
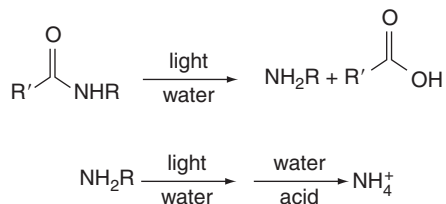
Figure 10.3 Possible mechanisms of photochemical ammonia formation from amides/peptides. The ketone is listed as a possible photosensitizer (Wang *et al.*, 2000).

Most of the published results on photammonification were obtained via a ''black box'' approach, i.e. without detailed inquiry of underlying mechanisms. The few exceptions are papers by Wang *et al.* (2000) and Tarr *et al.* (2001). These authors proposed chemical reactions behind photoammonification (Fig. 10.3), which include a ketone as a photosensitizer.

One remarkable finding is an initial increase in ammonium in river water immediately after the end of irradiation of water followed by a decrease during prolonged incubation (Wang *et al.*, 2000). Since ammonium concentration did not change in nonirradiated samples, authors suggest an abiotic mechanism, involving long-lived, photo-induced transients. However, since these authors used glass fiber filters with a nominal 0.5-μm cut off, that is not effective in bacteria removal, an alternative explanation to their result may be a biotic process. Still, a similar phenomenon (initial increase and subsequent decrease in ammonium concentration) was observed by Kieber *et al.* (1997), upon addition of ammonium after irradiation of seawater enriched with fatty acids. In this case, the samples were filter sterilized with 0.2-μm membrane filters (Fig. 10.4).

In addition to reports on ammonium changes due to photodegradation of DON there is an intriguing study by Kieber *et al.* (1999) demonstrating formation of nitrite during photodegradation of dissolved organic matter. An important implication of this finding is the existence of oxidized (+3), i.e. nonproteinaceous nitrogen within structures of natural, biogenic matter. The possibility that the observed nitrite is a result of photo-oxidation of organic nitrogen seems unlikely since this would generally require semi-conductor catalysis (e.g. Hidaka *et al.*, 1997; Nohara *et al.*, 1997). Kieber *et al.* (1999) found that nitrite photoproduction increased with the addition of humic substances to Gulf Stream water, suggesting an organic origin of the nitrite. Spokes and Liss (1996) noted in irradiated solutions of nitrate that increased humic substances concentrations resulted in an increase in nitrite. In this case the authors postulated both enhancement of nitrate reduction by reactive by-products of photodegradation of humic substances and/or release of nitrite from organic matter.
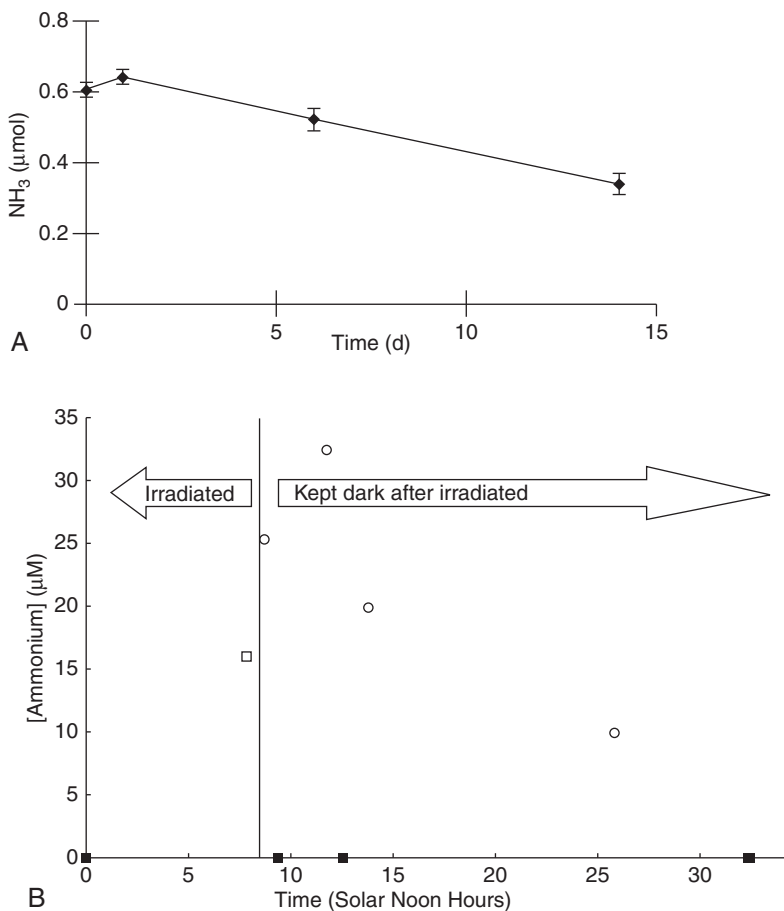
Figure 10.4 Comparison of changes in ammonia concentration observed during post–photolysis in sea water enriched with fatty acids (A) (after Kieber *et al.*, 1997) and river water (B) (Wang *et al.*, 2000) (in this case samples were either kept dark throughout experiment ■, were irradiated □, or were first irradiated and then kept dark ○).

3. PHOTOCHEMICAL TRANSFORMATIONS OF ORGANIC COMPOUNDS AND EFFECTS ON BIOAVAILABILITY

A number of organic compounds have been identified as products of DON photodegradation. In the majority of published papers, they are characterized in bulk as amines measured as fluorescent ortho–phtaldialdehyde derivatives (Lindroth and Mopper, 1979). Figure 10.5 shows one of the few cases where organic products of DON phototransformation are presented as chemical moieties identified at the molecular level.
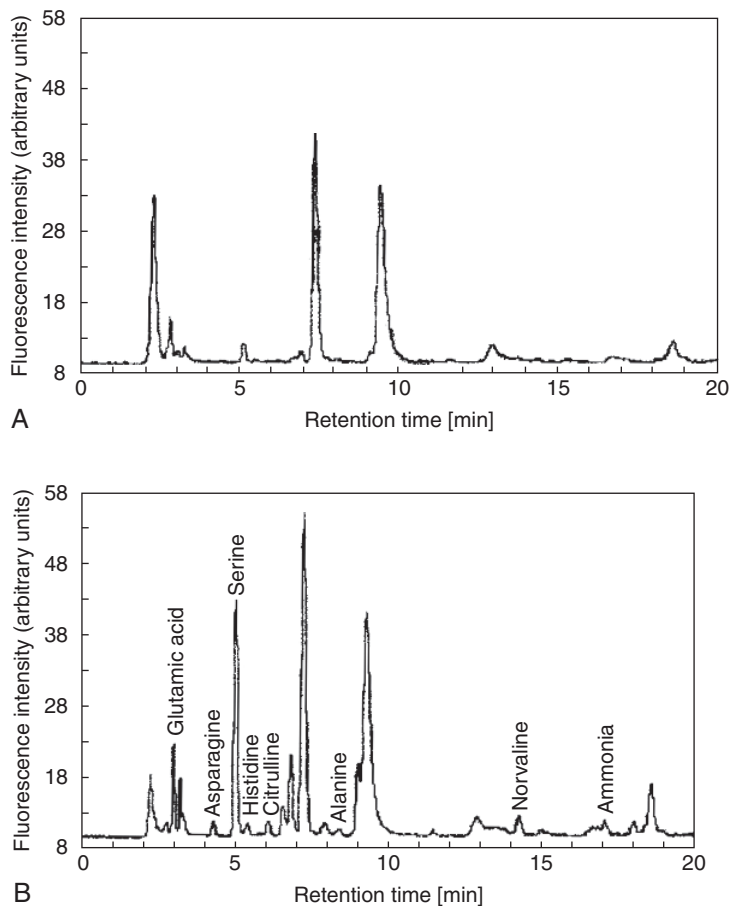
Figure 10.5 Amino acid photoproduction from Suwannee River fulvic acid (30 mg L^{-1}). Chromatograms are of OPA derivatized samples before (A) and after (B) 31 h irradiation in a solar simulator. Labeled peaks increased in size after irradiation are labeled with names of amino acids having the same retention time (Tarr *et al.*, 2001).

Published results on organic nitrogen products of photodegradation of dissolved organic matter are compiled in Table 10.2. Similarly to the photoammonification data, the publications include observations of increased, unchanged, as well as decreased concentrations of amines.

In addition to chemical analysis of irradiation products, bioassays have been employed to estimate how photochemical reactions change the availability of organic substances for bacterial growth. In this way, information is gained on the environmental role of photoproducts even though their identity and concentration remain unknown. This approach has yielded a large body of results on photochemical effects on dissolved organic carbon bioavailability (see e.g. Mopper and Kieber, 2002 for a review). In general, these effects are positive for DOM dominated

Table 10.2 Published results on photoproduction of amines from dissolved organic matter

Irradiated sample	DOC (μM)	DON (μM)	Absorption coefficient (m^{-1})	Irradiation conditions	Irradiation effects	Reference
Epilimnion lake water	∼800	∼16	Not available	Sunlight, 7 h exposure	Increase in DFAA (0.1 μM) and DCAA (∼0.5 μM)	Jørgensen et al. (1998)
Hypolimnion lake water	∼950	∼6	Not available	Sunlight, 7 h exposure	Increase in DFAA (∼0.05 μM), no change in DCAA	Jørgensen et al. (1998)
Baltic Sea water	Not available	10–30	Not available	Artificial (UV range), 12 h	Up to 59% increase or no change in DCAA; up to 65% increase no change; and up to 45% decrease in DFAA	Jørgensen et al. (1999)
Concentrate of river water humic substances dissolved in artificial seawater – 28 × Skidaway River	2352	56	a$_{350}$ ∼40	Sunlight, 7 h exposure	increase in bulk primary amines ∼0.3 μM	Bushaw-Newton and Moran (1999)

Concentrate of river water humic substances dissolved in artificial seawater—2.8 × Satilla River Estuary	2200	34	a$_{350}$ ~58	Sunlight, 7 h exposure	Increase in bulk primary amines ~0.06 μM	Bushaw–Newton and Moran (1999)
Suwannee River fulvic acid solution	~7200	Not available	Not available	Artificial, ~45 h exposure	0.7 μM increase in serine	Tarr *et al.* (2001)
Satilla River (modified)	4066	107	a$_{350}$ 128	Artificial sunlight, 8 h exposure	No change in primary amines	Koopmans and Bronk (2002)
Ground water	4224	66	Not available	Sunlight, 7 h exposure	0.63 μM increase in primary amines	Koopmans and Bronk (2002)
Ground water	1196	26	a$_{350}$ 21.8	Artificial sunlight, 8 h exposure	0.33 μM decrease in primary amines	Koopmans and Bronk (2002)
Ground water (diluted)	612	37	a$_{350}$ 19.7	Artificial (UV range), 36 h exposure	~0.03 μM increase in glycine and alanine; no change in serine	Buffam and Glathery (2003)

by humic substances, and negative for freshly synthesized algal organic matter (Tranvik and Bertilsson, 2001). Also when it comes to DON, both positive and negative effects of solar exposure on bacterial utilization have been observed.

In a concentrate of DOM from a Sphagnum bog, which was extensively rinsed by a tangential flow ultrafiltration approach to remove inorganic nutrients, Tranvik *et al.* (2000) found that bacterial utilization of DON was stimulated by pre-exposure of the organic matter to simulated sunlight. In bacterial cultures that received either pre-exposed or dark-treated DOM free from inorganic nutrients, together with an additional source of organic carbon (glucose) and inorganic phosphorus, to make the cultures limited by nitrogen and void of any N source other than the DON, bacterial growth was enhanced by light exposure to an extent that could not be explained by photoammonification. Hence, it appears that some DON compounds were transformed by light into more available organic forms.

DON, in addition to structural nitrogen of humic substances and humic-bound proteinaceous matter, consists of highly labile amines, amino acids, and proteins. Proteins generally do not absorb radiation within the solar spectrum that reaches the biosphere (>290 nm). In one of the first bioassays with chemically defined compounds, peptone lost its bioavailability upon irradiation (Naganuma *et al.*, 1996). The results, however, were obtained with unrealistically high substrate concentrations. Similar experiments, but with several orders of magnitude lower organic nitrogen concentrations, were made by Reitner *et al.* (2002). The measurable, negative change in bacterial growth was observed solely in a solution of tryptophan (one of the few amino acids able to absorb solar radiation) while there was no impact of irradiation on light transparent alanine.

This result suggests that phototransformation of biolabile substances proceeds mainly via direct photoreactions, while photo-induced interactions with other light-absorbing constituents of natural waters are of negligible importance. In contrast, in experiments with fresh algal extract irradiated in presence of humic substances (Tranvik and Kokalj, 1998), irradiation decreased susceptibility of the substrate to subsequent mineralization by bacteria. The decreased respiration was demonstrated to be caused by photo-induced, physico-chemical interactions between algal extract and humic substances.

Accordingly, photochemically induced decreased bioavailability of DON and other DOM may result from directly absorbed radiation (e.g. Reitner *et al.*, 2000) as well as indirectly, e.g. via photochemically induced interactions with other substances (e.g. Tranvik and Kokalj, 1998).

Most of the organic nitrogen in natural waters is bound to or built into structural components of humic substances (Bronk, 2002; Schnitzer, 1985). It has been demonstrated both in laboratory experiments and in field observations that proteinaceous matter bound to humic substances becomes bioresistant (Carlson *et al.*, 1985; Hubberten *et al.*, 1994). In pioneering studies on DON phototransformation Amador *et al.* (1989, 1991) showed that a significant part of amino acids associated with humic matter becomes bioavailable after irradiation. On the other hand, long-term irradiation of a mixture of protein and DOM compounds resulted in decreased bioavailability (Keil and Kirchman, 1994). The loss of bioavailability of the protein required presence of DOM, supporting that the DOM either interacted directly

with the protein, or mediated indirect photoreactions. Interestingly, the change in protein quality was detected only as decreased bacterial activity in bioassays, but could not be discerned as a change in amino acid composition of the protein.

4. Recommendation for Future Research

Despite the remarkable efforts put into research on environmental aquatic nitrogen photochemistry there is still no consensus on its environmental role. Published data so far do not allow testing of fundamental issues such as the proportionality between absorbed energy and the extent of photoreactions. The basic necessary data for such studies include spectral characteristics of irradiated samples as well as the quality and dose of light. This is particularly important in experiments using natural sunlight where share of UV radiation changes both quantitatively and qualitatively. In such experiments, data on the length of irradiation and the intensity of radiation determined for the full spectrum are not sufficient.

The observed apparent discrepancies among different studies of nitrogen photochemistry may be an effect of, according to Mopper and Kieber (2002), "limitations in the analytical methodologies used to quantify NH_4^+." In the majority of studies, modifications of the same phenolhypochlorite method (Solòrzano, 1969) were used for ammonium analysis, and the reported standard deviations ranges from 0.01 μM to above 0.5 μM. Progress in the understanding of the ecological role of photo-ammonification depends on careful analytical procedures, including the use of proper controls and methods that allow hing sensitivity and reproducibility in the submicromolar range.

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NITROGEN AND MARINE EUTROPHICATION

Hans W. Paerl *and* Michael F. Piehler

Contents

1. INTRODUCTION

The availability of nitrogen (N) most frequently limits autotrophic production of organic matter in the world's estuarine, near-shore coastal and open ocean waters (Dugdale, 1967; Nixon, 1986, 1995; Ryther and Dunstan, 1971). As such, the fertility of these waters is often closely controlled by N inputs, provided either internally by regeneration of pre-existing N and biologically-fixed atmospheric N_2, or supplied externally (i.e., ''new'') as combined N sources delivered via surface runoff, sub-surface groundwater or atmospheric deposition (Fig. 11.1). Because marine ecosystems lose some fixed N via denitrification and burial, ''new'' N supply is essential for compensating for these N losses, and ensuring productive conditions. However, during the past century humans have significantly altered the balance between ''new'' N inputs and N losses in the marine environment through large-scale use of synthetic N fertilizers in agriculture, rapid expansion of industrial and transportation–related fossil fuel combustion and coastal urbanization (Codispoti *et al.*, 2001; Rabalais, 2002; Vollenweider *et al.*, 1992). Over this time frame, terrigenous discharge and atmospheric N emissions have increased 10-fold (Holland *et al.*, 1999; Howarth *et al.*, 1996) and continue to grow as human development expands in coastal watersheds (Vitousek *et al.*, 1997).
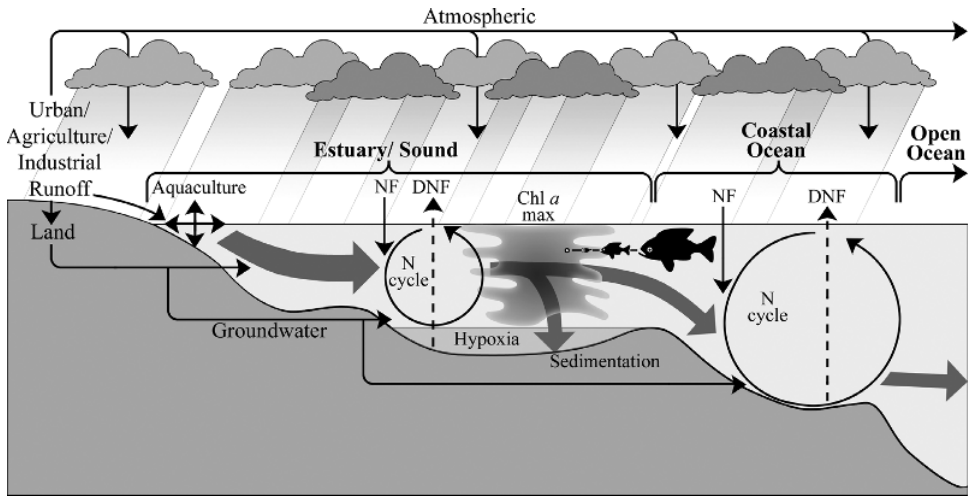
Figure 11.1 Schematic diagram illustrating sources, transformations and fates of nitrogen (N) along the estuary to ocean continuum. Surface, subsurface and atmospheric pathways of externally-supplied or new N inputs attributable to anthropogenic activities are shown as are internal N cycling. The combined anthropogenic N inputs are shown as a thick arrow (upstream), which decreases in thickness downstream as a portion of the N inputs settles to the bottom sediments and is buried and/ or denitrified (DNF). NF represents nitrogen (N₂) fixation, a biologically-mediated new N input. The linkage of anthropogenically-enhanced N inputs to accelerated primary production or eutrophication, and its trophic and biogeochemical fate are also shown. In many estuarine and sound systems, primary production and phytoplankton biomass are maximal in mid-system locations, where adequate new N loads and decreasing rates of flushing (increasing residence times) overlap. The resultant chlorophyll *a* maximum (Chl *a* max) is characteristic of estuarine systems in which residence times are long enough to allow periodic phytoplankton blooms to accumulate.

For at least 50 years, researchers have recognized this growing imbalance, especially in estuarine and coastal waters where N over-enrichment has fueled accelerated primary production, or eutrophication, which Nixon (1995) defined as ''an increase in the rate of supply of organic matter to an ecosystem.'' From a food web perspective, eutrophication is a condition where nutrient-enhanced primary production exceeds the ability of higher ranked consumers to utilize it. D'Elia (1987) characterized this condition as ''too much of good thing,'' or over-fertilization of N-limited marine ecosystems with ''new'' N, the bulk of it being anthropogenic (Galloway and Cowling, 2002; Howarth *et al.*, 1986; Vitousek *et al.*, 1997). Symptoms of N–driven eutrophication include: (1) subtle increases in plant production, to (2) changes in primary producer community composition, (3) rapidly accelerating algal growth, (4) visible discoloration or blooms (Jørgensen and Richardson, 1996; Paerl, 1988; Smetacek *et al.*, 1991), (Fig. 11.2), (5) losses in water clarity, (6) increased consumption of oxygen leading to ecosystem–scale oxygen depletion (hypoxia, anoxia) (Hagy *et al.*, 2004) (Fig. 11.3), which is stressful to resident fauna and flora, and can lead to (7) elimination of habitats (Diaz and Rosenberg, 1995; Rabalais and Turner, 2001). Lastly, excessive N enrichment, relative to other nutrient inputs, can alter nutrient input balances and cycling, including shifts to phosphorus and silicon limitation of
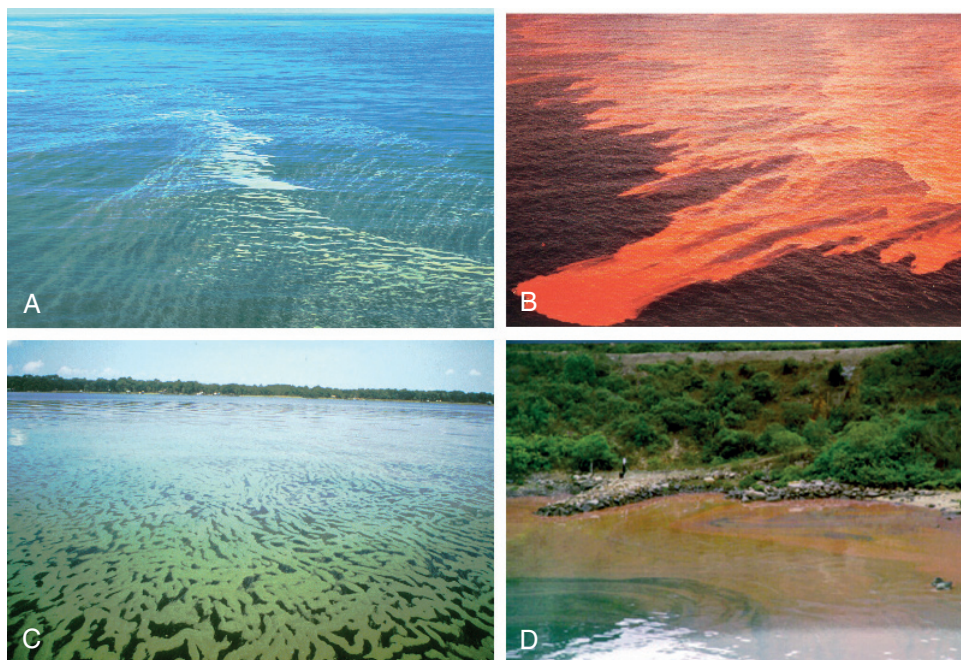
Figure 11.2 Estuarine and coastal phytoplankton blooms symptomatic of nitrogen-enhanced primary production or eutrophication. Clockwise, starting with upper left; (A) cyanobacterial blooms in the Gulf of Finland, Baltic Sea (photo courtesy of P. Moisander); (B) dinoflagellete red tide bloom off the coast of Japan (photo courtesy of ECOHAB Program); (C) dinoflagellate/ diatom bloom near the coast of Hong Kong, China (photo courtesy ECOHAB Program), and (D) cyanobacterial bloom on the St. Johns River Estuary, Florida (photo courtesy J. Burns).

primary production in receiving estuarine and coastal waters (Dortch *et al.*, 2001; Lohrenz *et al.*, 1999; Sylvan *et al.*, 2006).

Anthropogenic or cultural eutrophication has been closely linked to population densities in coastal watersheds (Nixon, 1995; Peierls *et al.*, 1991; Vitousek *et al.*, 1997; see also Chapter 9 by Seitzinger and Harrison, this volume; Chapter 18 by Boynton and Kemp, this volume). A significant, and in many instances increasing, proportion of "new" N input can also be attributed to remote sources delivered through airsheds and subsurface aquifers outside the immediate watershed (Galloway and Cowling, 2002; Jaworski *et al.*, 1997; Paerl, 1997). The area of an airshed can greatly exceed that of a watershed for a specific estuary or coastal region. For example, the airshed of the Baltic Sea includes much of Western and Central Europe (Hov *et al.*, 1994), and the airsheds of the US's two largest estuarine ecosystems, the Chesapeake Bay and Albemarle-Pamlico Sound, are from 15 to over 30 times the size of their watersheds (Paerl *et al.*, 2002) (Fig. 11.4). Thus, the airshed of one region may impact the watershed and receiving waters of another (Dennis, 1997) (Fig. 11.4). Furthermore, atmospheric N inputs do not stop at coastal margins. Along the North American Atlantic continental shelf and Mediterranean coast, atmospheric N inputs more than match riverine inputs (Herut *et al.*, 1999; Jaworski *et al.*, 1997; Paerl *et al.*, 2002), underscoring the fact that this is a regional, if not global, issue (Galloway and
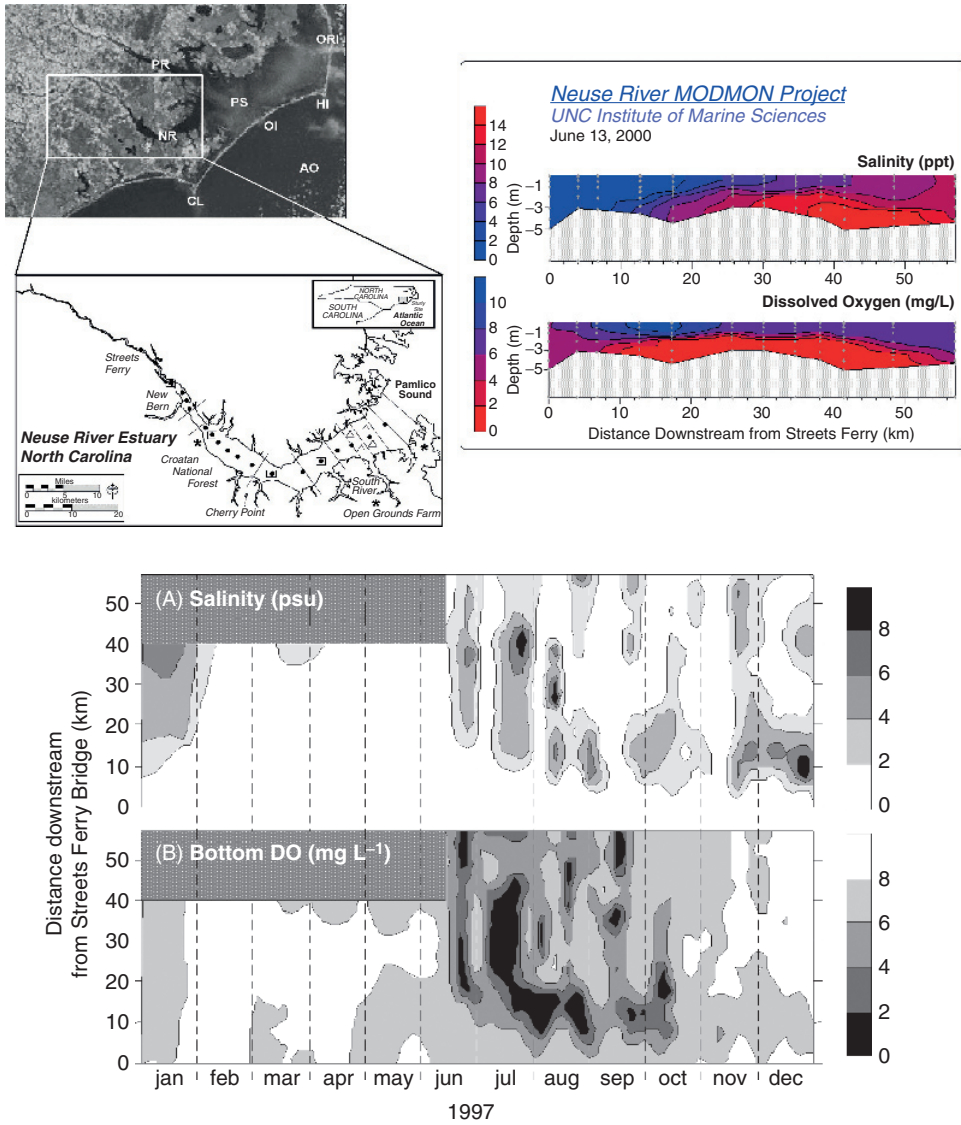
Figure 11.3 Low oxygen, hypoxic (less than 2 mg L^{-1} O_2) conditions in the bottom waters of the N-enriched, eutrophic Neuse River Estuary, located in coastal North Carolina (upper left hand side). Upper right hand side shows the lateral extent of hypoxia along the central channel of the estuary during summer (data from the Neuse River Monitoring and Modeling Program, www.marine.unc.edu/neuse/modmon, see Buzzelli et al., 2002). The lower part of this figure shows both the spatial and temporal extent of hypoxia in this estuary (adapted from Buzzelli et al., 2002).

PRINCIPAL OXIDIZED NITROGEN AIRSHEDS FOR:
NARRAGANSETT BAY, CHESAPEAKE BAY,
PAMLICO SOUND, TAMPA BAY, MOBILE BAY,
LAKE PONTCHARTRAIN

*DEVELOPED BY R. DENNIS, ATMOSPHERIC SCIENCES MODELING DIVISION:
ARL, NOAA, and NERL USEPA*
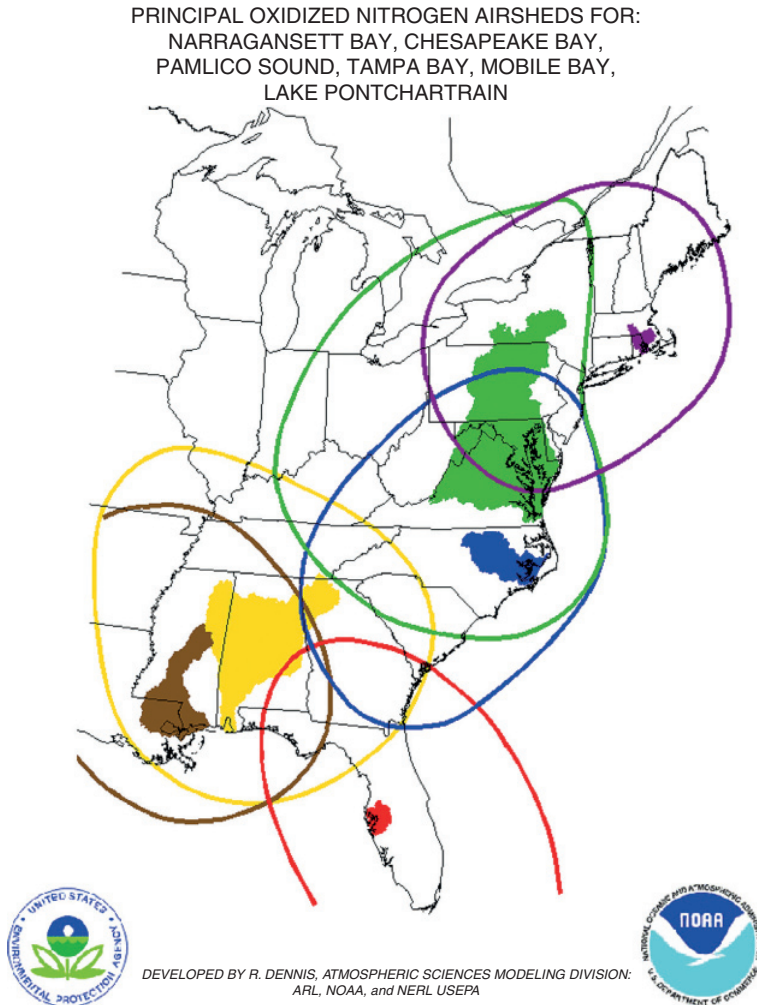
Figure 11.4 The spatial relationships between selected US Atlantic and Gulf of Mexico Coastal watersheds and airsheds. The airsheds are depicted from modeled depositional patterns of oxidized forms of N, NO$_x$ (*cf.* Paerl et al., 2002). Shown are the air- and watersheds for Narragansett Bay, Chesapeake Bay, Pamlico Sound, Tampa Bay, Mobile Bay, and Lake Ponchartrain. Note that because of their much larger sizes than watersheds, airsheds overlap for these systems. The airsheds also extend over the coastal ocean, indicating that NOx emissions in any of the watersheds have the potential to enrich coastal waters with "new" N. Developed by R. Dennis, Atmospheric Sciences Modeling Division, NOAA/ARL and NERL USEPA.

Cowling, 2002). For example, in truly oceanic locations (e.g., Bermuda), North American continental atmospheric N emissions (reduced and oxidized N) are commonly detected and significant (Luke and Dickerson, 1987; Prospero *et al.*, 1996). Likewise, islands in the North and mid Pacific receive N deposition originating on the Asian continent (Prospero *et al.*, 1989).

It has been estimated that riverine and atmospheric N inputs in the North Atlantic Ocean basin are at least equal to and may exceed N inputs by biological N_2 fixation (Howarth *et al.*, 1996; Paerl and Whitall, 1999). Therefore, our understanding of marine eutrophication dynamics needs to consider a range of scales including ecosystem, watershed, regional and global.

2. THE EVIDENCE FOR THE ROLE OF NITROGEN IN MARINE EUTROPHICATION

A vast number of studies conducted over the past 50 years have shown that N enrichment is a primary causative agent of marine (estuarine and oceanic) eutrophication (Dugdale, 1967; D'Elia *et al.*, 1986; Nixon, 1989, 1995; Ryther and Dunstan, 1971; Smetacek *et al.*, 1991). These include:

- *In situ* evidence of the spatial and temporal relationship of N inputs vs. primary production responses (Fig. 11.5)
- Nutrient addition bioassays where N enrichment has been shown to stimulate primary production (D'Elia *et al.*, 1986; Dugdale, 1967; Fisher *et al.*, 1992, 1999; Oviatt *et al.*, 1995; Paerl *et al.*, 1987, 2003; Pennock *et al.*, 1994; Piehler *et al.*, 2004) (Figs. 11.6 and 11.7)
- Paleoecological studies showing that historic increases in anthropogenic nutrient (N–dominated) loading have led to eutrophication (e.g., Chesapeake Bay, Cooper and Brush, 1993)
- Uptake studies which have shown that at ambient concentrations and supply rates, N limitation is widespread (Harrison and Turpin, 1982; Harrison *et al.*, 1987; Syrett, 1988)
- Correlative budgetary studies in which N loading or supply rates were directly related to daily or annual rates of primary production in diverse coastal ecosystems (Nixon, 1986, 1995) (Fig. 11.8)
- Stoichiometric analyses that have shown, relative to the availability of carbon (C), phosphorus (P), silicon (Si) and other nutrients, N often falls below the nutrient supply ratio needed to sustain balanced plant growth (i.e., Redfield ratio of 105:16:1 for C:N:P, Redfield, 1958; Smith, 1990)

Figure 11.5 Relationships, in time and space, between dissolved inorganic N (as nitrate, the dominant new N source), dissolved organic N, inorganic phosphorus (as orthophosphate) concentrations and phytoplankton biomass (as chlorophyll a) along the axis of the Neuse River Estuary, North Carolina. The locations shown on the y axis represent the distance between an upstream location at Streets Ferry Bridge and a downstream location below Cherry Point (both are shown as ◆ on map). Phytoplankton accumulate as distinct concentrations of biomass, which, depending on chlorophyll a levels, may be as visible blooms. During much of the year, nitrate concentrations decreased sharply concurrent with increased chlorophyll *a* concentrations forming the chlorophyll a maxima, while a similar relationship between orthophosphate and chlorophyll a is absent. This serves as in situ evidence that dissolved inorganic N supply is most likely controlling or "limiting" phytoplankton production. Dissolved organic N concentrations appear to closely track phytoplankton biomass, suggesting that phytoplankton may be a source of this form of N.
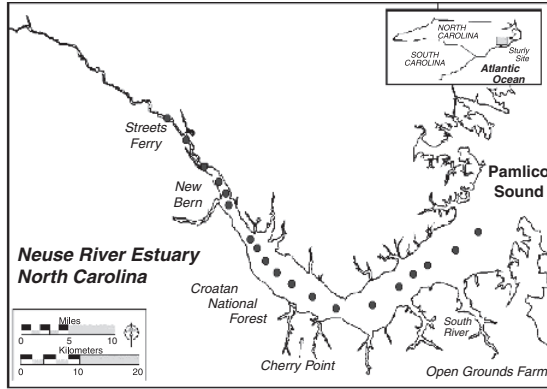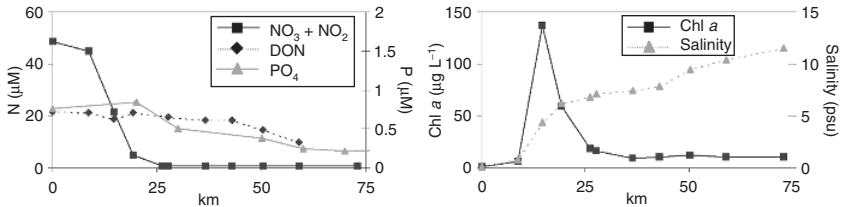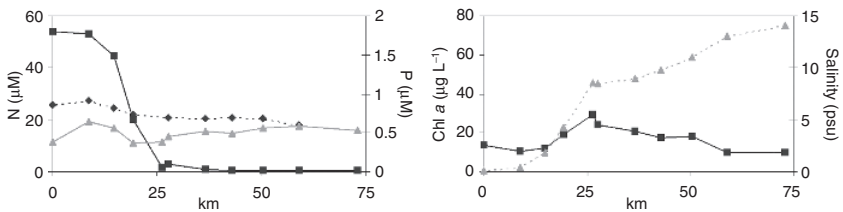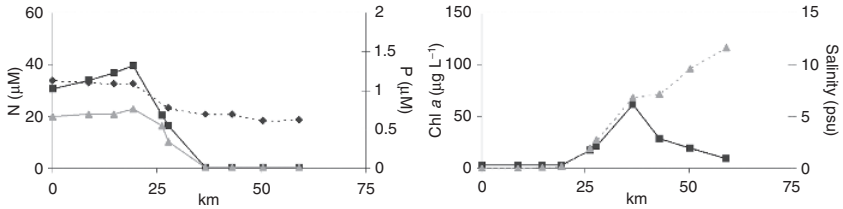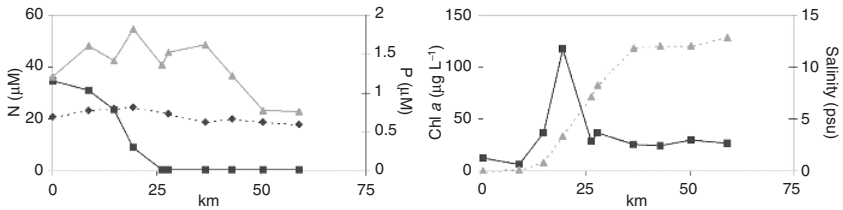
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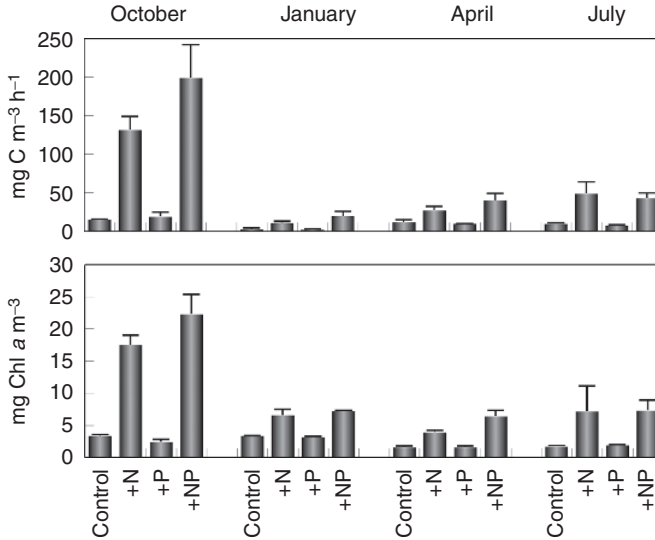
Figure 11.6 Results from seasonal in situ bioassays in the southwest basin of Pamlico Sound. Bars are means of 5 replicates and error bars are on standard deviation. The top panel shows the response of primary productivity of the natural phytoplankton community to the addition of nitrate (+N, 20 μM-N), phosphate (+P, 5 μM-P), nitrate and phosphate (+NP), and the un–amended control. The bottom panel shows the response of chlorophyll a to the same treatments. Relative to controls, strong N limitation was observed in Pamlico Sound despite high load of N to the upstream Neuse River Estuary.

- Specific case studies (e.g., Kaneohe Bay, HI, Chesapeake Bay, Neuse River–Pamlico Sound, Long Island Sound, Narragansett Bay, Baltic Sea, Coastal North Sea, Northern Adriatic Sea, Northern Gulf of Mexico) have shown that increasing N loads are directly linked to accelerated eutrophication (*cf.*, Boesch *et al.*, 2001a,b; Elmgren and Larsson, 2001, Fisher *et al.*, 1999; Nixon, 1995; Paerl *et al.*, 1998, 2004; Rabalais, 2002; Smith *et al.*, 1981; see also Chapter 18 by Boynton and Kemp, this volume)

Geochemists have pointed out that theoretically, nitrogen (N_2) fixation should compensate for N-limitation in the worlds oceans. According to this argument, P availability (which is assumed to control N_2 fixation) is ultimately limiting primary production (*cf.*, Doremus, 1982; Tyrell, 1999). This argument operates over geological time scales and requires predictable and consistent biology (i.e., N_2 fixation is solely and consistently controlled by new P inputs). However, the theory does not seem to be compatible with biological time scales and the complex environmental controls of N_2 fixation beyond phosphorus availability (Paerl, 1990; see also Chapter 3 by Carpenter and Capone, this volume and Chapter 7 by Mulholland and Lomas, this volume). In many estuarine and coastal systems, N_2 fixation does not automatically ''turn on'' when P is adequate and N is limiting. Experimental data indicate that other factors, including N:P supply ratios, iron (Fe) limitation, organic matter availability, physical constraints such as turbulence, irradiance, and potentially ''top down'' consumption processes control N_2 fixation (Howarth, 1988; Paerl, 1990). As a result, this argument has limited application to managing coastal eutrophication.
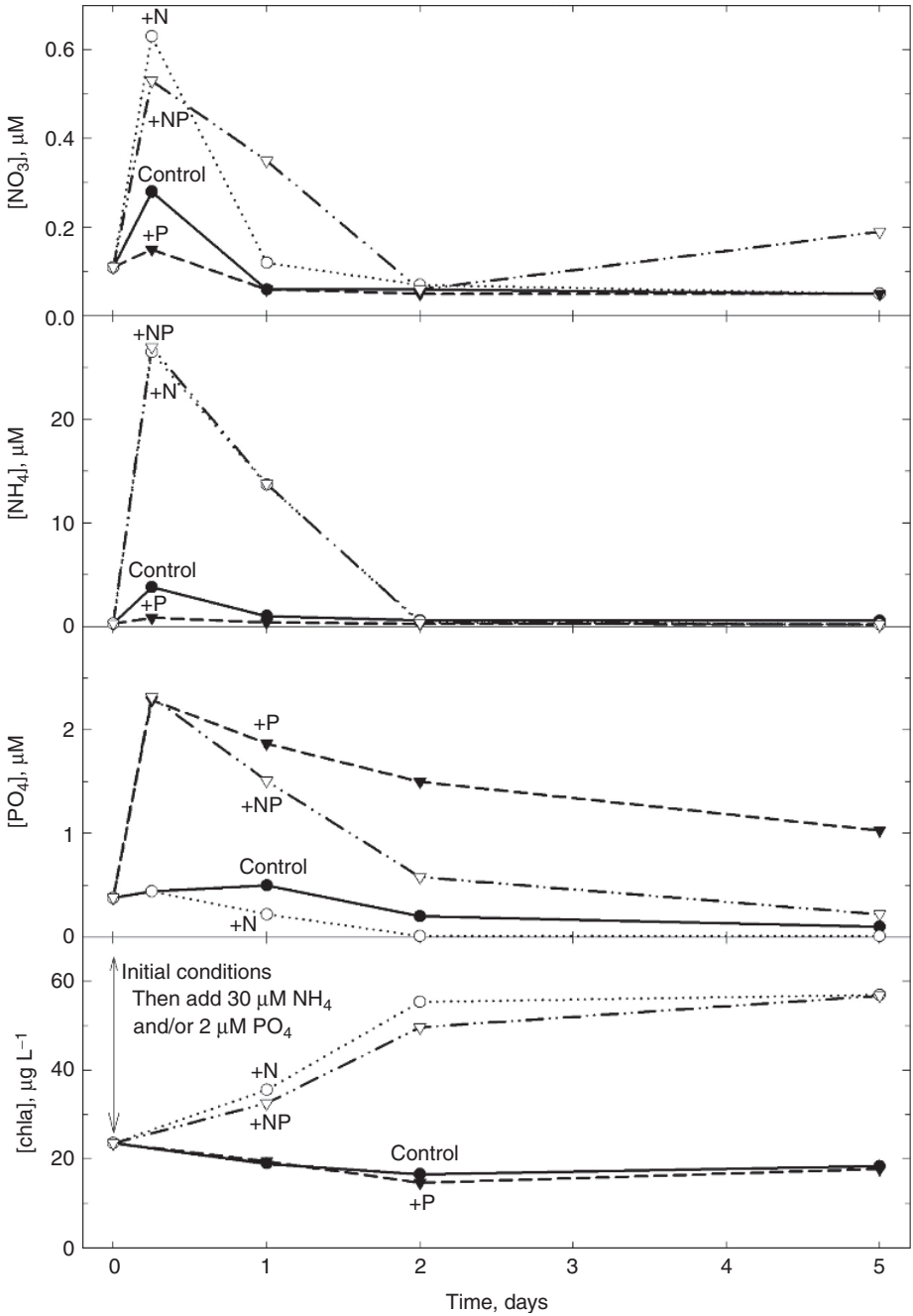
Figure 11.7 Time course of nutrients and chlorophyll *a* in the nutrient-addition bioassay performed on a Chesapeake Bay above-pycnocline water sample from station TF1.5 (Patuxent Tidal Fresh Region) in September 2000. Nutrients were added as one dose just after the initiation of the bioassay (0 days). N was added as NH_4^+ (NH_4Cl) and P was added as PO_4^{-3} (NaH_2PO_4). Added NH_4^+ was rapidly depleted, whereas excess P was present in all treatments except +N. The bioassay was incubated at 60% of surface irradiance. Chlorophyll a responded strongly to any N addition, but not in the control nor to P additions (adapted from Fisher and Gustafson, 2004).
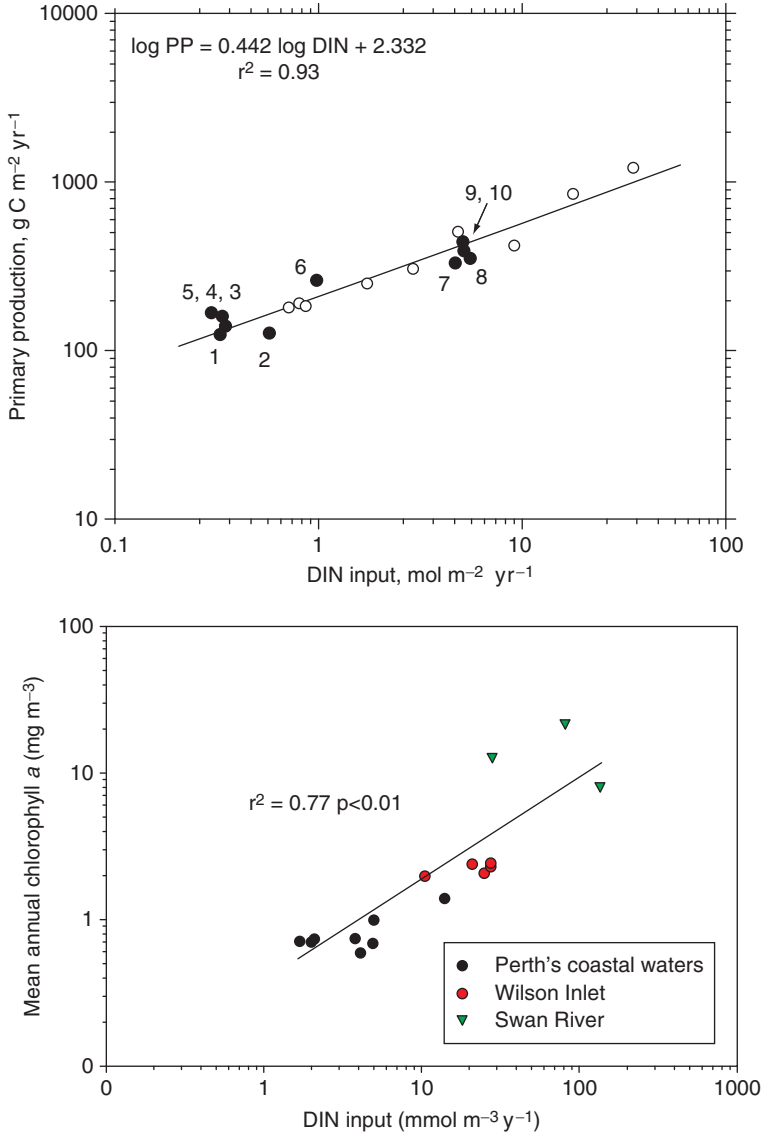
Figure 11.8 Upper frame: Direct relationships between dissolved inorganic N input and primary production in a various estuarine and coastal ecosystems★. Figure adapted from Nixon *et al.*, 1996. Lower frame: Direct relationship between dissolved inorganic N input and phytoplankton biomass, as mean annual chlorophyll *a* content of several Western Australian estuarine systems. Figure adapted from Twomey and Thompson, 2001.
★Details of systems: The open circles are for large (13 m³, 5 m deep) well-mixed mesocosm tanks at the Marine Ecosystems Research Laboratory (MERL) during a multi-year fertilization experiment (Nixon, 1995; Nixon *et al.*, 1986). Natural systems (solid circles) include; (A) Scotian shelf—DIN from Houghton *et al.* (1978), production from Mills and Fournier (1979); (B) Sargasso Sea—DIN from Jenkins (1988), production from Lohrenz *et al.* (1992) mean of 1989 and 1990 values of 110 and 144 g C m⁻² year⁻¹; (C) North Sea—DIN from Laane *et al.* (1993) assuming that the ratio of DIN/TN

Figure 11.8 cont'd in the input from the Atlantic equals that in the Channel, production from Chapter 9 by Seitzinger & Giblin (this volume); (D) the Baltic Sea—DIN and production from Ronner (1985), including DIN flux across the halocline; (E) North Central Pacific—DIN from Platt *et al.* (1984), production from Tupas *et al.* (1993) mean of 1992 and 1993 values of 150 and 185 g C m^{-2} year^{-1}; (F) Tomales Bay, CA, DIN and production from Smith (1991); (G) Continental shelf off New York—DIN and production from Walsh *et al.* (1987); (H) Outer continental shelf off southeastern U.S., DIN and production from Verity *et al.* (1993); (I) Peru upwelling—DIN calculated from annual mean upwelling rate of 0.77 m day^{-1}) and an initial 20 µM concentration of NO$_3$ in upwelled water (Walsh *et al.*, 1980), production off Chimbote from Guillen and Calienes (1981); (J) Georges Bank—DIN from Walsh *et al.* (1987). The equation is a functional regression.

The extent to which N loading promotes eutrophication varies greatly among marine ecosystems. Receiving waters exhibit varying sensitivities to N and other nutrient (P, Fe, Si) loads that are controlled by their size, hydrologic properties (e.g., flushing rates and residence times), morphologies (depth, volume), vertical mixing characteristics, geographic and climatic regimes and conditions. In addition, the magnitude and distribution of N in relation to other nutrient loads can vary substantially. In waters receiving very high N loads relative to requirements for sustaining primary and secondary production, other nutrient limitations may develop. This is evident in estuarine and coastal waters downstream of rivers draining agricultural regions that are highly enriched in N. Examples include the Po (Italy), Rhine (W. Europe), Yangtze (China) and Mississippi (USA), the Ganges (India) and the Nile (Egypt) rivers that over the past century have experienced very large increases in N loading (*cf.* Nixon, 2003; Rabalais, 2002). Here, excessive N loading may be saturating in-shore primary production, leading to either P and Si co-limitation or exclusive P and Si limitation (Conley, 2000; Dortch and Whitledge, 1992; Ferber, 2004; Lohrenz *et al.*, 1999, Sylvan *et al.*, 2006). Further offshore, receiving waters have remained N undersaturated and hence exhibit chronic N limitation (Justić *et al.*, 1995a,b; Rabalais *et al.*, 1996; Smetacek *et al.*, 1991). Because they have remained N limited even in the face of accelerated N loading, off-shore waters can support additional N-driven eutrophication (i.e., they are still in the initial stages of eutrophication) (*cf.* Codispoti *et al.*, 2001; Smetacek *et al.*, 1991).

N-driven eutrophication can exert feedbacks on internal N cycling, which may alter within-system N availability, N budgets and subsequent eutrophication potentials. Numerous studies have shown organic matter loading, sedimentation and the extent of bottom water/sediment hypoxia resulting from eutrophication can regulate key N tranformations, including nitrification and denitrification (Fear *et al.*, 2005; Heggie *et al.*, 1999; Henriksen and Kemp, 1988; Boynton and Kemp, 2000; Seitzinger and Giblin, 1996; Smith and Hollibaugh, 1989, 1998; Twilley *et al.*, 1999). These within-system feedbacks can significantly affect N availability, and hence subsequent eutrophication potentials (Eyre and Ferguson, 2002; Smith and Hollibaugh, 1998). For example, in the Baltic Sea the extent of hypoxia formation is thought to control denitrification rates and hence the ability of the system to depurate itself of fixed N (Elmgren and Larsson 2001; Wulff *et al.*, 2001). Lastly, top down effects such as grazing, and removal of grazers by overfishing (Jackson *et al.*, 2001) can significantly alter the flux, availability, utilization and manifestation of N and other nutrient inputs.

3. Nutrient, Physical and Climatic Controls of Marine Eutrophication

While anthropogenic N enrichment has been linked to N-driven eutrophication of estuarine and coastal waters (*cf.* Boesch *et al.*, 2001a,b; Nixon, 1995; Smetacek *et al.*, 1991), the manner and extent by which eutrophication affects production, nutrient cycling, trophodynamics, and habitat condition are complex products of nutrient inputs, physical features of receiving waters and climatic conditions. In arid regions, the generation, transport and fate of nutrients are quite distinct from wet regions. Similarly, polar, temperate and tropical coastal and oceanic regions can respond in contrasting manners to N and other nutrient inputs. Episodic climatic events, such as large storms and hurricanes, and extreme temperatures strongly modify the degree to which eutrophication is manifested in coastal waters.

As a result, marine waters exhibit highly individualistic responses to N loads over seasonal and longer (multi-annual, decadal) time scales. The degree to which these systems are exposed to tidal exchange and vertical mixing is critical for determining and predicting how they respond to specific N loads (Cloern, 1999, 2001; Nixon, 1995; Vollenweider *et al.*, 1992). Another variable is the manner in which N is delivered, ranging from acute pulsed events such as storms and associated flooding, to longer-term gradual (chronic) increases in N loading associated with more predictive seasonal, annual and interannual hydrologic cycles. There are striking contrasts in estuarine response to N inputs that reflect a range of hydrodynamic, optical and climatic conditions (Cloern, 1999, 2001; Sharp, 1994, 2001). North American examples include the contrast between strong tidally-driven estuarine systems like Delaware Bay and San Francisco Bay, moderately tidally-driven systems like Chesapeake Bay, micro-tidal, lagoonal systems like North Carolina's Pamlico Sound and Texas's Luguna Madre, and semi enclosed coastal systems like Florida Bay and the Long Island Sound (Bricker *et al.*, 1999). While these systems may receive comparable amounts of N, they exhibit differential biological responses. Interactions between the biological response and physical forcing features are significant. If the rate of flushing exceeds the growth and doubling rates of the resident phytoplankton communities, the system may be able to tolerate a relatively high N load without showing symptoms of eutrophication. In contrast, under similar N loading but relatively long residence time conditions, phytoplankton growth rates may be able to keep up with or exceed the rates at which populations are flushed from the system. Under these conditions, symptoms of eutrophication (algal blooms, hypoxia) are most strongly felt within the estuary, while under more highly-flushed, short residence time conditions, eutrophication may manifest itself more in downstream coastal waters (Fig. 11.9).

Variation in ecosystem sensitivity and response due to hydrologic variability can be seen in North Carolina's Pamlico Sound, the US's largest lagoonal ecosystem and second largest estuary (1700 km^2), draining numerous sub-estuaries. This system exhibits N limitation from its upstream oligohaline riverine components to the inlets
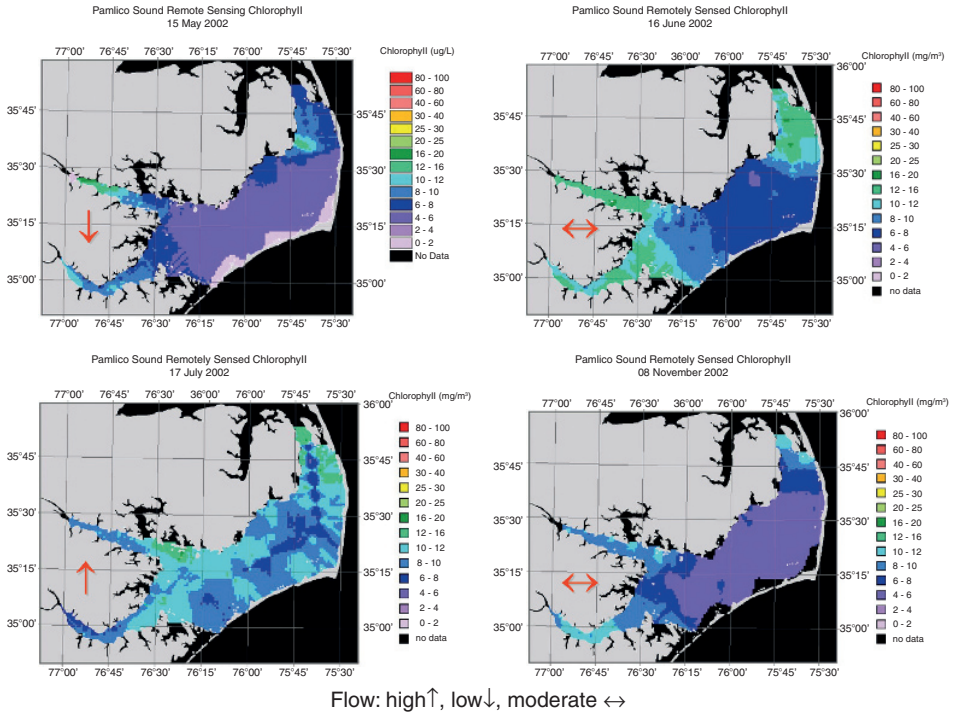
Figure 11.9 Spatial relationships between the phytoplanton biomass, as chlorophyll *a*, and estuarine freshwater discharge conditions in the Albemarle–Pamlico Sound estuarine system North Carolina, USA. Surface water chorophyll *a* concentrations were estimated using an aircraft-based SeaWiFS remote sensing system (Harding and Perry, 1997), calibrated by field samples that were analyzed fluorimetrically (Paerl *et al.*, 1995). Under relatively low flow and long residence time conditions, phytoplankton biomass is concentrated in the upstream reaches of the estuarine tributaries. Under moderate flow, phytoplankton biomass maxima extend further downstream. Under high flow (i.e., short estuarine residence time) phytoplankton biomass maxima are shifted further downstream into the open Pamlico Sound.

mediating exchange with the Atlantic Ocean (Peierls *et al.*, 2003; Piehler *et al.*, 2004; Twomey *et al.*, 2005). If high flow coincides with the period of maximum productivity (early spring through summer), the zone of maximum phytoplankton biomass accumulation (chlorophyll *a* ''maximum'') tends to be in the downstream estuarine segments (Valdes–Weaver *et al.*, 2006). In contrast, if low flow accompanies maximum productivity, or if drought conditions follow periods of high nutrient discharge, maximum biomass accumulation typically tends to occur in the upstream oligo- to mesohaline regions of the estuaries (Figs. 11.5 and 11.9). In either case, productivity may be enhanced by similar N loads, but depending on the means of N delivery (high vs. low flow discharge), ecosystem responses and impacts vary.

The Baltic Sea exemplifies the strong interactions between physical and chemical forcing features in terms of how eutrophication is expressed. There is general

agreement that excessive P and N loading have led to the eutrophication of this large brackish water system (Larsson *et al.*, 1985), although the history of productivity and algal boom responses to increasing levels of nutrient loading remains unclear (Elmgren, 1989; Elmgren and Larsson, 2001). On interannual and decadal time scales, the magnitudes, duration, biogeochemical and trophic ramifications of eutrophication vary considerably, depending on these interactions. Riverine and atmospheric inputs in the Eastern and the Southern Baltic regions have large impacts on amounts and ratios of new N and P loads controlling productivity (Conley *et al.*, 2002; Elmgren and Larsson, 2001; Savchuk and Wulff, 1999; Turner *et al.*, 1999). Furthermore, the freshwater runoff associated with large rivers draining the eastern, southern and northern Baltic influence both salinity and vertical stratification regimes throughout the sea. On the western side, dense, saltwater inflow from the North Sea is a key determinant of vertical salinity stratification, nutrient fluxes and budgets (Elmgren and Larsson, 2001; Humborg *et al.*, 2002; Savchuk and Wulff, 1999). The interaction of these physical–chemical forcing features in time and space determines the nature, distribution and duration of nutrient limitation, the magnitude and extent of manifestations of eutrophication, and the hypoxic volume of the Baltic basin. This interaction can vary substantially on interannual and decadal time scales (Elmgren and Larsson, 2001; Humborg *et al.*, 2002; Turner *et al.*, 1999). In single or consecutive years of high freshwater runoff combined with high nutrient delivery, productivity and biomass accumulation tends to be relatively high (e.g., spring non-cyanobacterial blooms, followed by large summer cyanobacterial blooms). High delivery of freshwater also maximizes vertical stratification, which in turn favors water column stability and buoyant cyanobacterial bloom species. Under these conditions maximum expression of eutrophication takes place (Wulff *et al.*, 2001). Conversely, relatively dry years tend to reduce N loading, and phytoplankton productivity and biomass accumulation are relatively low. The resultant ecosystem effect is a reduction in eutrophication potential (Conley *et al.*, 2002; Wulff *et al.*, 2001).

Another example of the interaction of physical forcings and nutrients modulating coastal eutrophication was provided by Bledsoe and colleagues (2004). In a 3-year study of the Suwanee River estuary in Florida, excessive phytoplankton growth was controlled by both nutrient loading and residence time. Maximum phytoplankton biomass was recorded during periods when loading from the river was elevated and prevailing on-shore winds decreased the flushing rate from the estuary. Their work illustrated the difficulties of managing nutrients in the context of mitigating natural circumstances such as meteorological conditions.

Very large climatic events, such as hurricanes and associated floods, can overshadow more subtle, longer-term (chronic) trends in eutrophication and water quality decline. The three sequential hurricanes (Dennis, Floyd and Irene) that struck coastal North Carolina during a six week period in fall 1999 and provided a striking example of the interacting effects of anthropogenic N loading and climatic forcing. These storms delivered up to 1 m of rainfall and caused record flooding in the largely agricultural watershed of the Pamlico Sound. The floodwaters rapidly mobilized nutrients and turned the Sound's sub-estuaries into raging rivers,

discharging nutrient enriched floodwaters directly to the Pamlico Sound (Paerl *et al.*, 2001; Peierls *et al.*, 2003). During normal hydrologic years, these N-limited estuaries remove much of the terrigenously-supplied N, either by uptake and assimilation or denitrification and dissimilation, to the extent that only a fraction of the externally supplied new N makes it past the estuarine filter into Pamlico Sound. During these years, symptoms of eutrophication (algal blooms, hypoxia and anoxia, fish kills) are largely confined to the estuaries themselves (Buzzelli *et al.*, 2002; Paerl *et al.*, 1998, 2006a,b,c) (Fig. 11.9). In contrast, floodwaters following large storm events flush the algal filter and nutrient-rich, low-salinity waters into Pamlico Sound and adjacent coastal waters (Paerl *et al.*, 2006a,c). From a eutrophication perspective, the productivity and algal bloom response takes place in the Sound and coastal waters during storm-prone periods, while during storm-free periods, the productivity response is more confined to the upstream estuarine regions (Paerl *et al.*, 2001; Peierls *et al.*, 2003, Valdes-Weaver *et al.*, 2006). Where and how this largely-anthropogenically generated N load manifests itself is a product of climatic forcing features such as storms and the resultant freshwater discharge patterns (see also Chapter 9 by Seitzinger and Harrison, this volume).

A single large summer–fall hurricane like Fran (1996) or Floyd (1999) can deliver about as much "new" N as the more predictable winter–spring wet period (Fig. 11.10). Thus, during a year supporting "normal" winter–spring runoff, accompanied by one or several summer–fall hurricanes, approximately twice the expected annual N load is delivered to downstream estuarine and sound waters. The eutrophication response to these large N loads can be seen in the form of above-average phytoplankton biomass production in the downstream waters of Pamlico Sound (Fig. 11.9) (Paerl *et al.*, 2006a,c). This demonstrates that the eutrophication gradient can be displaced in response to hydrologic discharge.

The manner and extent to which climatic conditions affect coastal N loading and eutrophication are, to some extent, dependent on land-based N sources and discharge characteristics. In rural agricultural watersheds, a large proportion (as much as 80% in coastal North Carolina) of anthropogenic N is discharged as diffuse, non-point source (NPS) loading. In contrast, in mixed urban and agricultural watersheds such as those in the Chesapeake Bay region, NPS loads account for about half the N discharge, while in largely urban watersheds such as those discharging to the Long Island Sound, point sources (PS) tend to dominate N discharge (80% PS, 20% NPS). NPS-dominated watersheds tend to be far more "leaky" in response to storm-driven N discharge events than PS dominated watersheds. On the other hand, point sources tend to dominate N discharge to estuarine and coastal waters during relatively dry periods (Howarth, 1998). The ecological and management ramifications of these contrasting scenarios are considerable. For example, if receiving waters have a tendency to develop blooms during high flow early spring-summer or fall periods, NPS controls are likely to be more effective at stemming eutrophication. On the other hand, PS controls may prove critical in controlling blooms under mid-summer, low flow conditions. In most instances, combined NPS and PS N input reduction is the most prudent and effective N management strategy for controlling eutrophication on a year-round and longer-term basis (see section VII) (Paerl *et al.*, 2006b).
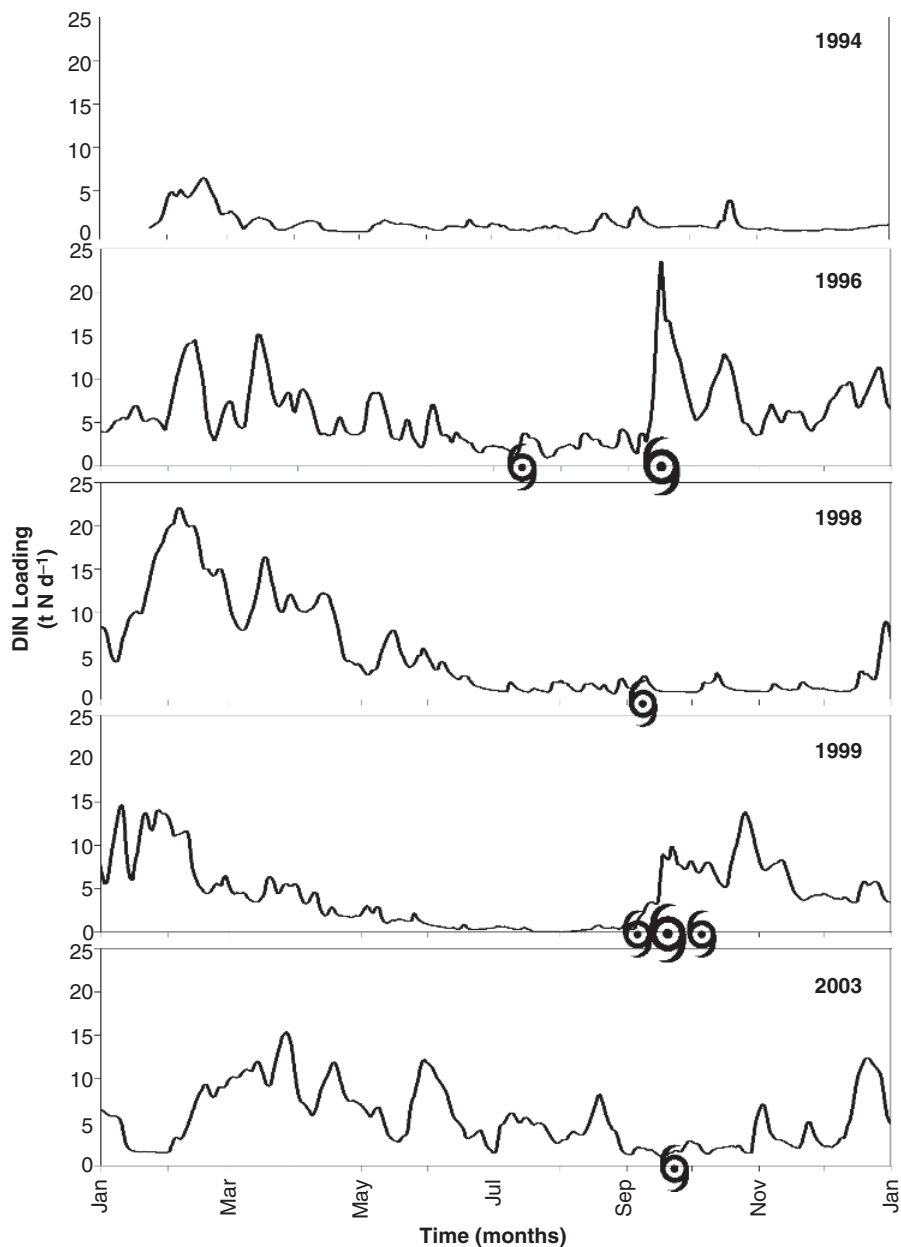
Figure 11.10 Annual patterns of dissolved inorganic nitrogen (DIN: nitrate/nitrite plus ammonium) loading to the Neuse River Estuary, NC. Years were chosen that represented different hydrologic conditions, including a drought year (1994), a year with relatively high rainfall during the typically rainy winter–spring periods, followed by low rainfall summer conditions (1998), years in which large summer–fall hurricanes affected the watershed (Fran in 1996, Dennis and Floyd, in 1999) and relatively wet years that were impacted by a coastal hurricane that delivered relatively little rainfall (Bonnie in 1998, Isabel in 2003). In years where hurricanes delivered large amounts of rainfall to the watershed (1996, 1999), hurricane-based N loads were on the same order of magnitude as spring runoff based N inputs. It is evident that rainfall amounts and periodicity have very large impacts on the delivery of land-based N to the lower estuary, receiving sound and coastal waters.

4. Is Nitrogen Nitrogen? Roles of Different Nitrogen Sources in Marine Eutrophication

Externally-supplied N comes in a various forms, including inorganic and organic, reduced (ammonium) and oxidized (nitrate, nitrite) inorganic N, all of which are potentially available to support new production and eutrophication. Laboratory experiments on phytoplankton isolates and bioassays with natural phytoplankton communities have indicated that these contrasting forms may be differentially and preferentially utilized, indicating that, depending on composition of the affected phytoplankton community, some forms are more reactive than others (Collos, 1989; Riegman, 1998; Stolte et al., 1994; see also Chapter 7 by Mulholland and Lomas, this volume). Phytoplankton community composition can also be altered by varying proportions and supply rates of different forms of N (Dortch, 1990; Harrington, 1999; Piehler et al., 2002; Pinckney et al., 1999; Stolte et al., 1994). In addition, specific N compounds may interact with light availability, hydrodynamics and other nutrients, most notably P, Si, Fe, and trace metals, to influence phytoplankton community growth rates and composition (Dortch and Whitledge, 1992; Harrison and Turpin, 1982; Smith, 1990; see also Chapter 38 by Hutchins and Fu, this volume and Chapter 37 by Kudela, this volume). These findings raise important questions about the quantitative and qualitative linkages between new N inputs and marine eutrophication:

- How does the composition of the new N pool influence new production and biogeochemical cycling in N-sensitive waters?
- To what extent does enrichment with certain N forms affect algal bloom potentials, specifically dominance by harmful algal bloom taxa (dinoflagellates, cyanobacteria, etc.)?
- Are some forms of N more potent catalysts of eutrophication than others?
- How are projected changes in human activities in coastal water- and air-sheds related to shifts in the composition of new N inputs, and can we link these shifts to predictable changes in the composition and ecological roles of primary producers catalyzing marine eutrophication?

One example of shifting N inputs is the proliferation of intensive livestock operations in coastal watersheds, which has led to large increases and changes in chemical composition of nitrogenous compounds discharged to estuarine and coastal waters via runoff, groundwater and atmospheric deposition (Galloway and Cowling, 2002; Howarth, 1998; Paerl, 1997). In general, coastal waters under the influence of these operations are experiencing increases in total N loading as well as a shift toward more reduced N (ammonium, organic N) relative to oxidized N (nitrate, nitrite) (Galloway and Cowling, 2002; Howarth et al., 2002). These increases, combined with increases in hypoxia and anoxia in receiving waters, are leading to more ammonium-rich conditions, which will favor algal species able to best exploit this N form. Alternatively, conversion of agricultural to urban lands tends to lead to more nitrate-enriched conditions, potentially favoring plant taxa best able to exploit this N form. Lastly, disruption or destruction of wetlands adversely affects their important N filtering capabilities, resulting in changes in both the amount and composition of N compounds that are processed by these ecosystems.

To improve our understanding of the biogeochemical and trophic roles "new" N composition plays in marine eutrophication, we must combine observational and experimental approaches focused on microalgal and higher plant communities responsible for eutrophication. Approaches include linking loading characteristics of specific N compounds and groups of compounds (e.g., dissolved organic matter) to microalgal growth responses in space and time. This approach relies on: (1) intensive monitoring of N inputs, their distributions and uptake characteristics in relation to microalgal community dynamics (Fig. 11.11), (2) nutrient manipulations (additions or reductions) where the community growth and compositional responses are assessed using various groups of species–specific growth/composition parameters, including microscopic observations, biochemical, molecular and automated identification and enumeration techniques (e.g., image analysis, diagnostic photopigment analyses, flow cytometry, genetic screening using probes, fluorescence *in situ*
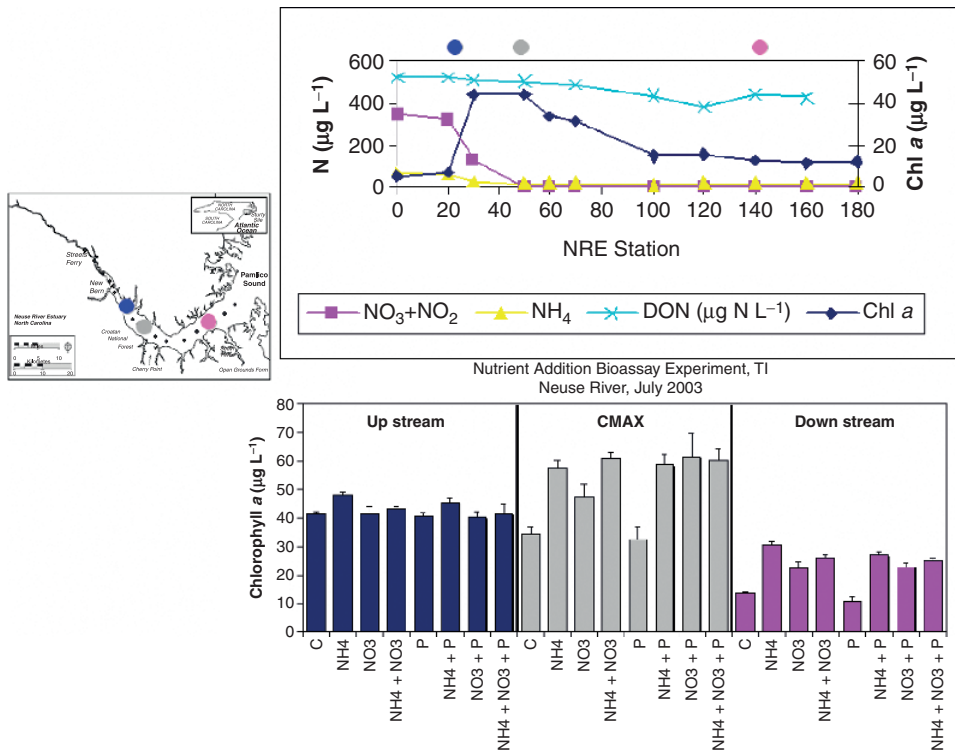
Figure 11.11 Results from a set of *in situ* nutrient addition bioassays conducted at three locations along the axis of the Neuse River Estuary that was routinely monitored for ambient nutrient (ammonium, nitrate, phosphate) concentrations and chlorophyll *a* as an indicator phytoplankton biomass. All nitrogen forms were added at 20 μM-N, while phosphate was added at 5 μM-P. The locations of bioassays are shown (symbols) on the map: (A) just upstream of the chlorophyll *a* maximum (C$_{max}$); (B) at the C$_{max}$ and (C) downstream of the C$_{max}$. Strong N limitation was encountered at the C$_{max}$ location. Downstream of the C$_{max}$, N limitation dominated and there was a strong preference for ammonium over nitrate as a DIN source.

hyridization, microautoradiography, microarrays), (3) a combination of the above, in which observational and experimental results are coupled to predictive modeling over a range of complexities, from species to communities to habitat and ecosystem levels.

The ability of some algal groups to utilize organic N, which often is the most plentiful new N source (Cornell *et al.*, 1995; Peierls and Paerl, 1997; Seitzinger and Sanders, 1999; see also Chapter 7 by Mulholland and Lomas, this volume), can also provide a competitive advantage (Antia *et al.*, 1991). For example, some heterotrophic dinoflagellates, cyanobacteria, diatoms, and brown algae (e.g., the brown tide species, *Aureococcus anophagefferens*) can assimilate diverse organic compounds over a range of naturally-occurring concentrations (Antia *et al.*, 1991; Gobler *et al.*, 2002; Kana *et al.*, 2004). In addition, members of these groups are capable of photoheterotrophy, which could be advantageous in illuminated surface waters where bloom species often thrive (Paerl, 1991). Lastly, it is well known that bloom-forming phytoplankton exchange a variety of organic and inorganic N compounds with bacterial and other algal epiphytes in consortial relationships (Paerl and Pinckney, 1996). Such exchanges can benefit the growth of both host and epiphyte, and may be particularly effective in the development and persistence of blooms in low-nutrient oligotrophic waters experiencing incipient eutrophication. Essential to these consortial associations is metabolic flexibility, particularly the ability to utilize and exchange a variety of N-containing compounds if and when they become available for supporting growth and bloom formation. This is relevant for planktonic and benthic marine cyanobacterial nuisance bloom genera (e.g., *Nodularia*, *Aphanizomenon*, *Lyngbya*), which can utilize inorganic and organic new N inputs and can also resort to N_2 fixation as an N source when external N sources are depleted. Using this "switch hitting" capability, these taxa are able to take advantage of intermittent or pulse nutrient loading events such as periods of riverine runoff following storm events, periodic upwelling and deepening of the mixed layer, atmospheric deposition (rainfall and dryfall) and groundwater loading events. This metabolic flexibility may help initiate and sustain massive blooms of these diazotrophic cyanbacteria in the eutrophied Baltic Sea, nutrient enriched coastal lagoons, and some estuaries (Paerl, 1988) as well as shallow water benthic environments (tropical reefs and intertidal flats) experiencing nutrient enrichment (La Pointe *et al.*, 1997).

Some allochthonous organic N compounds may also have chemotactic and allelopathic functions (Gallucci and Paerl, 1983; Keating, 1977; Legrand *et al.*, 2003). In these capacities, specific compounds that are excreted by some phytoplankton taxa may attract or repel other phytoplankton or bacterial taxa, and serve as cues for establishing specific associations between consortial and symbiotic partners. In this manner, organic N compounds (e.g., amino acids, peptides) may play a central and crucial role in determining microbial community composition, including establishment and proliferation of bloom species (Suikkanen *et al.*, 2004).

These results provide insight into the potential biogeochemical and trophic impacts of shifting compositions and quantities of new N compounds implicated in marine eutrophication. Both the amount and makeup of new N play critical roles in determining the magnitude and composition of primary producers. Structural and functional modifications of the primary producer community strongly influence

biogeochemical cycling and trophic (heterotrophs, grazers, herbivores and carni-
vores) responses to nutrient over-enrichment and eutrophication of estuarine, coastal
and open ocean waters.

5. THE ROLE OF NITROGEN IN RELATION TO OTHER NUTRIENTS

While excessive N loading has been implicated in marine eutrophication, it
also plays a role in the balance, availability and ecological manifestations of other
potentially-limiting nutrients, most notably P, Si and Fe (Hecky and Kilham, 1988;
see also Chapter 37 by Kudela, this volume and Chapter 38 by Hutchins and Fu, this
volume). N loading from anthropogenic sources has dramatically increased over the
past 50 years (National Research Council, 2000), to the extent that in some
receiving waters N over-enrichment has led to shortages in other nutrients required
for metabolic activity and growth. Under these conditions, N may be supplied in
excess of the stoichiometric nutrient ratios needed to support phytoplankton and
higher plant growth (i.e., Redfield ratio, Redfield, 1958). If N over-enrichment
persists for days to weeks, other nutrient limitations may, at times, dominate.
Examples can be found in the Northern Gulf of Mexico and the Northern Adriatic
Sea, regions receiving very high N loads discharged via the Mississippi and Po Rivers
respectively. Here, P limitation, N and P co-limitation and Si limitation (of diatom
growth) have been observed in the fresh and brackish water components of riverine
plumes that can extend well over 100 km into the receiving waters (Lohrenz *et al.*,
1999; Sylvan *et al.*, 2006). In these instances chronic increases in N loading from the
vast amount of agricultural land in the Mississippi and Po river basins appear to have
induced P and other nutrient limitations (Ferber, 2004, Justić *et al.*, 1995a,b). A
similar scenario is evident in the Chesapeake Bay, where elevated N loading
accompanying the spring maximal freshwater runoff period increases the potential
for P limitation (Fisher and Gustafson, 2004) (Fig. 11.12).

These shifts in nutrient limitation can lead to alterations in phytoplankton commu-
nity composition, such as a shift from diatoms towards flagellates and dinoflagellates
(Justić *et al.*, 1995b; Rabalais *et al.*, 2001; Turner *et al.*, 1998). Planktonic invertebrates,
shellfish and finfish consumers whose diets are highly dependent on the composition
and abundance of specific phytoplankton food species and groups may then be affected
(Turner *et al.*, 1998). In the Baltic and Black Sea basins, the combined effects of
enhanced watershed N loading and increased Si retention due to damming of rivers
has led to dramatic decreases in Si:N ratios in freshwater discharge to these seas. These
changes may affect nutrient cycling and food web dynamics (Humborg *et al.*, 2000).

Biogeochemical and trophic responses to altered nutrient loading stoichiometry
are intimately linked (Conley, 2000). For example, if phytoplankton composition
and biomass are altered in response to relatively high N:P and N:Si loads, the trophic
fate of this new production may change. In the case of diatoms, which are readily
grazed and packaged as fecal pellets by zooplankton, changes in their availability and
abundance may affect the downward flux and accumulation of such nutrient-rich
pellets. Furthermore, Si sedimentation via such pellets may be altered, resulting in
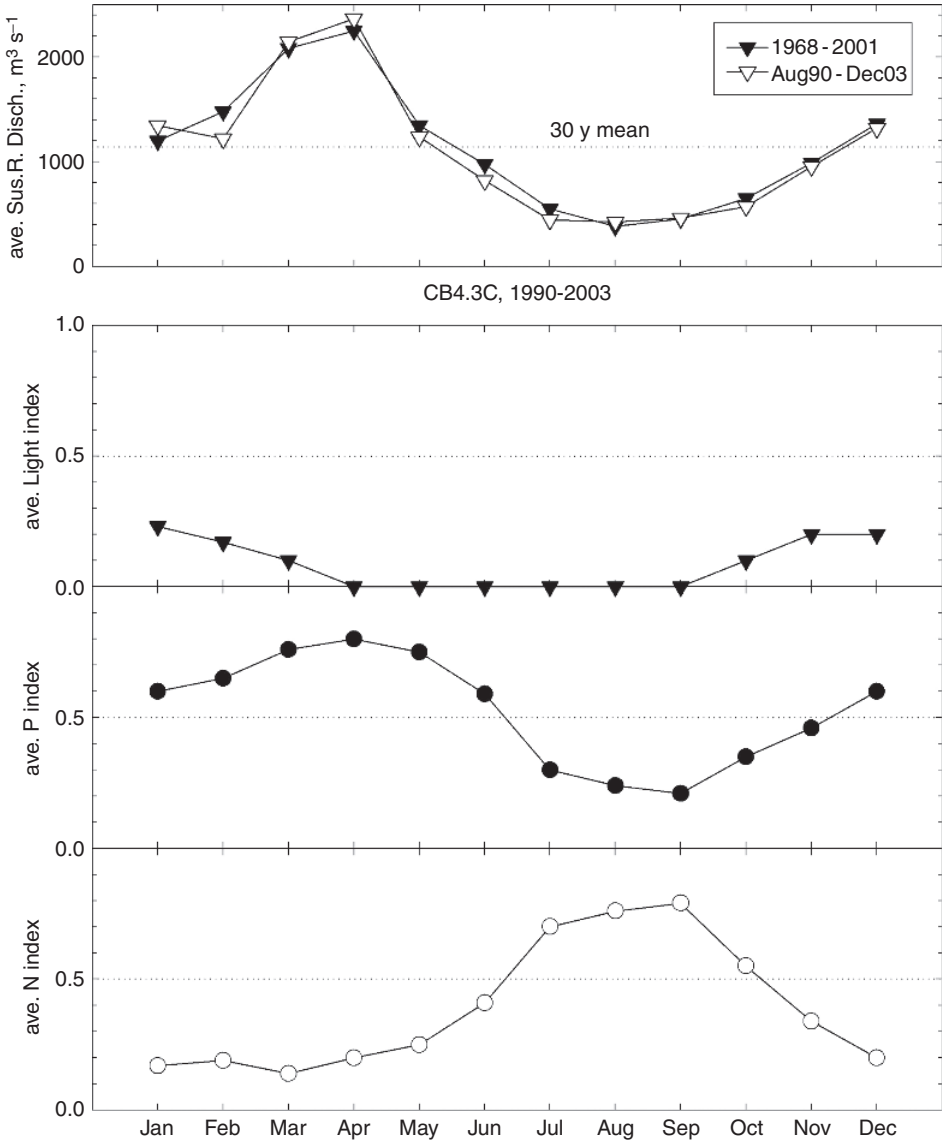
Figure 11.12 Seasonal variations in light, P, and N as limiting resources for phytoplankton at mesohaline Chesapeake Bay station CB4.3C during 1990–2003. Responses to nutrient addition bioassays were transformed to indices as described in Fisher *et al.* (1999). Values of the index >0.5 indicate the primary limiting resource. Upper panel is the average monthly Susquehanna River discharge during 1968–2001 (long term mean) and the monthly average discharge during the study period (Aug. 1990–Dec. 2003), with the 30 year mean discharge shown as the horizontal line (Figure adapted from Fisher and Gustafson, 2004).

changes in biogenic Si cycling in sediments and availability to future diatom populations. The composition of other phytoplankton groups may also be affected by anthropogenically-altered inputs and fluxes of growth-limiting nutrients. For example, taxa that are favored by increased levels of N supply relative to P and Si are likely to benefit (Dortch and Whitledge, 1992). These would include flagellates and dinoflagellates (Elmgren and Larsson., 2001; Humborg et al., 2000), and groups that include toxic and other bloom-forming nuisance species (Anderson and Garrison, 1997; Hodgkiss and Ho, 1997). Toxic, ungrazed nuisance species are likely to increase the downward flux of carbon and other nutrients, since they will be less effectively cycled in the water column. This, in turn may exacerbate hypoxia and anoxia potentials in vertically stratified waters. The ecosystem-level ramifications are multifold, including altered cycling of oxygen and nutrients, which would impact nutrient supply and availability to primary and secondary producers and trophic relations between them (including the microbial loop).

At nearshore and offshore oceanic locations, nitrogen enrichment can also affect primary production, nutrient cycling, and nutrient limitation. Nutrient addition experiments conducted on natural phytoplankton communities over a range of scales (microcosms to large-scale surface water fertilization experiments) have shown that N additions that mimic upwelling, atmospheric and riverine inputs are capable of stimulating primary production and may help initiate blooms (Paerl, 1997; Paerl et al., 1999; Zhang, 1994) (Fig. 11.13). These studies have also shown the potential for N-driven eutrophication to exist in open ocean waters. However, aside from some coastal seas such as the North Sea, Baltic, N. Adriatic, Northwest Pacific coastal regions, Sea of Japan and W. Mediterranean, the rates of N loading appear to still be too low to elicit the most obvious signs of eutrophication, such as noticeable ''greening'' of these oligotrophic waters and persistent algal blooms. The slow delivery of N and other potentially-limiting nutrients (e.g., Fe) to these waters most likely prevents such events from occurring. In contrast, acute N loading events, such as fertilizer spills from ships, riverine floodwaters extending over continental shelves (e.g., Gulf of Mexico, Amazon, Yangtze and Yellow Rivers), and experimental large-scale fertilization experiments (Kolber et al., 2002) are capable of eliciting significant increases in primary and potentially secondary production. Therefore, the potential for open ocean eutrophication exists and should be carefully monitored.

Fortunately, remote-sensing platforms specifically designed for detecting and quantifying phytoplankton biomass (as chlorophyll and other photopigments) are routinely collecting data over a range of temporal and spatial scales. In particular, NASA's satellite-based SeaWiFS ocean color sensing system can examine long-term, large-scale trends in estuarine, near-shore and oceanic primary production and producer biomass (http://seawifs.gsfc.nasa.gov/SEAWIFS.html) (Harding and Perry, 1997). This system, as well as aircraft and satellite-based multispectral (e.g., MERIS, MODIS) and hyperspectral (e.g., AVIRIS) remote sensing platforms offer an excellent opportunity to document coastal and open ocean phytoplankton (and higher plant) production baselines, against which change can be gauged over appropriate spatial and temporal scales.

An interesting result of open ocean nutrient limitation/enrichment research is the discovery that primary production in certain oligotrophic ocean gyres (Northern and
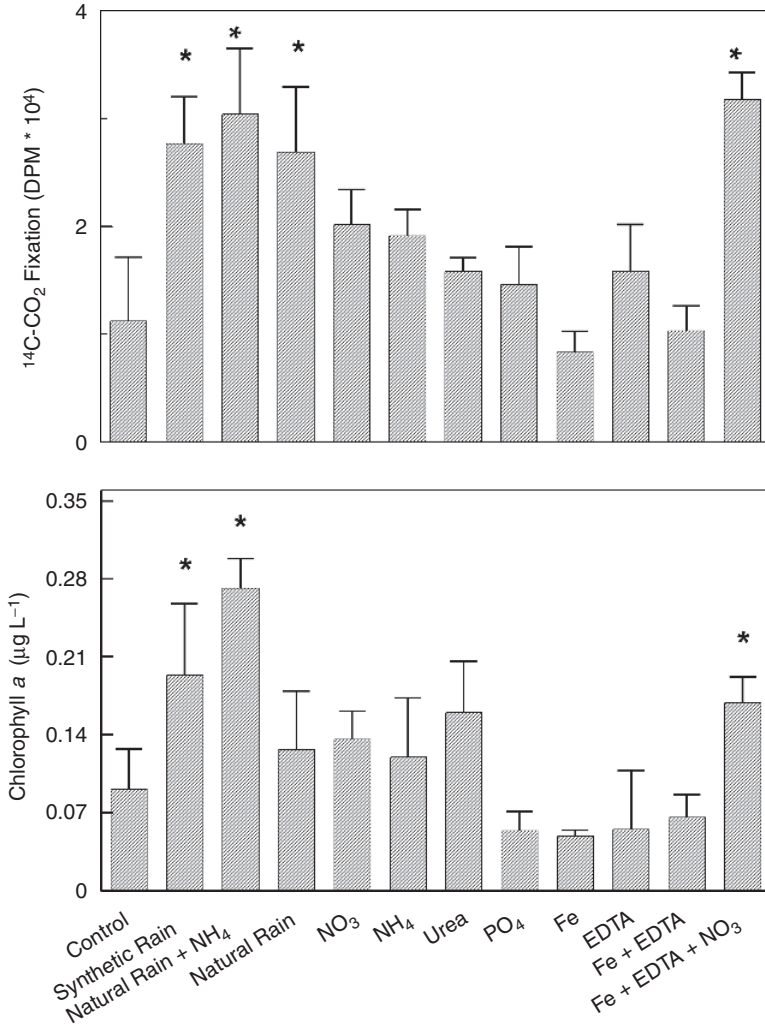
Figure 11.13 Effects of the addition of dissolved inorganic N (NH_4^+ and NO_3^-, 5 μM each), N-containing rainfall (collected at Morehead City, NC), iron (as EDTA-chelated and non-chelated $FeCl_3$; 0.2 μM each; EDTA, and the combination of NO_3^- and EDTA-chelated $FeCl_3$) on coastal Atlantic Ocean phytoplankton communities. *In situ* bioassays were conducted for 5 days on seawater collected approximately 25 km SE of Beaufort, NC in the summer of 1994. Phytoplankton growth responses are reported as photosynthetic CO_2 fixation and chlorophyll *a* accumulation. *Indicates treatments were significantly ($p < 0.05$) from controls, using Bonferroni *a posteriori* comparisons of the means. See Paerl *et al.* (1999) for details of the experimental procedures.

Central Pacific, Southern Ocean) can be Fe rather than N or P limited (Behrenfeld and Kolber, 1999; Martin *et al.*, 1994). These regions have dissolved inorganic N and P concentrations in excess of phytoplankton requirements, indicating that N enrichment is not likely to impact eutrophication potentials of these waters and that any eutrophication effect of N enrichment is reliant on parallel new Fe inputs (Kolber *et al.*, 2002).

New Fe inputs to these regions are largely atmospheric, originating from continental dust, volcanic sources, fossil fuel combustion and industrial sources (Duce *et al.*, 1996; GESAMP, 1989). For subtropical N. Atlantic oceanic waters, African Saharan dust storms appear to be a major Fe input source supporting algal bloom formation, including red tides (Walsh and Steidinger, 2001). New Fe inputs from this and other aeolian sources may in part control the assimilation of externally-supplied N as well internally-supplied N via N_2 fixation and upwelled regenerated N. A similar scenario has been proposed for a region of the Indian Ocean (Takeda *et al.*, 1995).

In more coastal/shelf regions, such as the W. Atlantic Gulf Stream off the US East Coast and off western Florida, the potential for iron limitation has also been demonstrated (Paerl *et al.*, 1999; Walsh and Steidinger, 2001). However, in these regions, Fe and N limitations appear to be closely linked and interdependent. For example, while dissolved inorganic nitrogen (DIN) additions, as either ammonium or nitrate, stimulated primary production and phytoplankton productivity (as CO_2 fixation) and biomass (as chlorophyll *a*) in oligotrophic Gulf Stream and Sargasso Sea waters off the North Carolina coast, per amount of N, ammonium often proved to be more stimulatory than nitrate (Paerl *et al.*, 1999) (Fig. 11.13). This differential response was attributed to the Fe required by phytoplankton to reduce nitrate (nitrate reductase) prior to its use as an N source to support growth. This requirement does not exist for ammonium assimilation. These results suggest that, in the case of nitrate enrichment, there may be a parallel Fe requirement, indicating that for eutrophication to proceed additions both nutrients are required. This synergistic enrichment may be provided by certain anthropogenic pollution sources, such as industrial and urban atmospheric emissions or riverine inputs enriched in both N and Fe (Church *et al.*, 1984; Duce *et al.*, 1986). Thus, the connection between human activities and marine eutrophication may need to include consideration of simultaneous multiple nutrient enrichment processes, which are influenced by agricultural, urban, and industrial activities.

6. HUMAN ACTIVITIES IN THE COASTAL ZONE AND EUTROPHICATION

Human activities in and modifications of coastal watersheds significantly affect estuarine and coastal N–driven eutrophication (Boesch *et al.*, 2001a,b; Vitousek *et al.*, 1997; see also Chapter 36 by Boyer and Howarth, this volume and Chapter 9 by Seitzinger and Harrison, this volume). Most prominent are coastal urbanization, human wastewater discharges, the destruction and modification of coastal wetlands, and large-scale agricultural and aquacultural operations (Fig. 11.1). As population density continues to grow in the coastal zone, these activities and associated pollution are becoming increasingly significant sources of N and other nutrients to coastal waters.

6.1. Human development of the coastal landscape

With increases in coastal population, and the accompanying shifts in land use (Vitousek and Mooney, 1997), come several changes that have significant impacts on the delivery of nitrogen to coastal waters. Sources of N change, and generally

increase, as human use of a coastal region intensifies. Wastewater, agricultural discharge, stormwater and industrial sources of N are among the major contributors. Another change that accompanies human development of the coastal landscape is an alteration of the transport mechanisms of N to coastal waters. Imperviousness, ditching, channelization of streams and rivers and removal of native vegetation all contribute to increased rates and quantities of N transport from the land to the adjacent water bodies. Finally, human activities in the coastal region often lead to significant loss of the natural landscapes that either retain or remove N. Wetland loss and watershed-scale landscape alterations are well documented, and contribute to the increased transport of N from human affected coastal regions. There is a cumulative impact on N transport to coastal waters from human development of the coastal landscape that includes the effects of increased sources, modified transport and altered biological processing of N (Fig. 11.1).

Sources of N that increase with human activity include sewage treatment plant discharges, fertilizers associated with agriculture and other activities (e.g., lawns, gardens, golf courses), increased atmospheric N deposition and increased stormwater carrying myriad non–point source pollutants associated with urbanization (Boesch et al., 2001a; Paerl, 1997). In the first stages of human development of coastal regions, the shifts in land use are generally from forest to agriculture and residential. Once an area is populated, the shifts in land use are then away from open space (forest and agriculture) toward residential and industrial (Beach, 2002). Through this sequence of land use alteration the N sources change and the trend is generally towards more and more N transported to coastal waters (Line et al., 2002).

Human modifications in the transport of water from land to coastal waters occur throughout coastal watersheds (Schueler and Holland, 2000). If sources of N are present, transport of water from the landscape to the aquatic system usually translates into enhanced N mobilization and loading. Urbanization leads to increased imperviousness through the presence of roads and rooftops, and imperviousness leads to accelerated transport of water from the landscape. However, imperviousness alone is not responsible for the changes in transport of stormwater and entrained pollutants (Beach, 2002). Poorly planned urban areas often exacerbate the problems associated with imperviousness through sprawling development. Development characterized by sprawl tends to have more and wider roads that have the added effect of more driving, and thus larger sources of N and other potential pollutants (Trobulak and Frissell, 2000). Improving the quality of planning during development can significantly reduce the negative impacts on water quality. A three phased approach that includes strategies to reduce the area covered by streets and parking lots, improved site design and conserve natural areas is required to address the impacts of urbanization on stormwater transport to adjacent waters (Schueler and Holland, 2000).

The retention and removal (i.e., attenuation) of N during transport through the watershed can be significantly affected by human activities. Ditching, channelization and increased imperviousness all facilitate movement of water, and thus N, to coastal waters. Reduced residence time often equates to reduced biological attenuation of the N load from a watershed. Larger quantities of faster moving waters are less likely to be effectively filtered in small headwater streams, wetlands and riparian areas. Human activities also lead to direct negative effects on natural systems that attenuate the N load from the landscape. Destruction and degradation of headwater streams

that often occurs in areas with intensive human use is likely to significantly reduce N removal and retention (Peterson *et al.*, 2001). In the US, wetland loss is on the order of 50% since the pre-settlement era and despite legal protections, this loss is likely to continue to increase (Mitsch and Gosselink, 2000). Even with laws in place to protect wetland loss, management of wetland resources presents a significant challenge (La Peyre *et al.*, 2001).

Wetlands are known sinks for N transported to them from watersheds (Reddy and Gale, 1994). The loss of wetland transformations of N (as opposed to removal of N) are also potential contributors to water quality by transforming N from highly biologically available inorganic forms to possibly less labile organic forms (Craft *et al.*, 1989). Riparian areas are also sites of significant biological removal of N via denitrification (Jacobs and Gilliam, 1985; Spruill, 2004). Their function is frequently either lost or significantly compromised by human activities, diminishing the ability of the landscape to remove N (Groffman *et al.*, 2003). Urbanization is common enough in coastal regions that nitrogen cycling research in the urban landscapes is required to understand the transport and transformation of N in these areas (Groffman *et al.*, 2002)

6.2. Agricultural activities

World-wide, coastal regions are experiencing unprecedented agricultural growth, in the form of conversion of forests, swamps and wetlands to row-crop farmland and the conversion of small family farms to intensive livestock (poultry, hogs, cattle) operations (see also Chapter 9 by Seitzinger and Harrison, this volume and Chapter 36 by Boyer and Howarth, this volume). The rapid rise in commercial fertilizer use accompanying agricultural expansion in coastal watersheds has accelerated N loading from these lands. This trend has been evident in developed regions of Europe, North America, and Australia for at least half a century (i.e., post World War II; Chambers *et al.*, 2001; Howarth, 1998; Nixon, 1995; Vitousek *et al.*, 1997). However, currently the most rapid rise in N loading from agricultural lands is occurring in developing nations in Asia, Africa, South and Central America. In addition, countries that have had traditional agricultural sectors (e.g., China, India) are using ever-increasing amounts of synthetic N fertilizers to bolster production. Not surprisingly, these countries are experiencing some of the largest increases in N discharge to N limited estuarine and coastal waters (Vitousek *et al.*, 1997). There is substantial evidence that symptoms of advanced eutrophication are proliferating in these waters, including a troublesome rise in harmful (toxic, hypoxia-inducing) algal bloom events (Hallegraeff, 1993; Richardson, 1997), hypoxia and anoxia (Rabalais, 2002).

In many watersheds along the US East and Gulf Coasts, as well as throughout Canada, agriculture is responsible for at least half the estuarine and coastal new N (and P) inputs (Table 11.1, Fig. 11.14) (Castro *et al.*, 2003; Driscoll *et al.*, 2003). This percentage is growing in regions that have yet to experience forest and wetland conversion to intensive agriculture. Clearly, this sector of human activity is a prime target for N input reductions. A variety of best management strategies aimed at reducing N losses from agricultural operations is either in place or being formulated for coastal watersheds in North America and Europe. In most mandated N reduction

Table 11.1 Comparison of P and N loading to Canadian surface and ground waters from various sources, 1996

Nutrient Source	Nitrogen (10^3/year)	Phosphorus (10^3t/year)
Municipality	80.3	5.6
Municipal wastewater treatment plants	11.8	2.3
Sewers		
Septic systems	15.4	1.9
Industry[a]	1.8	2.0
Agriculture[b] (residual in the field after crop harvest)	293	55
Aquaculture	2.3	0.5
Atmospheric deposition to water	182 (NO_3^- and NH_4^+ only)	N/A

[a] Industrial N loads are based on NO_3^- NH_4^+ and are thus DIN not TN; industrial loads do not include New Brunswick, Prince Edward Island or Nova Scotia. Quebec data are only for industries discharging to the St. Lawrence River.
[b] Agricultural residual is the difference between the amount of N or P available to the growing crop and the amount removed in the harvested crop; data are not available as to the portion of this residual that moves to surface or ground waters.
Source: Environment Canada.
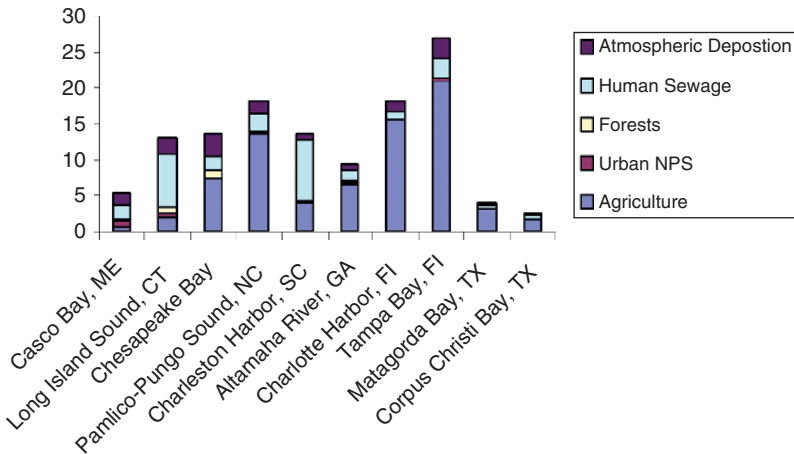
Figure 11.14 Relative contributions of atmospheric deposition, human sewage, forests, urban non-point sources and agriculture to "new" N loading in a set of representative US Atlantic (East Coast) and Gulf of Mexico estuaries. Figure adapted from Castro *et al.* (2003).

strategies that have be formulated for watersheds where agriculture constitutes a significant fraction of new N input, a 30–50% reduction of N discharge from agricultural lands can be expected. There are numerous efforts in place to meet such mandated reductions. They include; construction of riparian vegetative buffers, no-till farming practices, restrictions on timing and amount of N fertilizer application, use of organic fertilizer and the use of natural sources of new N, such as soybeans and other leguminous plants that are rotated with N demanding crops such as cereals (corn, wheat, rice), fiber crops (cotton, flax) and vegetables.

While nitrate is a dominant new N source from agriculture, ammonium and organic N can also be significant waste products, especially from animal operations where reduced N tends to accumulate in waste stored in "lagoons" or sprayed as fertilizer on surrounding fields. Discharge from agriculture is dominated by surface runoff (Boesch *et al.*, 2001a,b). Subsurface and atmospheric N inputs can also be large from intensive animal operations and feedlots (Paerl, 1997; Walker *et al.*, 2004; Whitall and Paerl, 2001). Each of these sources is significant enough to warrant their inclusion in basin-wide and regional N reductions aimed at improving water quality (e.g., EPA's Total Maximum Daily Loads or TMDLs; US EPA, 1999b).

6.3. Aquaculture/mariculture

As a means of sustaining coastal fisheries-based economies and as a source of animal-based protein, coastal aquaculture and mariculture (jointly termed aquaculture) have grown rapidly. In part, the need for and success of aquaculture can be attributed to the depletion of traditional estuarine and coastal fisheries in many regions of the world, including Asia, Europe, North and South America. Aquaculture has also proven more profitable and attractive than many sustenance fisheries because highly-desired, high-income specialty fisheries are often amenable to aquaculture. These include shellfish (oysters, clams, mussels), crustacean (shrimp, lobster, crab, crayfish), and finfish (salmon, trout, catfish, striped bass, tilapia) operations that are expanding worldwide. A vast majority of these operations depend on fertilization and/or input of food supplies to sustain productivity. As with intensive agricultural operations, ensuring adequate N supply is critical to maximizing aquacultural production. Invariably, this translates into administering high doses of fixed N, of which a significant fraction, ranging from a few percent to well over 50% is released to the surrounding waters (Leung *et al.*, 1999; Tovar *et al.*, 2000; Wu, 1995). While overall, aquaculture represents a much smaller N input source than agriculture, from an N retention perspective, aquaculture operations can constitute localized intensive sources of N fertilization and potential drivers of eutrophication in nearby N-limited waters. The unintentional eutrophicating effects of aquaculture have already become evident in coastal net-pen fish (salmon in particular) operations in semi-enclosed (i.e., poorly-flushed) lagoons and embayments (fjords) in Northern Europe (Denmark, Norway, Sweden, Scotland), North and South America (Western Canada, New England and the Canadian Maritime provinces, Colombia, Brazil and Chile. In particular, the Scandinavian fjords have experienced symptoms of advanced eutrophication in response to excessive fertilization associated with these as well as agricultural operations (Christensen *et al.*, 2000). Additionally, the

eutrophication related impacts that result from these operations are complicated by the use of antibiotics in feed formulations. Antibiotic introduction could affect microbial antibiotic resistance, and hence alter microbial community composition and activities in response to nutrient enrichment. In Asia and South America, brackish to full-salinity coastal lagoonal ecosystems are being used to produce shrimp, shellfish and a variety of finfish species. These systems represent concentrated nutrient sources adjacent to low-nutrient-adapted estuarine and coastal habitats, including mangroves, mudflats, marshes, coral reefs, and oligotrophic near-shore surface waters (Wu, 1995). Although little work has been done to detect and characterize ecological responses to the nutrient enriched effluent from these operations, previous experimental work indicates that both increases in benthic and planktonic microalgal production, epiphytization of seagrasses and corals, shifts in plant in producer and grazer community composition are among the responses accompanying eutrophication of receiving waters (D'Elia et al., 1981; Johannes, 1975; LaPointe et al., 1997; Laws and Redalje, 1979). The global expansion of aquaculture represents a potential threat to both water quality and habitat integrity of ambient waters, many of which support flora and fauna adapted to oligotrophic conditions.

7. The Future and Nitrogen Management

It is clear that N over-enrichment due to human population growth in coastal water- and air-sheds will remain a key driver of marine eutrophication (Peierls et al., 1991; Vitousek et al., 1997) well into the foreseeable future, especially in the rapidly-developing regions of Asia, Africa, and South America. The growing N "glut" that accompanies anthropogenic encroachment on the coastal zone will continue to enhance primary production and accelerate eutrophication. Because few receiving waters show any indication of becoming N saturated, the potential exists for coastal and oceanic waters to exhibit further N-enhanced eutrophication. While some upstream segments of estuarine ecosystems that have experienced a lengthy history of excessive N enrichment at times experience N saturation and show spring N & P co-limitation or even P or Si limitation (Fisher and Gustafson, 2004, Fisher et al., 1999; Justić et al., 1995a,b; Sylvan et al., 2006), they continue to show an overall high degree of sensitivity to N enrichment. This is especially true for the broader downstream mesohaline and euhaline portions of estuaries, where the combined effects of denitrification and relatively abundant and readily recycled marine P and Si supplies preclude limitation by these elements.

Therefore, preservation of acceptable water quality and reversal of eutrophication in these systems will invariably include reductions of current loads and setting limits on new N input, either alone or in combination with other nutrient (e.g., P) input reductions. Freshwater nutrient management has, in many instances, successfully arrested and reversed eutrophication by reducing P loadings (Edmondson, 1970; Likens, 1972; Vollenweider, 1982). Indeed, the upstream freshwater portions of many estuaries are also P-limited (Boynton and Kemp, 2000; Fisher et al., 1999), and P input constraints in these waters have been quite successful in reducing the unwanted symptoms of eutrophication (nuisance algal blooms, toxicity, hypoxia,

etc.) (Elmgren and Larsson, 2001; Paerl *et al.*, 2004, 2006b; Vollenweider *et al.*, 1992). As estuaries drain to the seas, both salinity and N limitation increase (Ryther and Dunstan, 1971; Nixon, 1986, 1995). However, reducing N alone can create favorable conditions for N_2 fixing phytoplankton and present the possibility of the return of N to the system through N_2 fixation (Horne, 1977; Piehler *et al.*, 2002). Therefore, along the freshwater-estuarine-coastal transitional zone, both N and P input constraints are likely to be needed if we are to control and manage marine eutrophication (Cloern, 2001; Conley, 2000; Paerl *et al.*, 2004, Sylvan *et al.*, 2006). This calls for addressing nutrient management along the integrative watershed-to coastal ocean (and beyond) scale; a scale that is no doubt challenging, but essential if we are to consider effective, long-term nutrient management aimed at preserving the entire suite of biotic resources involved in and affected by marine eutrophication.

Coastal waters are sensitive to both human and natural perturbations. For example, accelerating nutrient, sediment, and toxics inputs, may be accompanied by climatic, geochemical (i.e., volcanism, subsidence) and other forms of natural change. There is evidence that certain manifestations of climate change, including tropical storm and hurricane frequency, may also be increasing (Goldenberg *et al.*, 2001, Webster *et al.*, 2005). It is therefore useful to develop management approaches and indicators of ecosystem response that could help distinguish human from natural perturbations.

Determining and evaluating the ecological response of marine ecosystems to N enrichment and interacting physical-chemical perturbations is highly dependent on the indicators used and sampling sites selected. Benthic indicators may tell quite a different story from planktonic ones in ephemerally-stratified system (e.g., Chesapeake Bay, Pamlico Sound, Mobile Bay, Florida Bay). In well-flushed (short water residence time) systems, phytoplankton growth responses to nutrient enrichment may not be nearly as profound as those for benthic microalgae. Here, benthic microalgae and associated infauna may be more sensitive and meaningful indicators of ecosystem response to nutrient enrichment. In addition, indicators of community structure (i.e., diversity indices, keystone species) may gauge ecosystem conditions quite differently than indicators of function (primary and secondary production, respiration, nutrient cycling). Indices of biotic integrity (IBI) and habitat suitability indicators (HBI) are specific examples of indicators that in combination can assess structure, physical-chemical quality and biological measures.

To link N and other nutrient inputs to eutrophication dynamics across diverse coastal and oceanic systems, specific, broadly-applicable and integrative indicators that can couple biotic community structure to function in the context of ecological condition and change are needed (*cf.* Bartone, 2005; Committee on Environment and Natural Resources, 1997). Using case studies representing a range of impacted habitats, we need to develop and assess indicators of water quality, food web and habitat condition in response to a range of physical, chemical and biotic perturbations in marine ecosystems varying in water residence time, climatic regime and trophic state. Emphasis should be placed on microbial, plant and animal taxa and assemblages capable of providing qualitative and quantitative responses to nutrient and other stressors. These indicators should have sufficient detail to help clarify mechanisms underlying ecological change in response to external perturbations, with the ultimate goal of identifying indicators that will help researchers and

managers quantify roles of key organisms mediating production, energy flow, nutrient and oxygen cycling aspects of eutrophication. Combining these specific indicators with modeling efforts will help clarify and distinguish anthropogenic from natural stressors in diverse marine environments.

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NITROGEN UPTAKE IN THE SOUTHERN OCEAN

William P. Cochlan

Contents

1. INTRODUCTION

Nitrogen (N) is generally considered to be the most important macro-nutrient limiting primary production in neritic and oceanic regions of the ocean. This is not the case in the Southern Ocean, where elevated concentrations of nitrate (NO_3^-) persist throughout the growth season, and phytoplankton biomass remains modest—the so-called ''Antarctic Paradox'' (e.g., El-Sayed, 1987; Priddle *et al.,* 1998). In this respect, the Southern Ocean can be classified as a High Nutrient, Low Chlorophyll region (HNLC; Minas *et al.,* 1986). The exact mechanisms responsible for the relatively low biomass and lack of NO_3^- depletion common to HNLC areas are still unknown, but explanations have generally been divided into either ''top-down'' (ecological) or ''bottom-up'' (physiological) controls; in other words those controlling ''yield'' versus ''rate.'' However, the Southern Ocean should not be considered as a single marine ecosystem, and this vast polar ocean is much more heterogeneous than originally thought. Seasonal and spatial dynamics of nutrients and primary productivity are strongly influenced by the presence of complex frontal features associated with the major current systems and bottom topography, and the annual advance and retreat of sea ice, resulting in considerable variability in phytoplankton production and the relative use of N substrates.

The Southern Ocean encompasses a region that is 10–20% of the world ocean (Laws, 1985; Boyd, 2002), and comprises two major current systems: the strong,

eastward flowing Antarctic Circumpolar Current (ACC) and the more southerly, but westward flowing Antarctic Coastal Current. The pelagic ecosystem can be divided into a series of distinct environments (or biomes) using a number of different criteria, including the distribution of the essential micronutrient iron (Fe) regulating phytoplankton growth (cf. Longhurst, 1998). Due to the complexity of the region, there is often duplication of Southern Ocean terminology resulting in some confusion. I refer the reader to a comprehensive review of the Southern Ocean and its biomes by Longhurst (1998) for a more complete description of the physical and biological characteristics of Southern Ocean oceanography.

The biogeochemical provinces of the Southern Ocean, as defined by Tréguer and Jacques (1992), are the Polar Front Zone, the Permanently Open Ocean Zone, the Marginal and Seasonal Ice Zones, and the Coastal and Continental Shelf Zone (Fig. 12.1). Note that the Marginal Ice Zone is a seaward subzone of the Seasonal Ice Zone delimited by the recent melting of ice, a seasonally migrating band of recently exposed surface waters within the Seasonal Ice Zone (Moore *et al.,* 2000). The
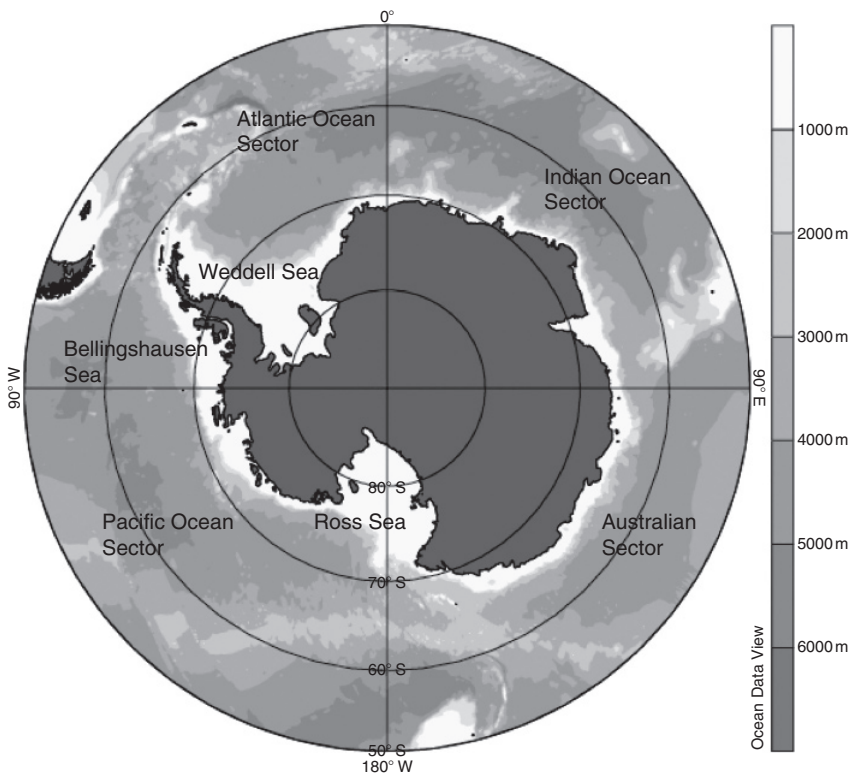
Figure 12.1 The sectors of the Southern Ocean and the four biogeochemical provinces: the Polar Front Zone, the Permanently Open Ocean Zone, the Marginal and Seasonal Ice Zones, and the Coastal and Continental Shelf Zones.

terminology of Tréguer and Jacques (1992) is commonly used in the polar N literature, and will be employed throughout this chapter.

The concept of new and regenerated production has been used for over three decades as a basis to link surface N dynamics with biogenic particle flux (Dugdale and Goering, 1967; Eppley and Peterson, 1979). Under steady-state conditions and averaged over appropriate time scales, new production is the proportion of total production that can be exported from the euphotic zone, mainly through the sinking of particles, without depleting the system; this proportion is also known as the f-ratio, the ratio of new to total production (Eppley and Peterson, 1979). Traditionally, new production has been equated with the uptake of ^{15}N-labeled NO_3^-, which is allochthonous (from outside of the system) in open water systems, and regenerated production with the uptake of ^{15}N-labeled ammonium (NH_4^+)and more recently ^{15}N-labeled urea, which are both autochthonous in that their presence relies on metabolic recycling in the upper water column. Recent evidence, including substantially higher N_2 fixation rates, aeolian inputs of organic and inorganic N, nitrification within the euphotic zone, and $DO^{15}N$ release may require the original model to be reevaluated for some regions (e.g., Bronk $et\ al.,$ 1994). In practice, f-ratios are generally estimated as the proportion of NO_3^- uptake to the total N (NO_3^- plus NH_4^+ and sometimes urea) uptake.

In the Southern Ocean, the f-ratio has been estimated for surface waters using ^{15}N uptake techniques in a number of investigations since 1977. Studies include those conducted in the Indian Ocean Sector (Collos and Slawyk, 1986; Lucas $et\ al.,$ 2007; Mengesha $et\ al.,$ 1998; Probyn and Painting, 1985; Semeneh $et\ al.,$ 1998; Slawyk, 1979), the Ross Sea (Cochlan and Bronk, 2001, 2003; Cochlan $et\ al.,$ 2002a; Goeyens $et\ al.,$ 2000; Hu and Smith, 1998; Lipizer $et\ al.,$ 2000; Nelson and Smith, 1986; Olson, 1980), the Scotia Sea (Cota $et\ al.,$ 1992; Glibert $et\ al.,$ 1982; Goeyens $et\ al.,$ 1991a,b; Koike $et\ al.,$ 1986; Olson, 1980; Owens $et\ al.,$ 1991; Rönner $et\ al.,$ 1983) the Weddell Sea (Cota $et\ al.,$ 1992; Goeyens $et\ al.,$ 1995; Kristiansen $et\ al.,$ 1992; Rönner $et\ al.,$ 1983; Smith and Nelson, 1990), the Antarctic Peninsula (Cochlan $et\ al.,$ 1993; Probyn and Painting, 1985); the Bellingshausen Sea (Waldron $et\ al.,$ 1995; Bury $et\ al.,$ 1995), the Australian Sector (Savoye $et\ al.,$ 2004; Elskens $et\ al.,$ 2002), and the Pacific Ocean Sector (Cochlan $et\ al.,$ 2002b; Coale $et\ al.,$ 2004; Sambrotto and Mace, 2000).

While this list is not exhaustive, a few notable trends have emerged. First, despite the abundance of NO_3^-, the Permanently Open Ocean Zone generally supports low rates of N uptake (except in proximity to islands), and the rates are dominated by regenerated N, resulting in low f-ratios (averaging ~0.2–0.5) and no measurable declines in surface NO_3^- concentration. Second, in the Coastal and Continental Shelf Zone and the Seasonal Ice Zone, N uptake patterns are more similar to those found in temperate upwelling zones, but with much lower uptake rates. Here, water column stabilization and cell seeding from retreating sea ice allow for considerable bloom development and substantial declines in surface NO_3^- with initial N-nutrition dominated by new N, resulting in high f-ratios (averaging ~0.7–0.9). Third, the seasonal increase in regenerated production in both regions is generally accompanied by an increase in surface concentrations of NH_4^+, and a transition from a predominately diatom- to a flagellate-dominated community, although not always. Others

have previously reviewed the dynamics of N nutrition in the Southern Ocean for both open ocean and marginal ice zones (e.g., Bury *et al.,* 1995; Goeyens *et al.,* 1995; Jacques, 1991; Tréguer and Jacques, 1992), including their relationship to ambient concentrations of silicic acid ($Si(OH)_4$, abbreviated Si; Goeyens *et al.,* 1998), and thus will not be repeated here. In this chapter, I will instead attempt to summarize the basic environmental factors regulating N uptake in the Southern Ocean, rather than concentrate on regional and/or seasonal differences. These environmental factors include: iron and light availability, NH_4^+ concentration and its inhibitory effects on NO_3^- uptake, the low ambient seawater temperature, and the role of heterotrophic bacteria as competitors for N in the Southern Ocean.

2. Environmental Factors Regulating Nitrogen Uptake

2.1. Micro-nutrient iron

Iron (Fe) is now recognized as an important factor regulating the productivity of many areas of the ocean, specifically those regions characterized as HNLC areas where rates of primary productivity and concentrations of biomass are lower than expected based on the availability of NO_3^-, phosphate, and Si. The key aspect of the nitrogenous nutrition of phytoplankton in these HNLC regions is the failure of primary production to fully utilize the abundant NO_3^- reserves during the growing season. In the open ocean regions of the Southern Ocean, the apparent "Antarctic Paradox" has been observed for decades, and Fe's possible role as a bio-limiting factor in the underutilization of surface macro-nutrients and production of modest biomass has been long suggested (Gran, 1931; Hart, 1934 as cited by de Baar *et al.,* 1990). Indirect evidence for the role of Fe in the Southern Ocean was first provided by de Baar *et al.* (1995) who demonstrated a positive relationship between the magnitude of *in situ* phytoplankton biomass and ambient Fe concentrations in the vicinity of the Antarctic Polar Front in the South Atlantic sector during a natural Fe fertilization event during austral spring 1992. However, the first studies to provide direct evidence of Fe-stimulated phytoplankton growth were conducted in the Southern Ocean in 1988 in the Weddell/Scotia Seas (de Baar *et al.,* 1990; Buma *et al.,* 1991), and later in 1990 in the Ross Sea (Martin *et al.,* 1990; 1991) and the Drake Passage in 1991 (Helbling *et al.,* 1991) using shipboard Fe-enrichment experiments.

   The early, multi-day, shipboard incubation experiments exhibited Fe-enhanced phytoplankton biomass (relative to un-enriched controls) over time, but generally long lag periods were observed. Both Fe-enriched and control treatments showed significant increases in chlorophyll *a*, reaching levels not normally observed *in situ*, presumably due to reduced grazing pressure and a more favorable light environment within the bottles (e.g., de Baar, 1994). These and more recent deck–board experiments (e.g., Sedwick and DiTullio 1997; Sedwick *et al.,* 1999) report Fe-enhanced draw-down rates of NO_3^- over time, with NO_3^- often declining to levels below detection (e.g., 0.03 μM) after many days of incubation, a situation rarely observed

in the Southern Ocean except at intense ice-edge (e.g., Nelson and Smith, 1986) and coastal blooms (e.g., Holm-Hansen *et al.,* 1989; Kocmur *et al.,* 1990), or in strongly stratified fjords (e.g., McMinn *et al.,* 2000). However, it has been suggested that due to the concomitant increase in biomass following Fe enrichment, such enhanced rates of NO_3^- utilization are not necessarily due to any actual increase in the NO_3^- uptake capability of the resident phytoplankton. In a re-analysis of the NO_3^- depletion data from the Ross Sea Fe-experiments of Martin *et al.* (1990), Dugdale and Wilkerson (1990) contend that Fe had no effect on specific NO_3^- uptake rates, despite the enhancement in NO_3^- depletion rates, and when absolute rates were normalized to an appropriate biomass parameter (particulate N) the resultant specific uptake rates of NO_3^- in the Fe-treatments did not differ from those of the controls.

Since then, a number of similar deck-board experiments have been conducted using ^{15}N isotopes to estimate specific N uptake rates from short-term incubations (3–24 h) determined at either the beginning (Timmermans *et al.,* 1998), the end (Franck *et al.,* 2000), both (van Leeuwe *et al.,* 1997), or throughout (Cochlan *et al.,* 2002a) multi-day, experimental bioassays. In each of these studies, specific NO_3^- uptake rates were enhanced following the addition of Fe. In the HNLC waters just north of the sub-Antarctic Front and south of the Antarctic Polar Front in the Pacific region of the Southern Ocean, Timmermans *et al.* (1998) report significant (1.25–1.45-fold) increases in specific $^{15}NO_3^-$ uptake rates even after the first day (27 and 29 h), whereas specific $^{15}NH_4^+$ uptake rates (only done south of the Antarctic Polar Front), remained unaltered by Fe additions. In the ACC/Polar Front Zone/Weddell Sea regions, modest (0–1.3-fold) increases are reported by van Leeuwe *et al.* (1997) for specific $^{15}NO_3^-$ uptake rates conducted at the beginning of their experiments, with (1.4–1.7-fold) increases found at the end (9–12 d) of their bioassays. In the Pacific sector of the Southern Ocean, similar increases (1.6–1.8 and 1.1–2.5-fold) are reported for specific $^{15}NO_3^-$ uptake rates determined 10–16 d after Fe enrichment in the high Si (also HNLC) waters south of the Antarctic Polar Front during austral spring and summer, respectively, whereas greater (2.9–4.4-fold) increases were found in the low Si waters north of the Antarctic Polar Front (Franck *et al.,* 2000). In the Ross Sea, Fe-enrichment experiments were conducted close to the Ross Ice Shelf and further north in HNLC waters (near the original Martin experiments) during austral summer 1997 (Cochlan *et al.,* 2002a). During this study, specific NO_3^- uptake rates were also enhanced by Fe additions, although as found by van Leeuwe *et al.* (1997) uptake rates for the Fe-treatments did not differ substantially from the uptake rates observed in the controls during initial sampling periods. It is not until the later sampling periods (i.e., after 3 d), that the specific NO_3^- uptake rates were >1.9-fold and >2.6-fold higher than rates measured in the controls for the continental shelf and HNLC sites, respectively. Specific uptake rates of NH_4^+ were not enhanced by Fe at their HNLC site (actually they were negatively correlated with increasing Fe concentration), but the 30–40% increase in NH_4^+ uptake in the higher Fe enrichments during the later sampling periods for the continental shelf station was explained as a concentration-dependent function of the increasing NH_4^+ concentration found during that time. In the Ross Sea study, specific NO_3^- uptake rates also were reported to be significantly correlated to the

Fe concentrations measured at the start of the bioassays, and based on the estimated half-saturation constant (K_s) of 0.09 nM Fe (by employing Michaelis–Menten type kinetics), the availability of Fe during that time (ambient dissolved Fe concentration of 0.03–0.04 nM) would appear to limit NO_3^- uptake by phytoplankton. These studies all demonstrate a clear enhancement of specific and absolute NO_3^- uptake rates by Fe during short-term incubations, and are supported by similar results conducted in other HNLC regions including the equatorial Pacific Ocean (Coale et al., 1996; Cochlan, 2001; Price et al., 1991, 1994) and the eastern (Boyd et al., 1996, 1998; Marchetti et al., 2006) and western (Kudo et al., 2005, in review) subarctic Pacific Ocean.

The short-term N uptake studies, together with the overall enhancement in biomass accumulation and macro-nutrient (NO_3^- and phosphate) decline seen during multi-day, Fe-enrichment shipboard incubation experiments, provide strong support for Fe-limitation in HNLC areas of the Southern Ocean. However, even though such long-term incubation experiments reflect many of the in-situ chemical and biological processes occurring in the natural ecosystem, loss terms such as grazing by larger zooplankton, and sinking/sedimentation of diatoms, are altered within bottles and may have a significant effect in natural ecosystems. Consequently, extrapolation of these experiments as accurate representations of the in situ response have been strongly criticized (e.g., Banse, 1991; Dugdale and Wilkerson, 1990, 1991; Frost, 1991). In contrast, in situ experiments provide a cohesive link between the addition of a potentially limiting resource and the biological response, without uncertainties such as light field alteration, reduced turbulence, and exclusion of grazers (Carpenter et al., 1995; Hecky and Kilham, 1988; Watson et al., 1991).

Twelve such in situ Fe-enrichment experiments have now been conducted in HNLC regions of the ocean (see comparative reviews by de Baar et al., 2005, and Boyd et al., 2007), including six in the Southern Ocean with varying degrees of enhanced phytoplankton biomass and draw-down of NO_3^- following Fe enrich-ment. The first in situ Fe experiment in the Southern Ocean—Southern Ocean Iron Release Experiment (SOIREE; Boyd et al., 2000) was conducted at the end of the austral summer in February 1999 south of the Antarctic Polar Front in the Australian sector in a region of relatively low Si (10 µM), but high NO_3^- (25 µM). By the final day (day 13) of this mesoscale open ocean experiment, chlorophyll a concentration had increased 5-fold and both NO_3^- and Si had decreased by 3 µM (Frew et al., 2001); no rates of N uptake are available. The Carbon Dioxide Uptake Southern Ocean (CARUSO)/Eisen Experiment (EisenEx) followed in austral spring 2000 in the core of a cold Antarctic eddy which had spun off the Polar Frontal jet into the Atlantic sector of the sub-Antarctic region. Twenty days since the first Fe infusion, the draw-down in NO_3^- and Si concentrations was 1.61 µM and 4 µM, respectively (Bozec et al., 2005). In 2004, two in situ Fe experiments were performed in the Southern Ocean. The European Iron Fertilization Experiment (EIFEX) was con-ducted within a mesoscale eddy at the Antarctic Polar Front in the Atlantic sector of the Southern Ocean for five weeks starting in during January (Hoffmann et al., 2006; Jacquet et al., 2008), and the SOLAS Air–Sea Gas Experiment (SAGE; Hall et al., 2005) was conducted east of New Zealand in sub-Antarctic waters around an eddy during March–April. Although chlorophyll biomass increased by 2-fold (SAGE)

and > 5-fold (EIFEX) during these experiments, estimates of NO_3^- depletion during the Fe-enriched blooms are not yet published. The only currently available $^{15}NO_3^-$ uptake rates, as opposed to net draw-down estimates, determined during polar, open ocean Fe enrichment experiments are those from the Southern Ocean Fe Experiment (SOFeX; Coale et al., 2004, Cochlan et al., 2002b, 2004) conducted in late January–February 2002.

During SOFeX, two separate patches north and south of the Antarctic Polar Front were enriched with Fe; the northern patch had low ambient Si concentrations (<3 μM) and high ambient NO_3^- concentrations (20 μM), and the southern patch had elevated ambient concentrations of both Si (~60 μM) and NO_3^- (28 μM). In both the northern and southern patches, there were clear decreases in the ambient NO_3^- concentration (1.4 and 4.1 μM; Hiscock and Millero, 2005) and 10–20-fold increases in phytoplankton chlorophyll a (Coale et al., 2004) following Fe enrichment. Although it is not surprising that both NO_3^- and NH_4^+ absolute uptake rates also increased as a result of the biomass increase, the dramatic enhancement of the specific uptake rates of NO_3^- relative to outside control waters (average increase was ~6–7-fold), and only a modest increase (average increase was ~2-fold) in specific rates of NH_4^+ or urea (Cochlan et al., 2002b, 2004; Cochlan and Kudela, 2006), is clearly indicative of faster rates of NO_3^- consumption per unit phytoplankton biomass, a result similar to those reported for Fe-amended bottle experiments discussed above. During SOFeX, the proportion of NO_3^- uptake to the total N (NO_3^-, NO_2^-, NH_4^+ and urea) uptake was measured throughout the experiment, and the resulting f-ratio increased in the southern patch from 0.1–0.2 to ~0.5–0.6, and from ~0.2 to ~0.3–0.4 in the northern patch (Fig. 12.2). The increased f-ratio in both regions clearly indicates that alleviation of Fe-limitation allows for the greater relative utilization of NO_3^- reserves that would otherwise be underutilized for growth in the surface waters south and north of the Antarctic Polar Front zone, and confirms the role for Fe-limitation in the creation of HNLC waters in the Southern Ocean.

Recent experiments in the Southern Ocean also have implicated zinc as a co-limiting nutrient, primarily through the regulation of Si uptake by diatoms (Franck et al., 2000, 2003), but not NO_3^- (see also Kudela, this volume). Iron and zinc-limitation have also been linked to increased Si:NO_3^- depletion ratios and increased silicification of diatoms (Hutchins and Bruland, 1998; Takeda, 1998), which are disproportionately represented in previous Fe-enriched blooms in both in vitro (e.g., Martin et al., 1989) and in situ experiments (e.g., Boyd et al., 2000; Coale et al., 1996). Although phytoplankton do not have an absolute zinc requirement during the uptake and assimilation of NO_3^- and NH_4^+, zinc is required for Si uptake (e.g., De La Rocha et al., 2000; Rueter and Morel, 1981), and for the activities of the metallo-enzymes, carbonic anhydrase, and alkaline phosphatase (e.g., Anderson et al., 1978). However, under zinc limitation, other elements may substitute for zinc in these enzymes both in vivo and in vitro (e.g., Lee and Morel, 1995; Morel et al., 1994; Price and Morel, 1990). During the shipboard incubations conducted just north and south of the Antarctic Polar Front, zinc alone had no effect on NO_3^- uptake (Franck et al., 2000), and in the offshore HNLC region of the Ross Sea, Cochlan et al. (2002a) found that the N uptake response to zinc was relatively
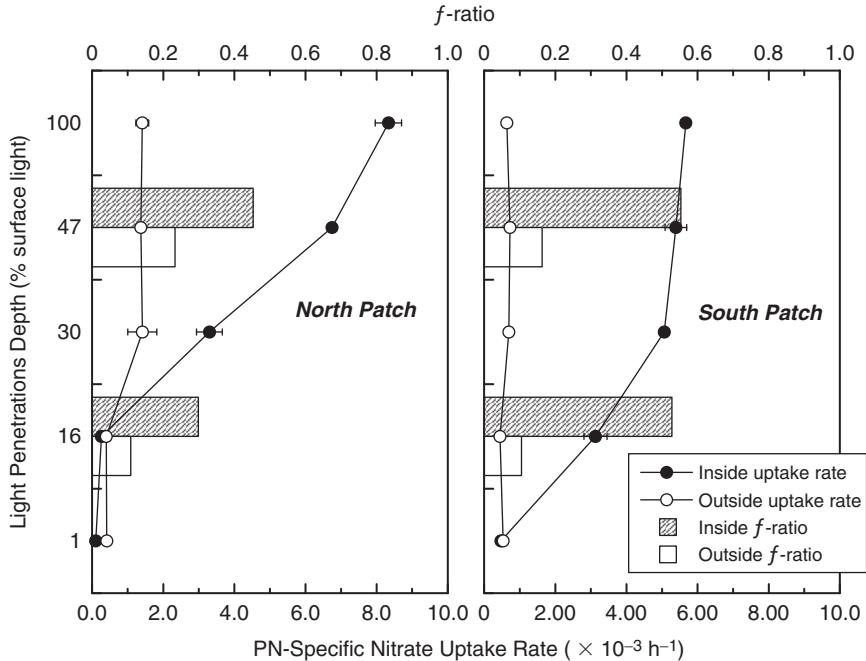
Figure 12.2 SOFeX depth profiles of biomass (PN)-specific NO_3^- uptake rates, determined during 24-h incubations in Plexiglas® acrylic incubators under simulated *in-situ* light and temperature conditions. Ultra-clean trace-metal techniques were used for sample collection within and outside (control waters) of the Fe-enriched patch north and south of the Antarctic Polar Front zone. The f-values [$f = V_{NO_3}/(V_{NO_3} + V_{NH_4} + V_{NO_2} + V_{Urea})$] were determined at the isolume depths of 47 and 16% surface irradiance, using tracer-level isotopic enrichments, and are not corrected for the effects of isotopic dilution. Error bars represent the range of duplicate samples ($n = 2$). Corrected from Coale *et al.* (2004).

modest, presumably due in part to the relatively high dissolved concentrations of zinc present ($\sim$2 nM). However, during the later sampling periods, zinc addition at the HNLC site resulted in a 40% increase in the specific uptake rates of NO_3^- but not NH_4^+, whereas the specific uptake rates of NO_3^- and NH_4^+ increased by 4–16% and 18–49%, respectively, at a continental shelf site close to the Ross Ice Shelf.

In contrast to zinc, the crucial role of Fe in the bioenergetics of carbon (C) and N metabolism is well recognized (e.g., Morel *et al.,* 1991; Sunda, 1989). Substantial amounts of Fe are required in both photosynthetic and respiratory electron transport chains (e.g., Raven, 1988), the synthesis of chlorophyll (Chereskin and Castelfranco, 1982), and the assimilation of NO_3^-. Theoretical calculations based on Fe utilization efficiencies and cellular metabolic Fe demands, predict that phytoplankton growing on NO_3^- require 60% more Fe than those growing on NH_4^+ (Raven, 1988, 1990), and greater cellular Fe requirements for NO_3^- growth have indeed been demonstrated for laboratory cultures of diatoms (Maldonado and Price, 1996). The extra Fe is needed to reduce NO_3^- to NH_4^+ before it can be incorporated into amino acids. This process requires the assimilatory enzymes nitrate reductase (requires one

atom of Fe) and nitrite reductase (requires five atoms of Fe), and either ferredoxin (an Fe-containing e^- donor) or flavodoxin (a non-ferrous e^- donor; Doucette $et\ al.,$ 1996; La Roche $et\ al.,$ 1993), plus the need for greater quantities of reducing power (8 mol e^-/mol N) derived from Fe-dependent photosynthetic redox reactions. Thus, the utilization of NH_4^+ over NO_3^- observed in HNLC regions such as the Southern Ocean can be assumed to be a more energetically efficient strategy for obtaining N in response to Fe-limitation.

2.2. Nitrogen concentration effects

For nearly 40 years, the kinetics of N uptake of both cultured and natural assemblages have been determined using the Michaelis–Menten formulation for enzyme kinetics:

$$V = \frac{V_{max}\ S}{K_s + S}$$

where V is the particulate N (PN) specific uptake rate, V_{max} is the maximal specific uptake rate, S is the substrate concentration, and K_s is the half-saturation constant at which V is equal to half the maximum uptake rate (i.e., $V = V_{max}/2$) (Dugdale, 1967). The substrate affinity at low concentrations (i.e., $S < K_s$) can be determined from the initial slope (α) of the Michaelis–Menten plot, and is generally calculated as $\alpha = V_{max}/K_s$; the derivative of the equation, with respect to S, as S approaches zero (Button, 1978; Healey, 1980). Only a few kinetics studies have been conducted in the Southern Ocean, and although some results are suggestive of a rectangular hyperbolic relationship between N concentration and uptake (e.g., Collos and Slawyk, 1986; Glibert $et\ al.,$ 1982; Mengesha $et\ al.,$ 1998), not enough concentrations were employed in these studies to estimate statistically, meaningful kinetic parameters. Kinetic parameters are not available for NO_3^- uptake because of the elevated NO_3^- concentrations (>20 μM) routinely present in the surface waters of open ocean regions, and in marginal ice and continental shelf zones at the beginning of the growth season (e.g., Tréguer and Jacques, 1992).

Kinetic parameters for NH_4^+ and urea uptake in the Southern Ocean have only been estimated for phytoplankton assemblages in the western Ross Sea during a 1996–1997 seasonal study covering austral spring and summer (Cochlan and Bronk, 2001). Their results demonstrate that, with the exception of a pack ice station with high ambient NH_4^+ concentrations, the affinity estimates of Ross Sea phytoplankton for NH_4^+ are greater than those found in other marine systems, as demonstrated by K_s values <0.40 μM and isotope-corrected α values that range from 12.1 to 36.8×10^{-3} h^{-1}/μM. In the pelagic waters of the Arctic, K_s values for NH_4^+ are generally greater and range from <0.1 to 2.2 μM (Table 12.1), similar to those values reported for temperate neritic diatoms and flagellates (1.82 ± 0.09 μM; Eppley $et\ al.,$ 1969) and natural assemblages of coastal phytoplankton ($\sim$ 0.5–2.0 μM; Kudela and Cochlan, 2000). However, the lower K_s values measured in the open Ross Sea (0.04 to <0.4 μM) are more similar to those reported for oceanic

Table 12.1 Summary of half-saturation constants (K_s), determined for natural phytoplankton assemblages from polar regions

Region	K_s-NH_4^+	K_s-NO_3^-	K_s-Urea	Source
Barents Sea				
$[NO_3^-] \geq 1\ \mu M$	1.3	1.8	0.2	Kristiansen et al. (1994)
$[NO_3^-] \leq 1\ \mu M$	0.1–0.2	0–2.2	0.0–0.1	Kristiansen and Farbrot (1991) Kristiansen and Lund (1989)
Greenland Sea[a]	2.2	0.29–2.7	–	Muggli and Smith (1993)
Barrow Strait, NWT (ice algae)	1.6	<4.0	0.9	Harrison et al. (1990)
Eastern Canadian Arctic				
$[NO_3^-] < 1\ \mu M$	0.17	0.87	0.30	Smith and Harrison (1991)
Toolik Lake, Alaska				
$[NO_3^-] < 1\ \mu M$	0.05–0.49	0.05–0.30	–	Whalen and Alexander (1986)
Western Ross Sea				
$[NO_3^-] > 10\ \mu M$	0.04–0.33[b] 0.12–1.60[c]	★	0.12	Cochlan and Bronk (2001)

Isotope-dilution corrected NH_4^+ uptake rates were used in the Ross Sea study to determine K_s; all other sources report uncorrected rates. Half-saturation constants reported in units of μM-N (Note: 1 μM urea = 2 μM urea-N).
[a] *Phaeocystis pouchetii* bloom.
[b] Estimates determined from isotopic-dilution corrected rates.
[c] Estimates determined from uncorrected rates.
★ Ambient NO_3^- concentrations were too high to determine K_s.

phytoplankton species (0.1–0.5 μM; Eppley *et al.,* 1969) and oligotrophic oceanic regions (0.03–0.6 μM; Kudela and Cochlan, 2000; MacIsaac and Dugdale, 1969).

The half-saturation constant for urea (K_s = 0.12 μM-N) was only estimated for one station, composed primarily of diatoms and nanoflagellates, and is similar to those determined for the Barents Sea in the Arctic (0–0.120 μM-N; Kristiansen and Farbrot, 1991; Kristiansen *et al.,* 1994). Note that the urea concentrations reported here (and in Table 12.1) are in terms of N (i.e., 2 μM urea-N is equivalent to 2.0 μg-at N L^{-1} which equals 1 μM urea). The K_s values from both polar regions are lower than the few values reported for cultures of neritic marine diatoms (0.4–2.0 μM-N; McCarthy, 1972; Rees and Syrett, 1979), pico- and raphidophycean flagellates (0.4 μM-N; Cochlan and Harrison, 1991a; Herndon and Cochlan, 2007), and dinoflagellates (0.9–6.6 μM-N; Fan *et al.,* 2003; Kudela *et al.,* 2008), but very similar to the range reported for natural assemblages in non-polar, oceanic areas (0.02–0.27 μM-N; cf. Kudela and Cochlan, 2000). Based on these kinetic parameters,

although the Ross Sea is a NO_3^-–rich region populated by relatively large phytoplankton, the half-saturation constants are more characteristic of oligotrophic waters dominated by picoplankton assemblages. In this respect, the Ross Sea phytoplankton communities are similar to oceanic phytoplankton in that they possess a relatively efficient means of acquiring these reduced N forms, even though they are normally available in very low concentrations in the euphotic zone of the open waters of the Southern Ocean.

Elevated surface concentrations of NH_4^+ in the Southern Ocean are only generally observed in the vicinity of land masses and ice-edge zones, and range from >1 to ∼4 μM (e.g., Koike *et al.,* 1986; Owens *et al.* 1991); concentrations that would easily saturate the uptake capability of the Ross Sea phytoplankton. Ambient concentrations of urea, although not as frequently measured, can reach 1 μM-N in the open surface waters of the Pacific sector (Sambrotto and Mace, 2000) and the Marginal Ice Zone of Bellingshausen Sea (Bury *et al.,* 1995) with much greater concentrations beneath (1.6–2.8 μM-N) and within (6–18 μM-N) the sea ice. However, surface concentrations of urea in the Ross Sea are very low, year-round (≤0.1 μM-N) and only reach 0.3–0.4 μM-N during the austral fall (Cochlan and Bronk, 2003; US JGOFS database—current URL: http://usjgofs.whoi.edu/index.html) still far below the concentrations necessary to saturate urea uptake.

2.3. Nitrogen substrate interactions

An important factor to consider when examining the nitrogenous nutrition of phytoplankton is the availability of different N substrates for growth and the uptake interactions between them. A commonly held belief in phytoplankton ecology is that phytoplankton prefer to use NH_4^+ over NO_3^-, presumably as an adaptation in terms of energetic savings (e.g., Thompson *et al.,* 1989), and that little or no NO_3^- uptake occurs when ambient NH_4^+ exceeds a certain threshold value. Classically, this concentration has been reported as *ca.* 1 μM (McCarthy, 1981), although in an extensive review of field studies Dortch (1990) found that NH_4^+ inhibition of NO_3^- uptake is rarely so severe, and that it is a highly variable phenomenon dependent on a number of factors including nutrient history, light, and the taxonomic composition of the phytoplankton assemblage. For example, whereas NH_4^+ concentrations as low as 0.1–0.3 μM completely inhibit NO_3^- uptake in subarctic Pacific assemblages dominated by autotrophic flagellates (Booth, 1987; Wheeler and Kokkinakis, 1990), NH_4^+ concentrations as high as 0.6 μM have little effect on NO_3^- uptake by diatom–dominated assemblages of recently upwelled waters off Oregon (*f*-ratios averaged 0.83; Kokkinakis and Wheeler, 1987).

In the Southern Ocean, a number of temporal and spatial correlation studies have shown lower *f*-ratios with elevated NH_4^+ concentrations where the reduction in the *f*-ratio may be due to increased NH_4^+ uptake rates and/or reduced NO_3^- uptake rates (e.g., Goeyens *et al.,* 1995; 1998; Lucas *et al.,* 2007; Owens *et al.,* 1991). However, there have been few inhibition (suppression) studies in the Southern Ocean where either single or multiple NH_4^+ concentrations are experimentally increased to evaluate the effects on NO_3^- uptake rates. The first such study was conducted at two stations in the Scotia Sea by Glibert *et al.* (1982), and an inhibitory effect was

only seen at one station; an $\sim$30% inhibition of $^{15}NO_3^-$ daily uptake rates was observed with a 5 μM addition of NH_4^+. However, Reay et al. (2001) reported significant inhibition of daily $^{15}NO_3^-$ uptake rates both north and south of the Antarctic Polar Front in the northern Scotia Sea (near South Georgia Island) at all seven stations where they enriched samples with 1 μM of NH_4^+. Three other studies in the Southern Ocean have demonstrated that NO_3^- uptake can be strongly suppressed by experimentally elevating NH_4^+ using a series of concentrations. Both linear and non-linear (convex) responses have been observed; non-linear responses have been described by either a three-parameter exponential function (Varela and Harrison, 1999) and/or an inverse Michaelis–Menten hyperbola (Harrison et al., 1996) characterized by the kinetic parameters I_{max} (potential maximal inhibition) and K_I (half-saturation constant of inhibition). The first of these studies was conducted in the Indian sector by Mengesha et al. (1998) at an ice-edge station (conducted twice) dominated by diatoms and a picoflagellate-dominated assemblage further north in the permanently open ocean zone. Their pooled data of both ambient and added NH_4^+ effects on NO_3^- uptake demonstrate an 88% maximum reduction in NO_3^- uptake in the spring, and that an NH_4^+ concentration of 0.21 μM (K_I) would reduce NO_3^- uptake by 50%. However, since the ambient NH_4^+ concentrations during spring were low relative to the K_I, inhibition at that time was considered minimal. With the seasonal increase in ambient NH_4^+ concentrations in this region, their derived parameters would suggest increased inhibition of NO_3^- uptake rates further on in the growing season, a suggestion supported by lower specific rates of NO_3^- observed during the summer, although inhibition results were not reported.

In the western Ross Sea, a similar study was conducted in 1996–1997 during three cruises spanning pre-bloom, bloom, and bloom decline conditions (Cochlan and Bronk, 2003). At all but two of the seven stations, one being in the NH_4^+-rich waters under the pack ice, the maximal realized inhibitions (mean I_{max} = 0.63, range from 0.40 to 0.83) and half-inhibition concentrations (mean K_I = 0.10 μM, range from 0.04 to 0.22 μM) suggest that NO_3^- uptake could be reduced by an average of 63%, and that an ambient concentration of only 0.10 μM would reduce the NO_3^- uptake rate by 50%. However, even with their highest NH_4^+ additions (5–10 μM) there was never complete inhibition of NO_3^- uptake. Their analysis also suggests a seasonal progression in NH_4^+ inhibition of NO_3^- uptake, with the greatest inhibition occurring during the summer period despite the low ambient NH_4^+ concentrations present, in contrast to the Indian sector study. Using the derived NH_4^+ inhibition constants and kinetic uptake parameters determined during concurrent NH_4^+ uptake experiments (Cochlan and Bronk, 2001), they also estimated the proportion of new to total production (the f-ratio) at in situ and elevated NH_4^+ concentrations during the year for the western Ross Sea. In this simple modeling effort, they were able to differentiate between the two well-known effects of NH_4^+ on phytoplankton nitrogenous nutrition: substrate-dependent NH_4^+ uptake and inhibition of NO_3^- uptake, and suggest that the most important factor influencing the proportion of new and regenerated production in the Ross Sea is the concentration-dependent NH_4^+ uptake rate, not the inhibition of NO_3^- uptake rates by increasing ambient NH_4^+ concentration that has been suggested for other coastal and open ocean regions of the Southern Ocean. Similar inhibitory experiments were

conducted in the Australian sector (sub-Antarctic and Antarctic Polar Front zones) during 1998 by Elskens *et al.,* (2002), who found that the maximum apparent inhibition of NO_3^- uptake ranged from 33 to 81%; again even at the highest NH_4^+ concentration (1 μM), NO_3^- uptake was never totally suppressed.

The inhibitory effect of NH_4^+ on the uptake of NO_3^- has been suggested by Jacques (1991) to invalidate the applicability of the new and regenerated production paradigm to the Southern Ocean. However, to what extent elevated NH_4^+ concentrations repress the utilization of NO_3^- is still a poorly understood question in the Southern Ocean, and our knowledge is based primarily on a compilation of correlative studies of NO_3^- uptake and ambient NH_4^+ concentration with little regard to the phytoplankton species composition of the assemblage. The role of Fe in this interaction also is unclear, but given the greater Fe demand for phytoplankton growth on NO_3^- versus NH_4^+ (e.g., Maldonado and Price, 1996), modeling efforts have suggested a close interaction between Fe and NH_4^+ in regulating new (NO_3^-–based) production (Armstrong, 1999; Elskens *et al.,* 2002). These studies predict that in conditions of abundant ambient NO_3^- and Fe-limitation, the effect of experimentally increasing NH_4^+ on NO_3^- uptake rates will be minimal, whereas in conditions of enhanced Fe availability, abundant NO_3^- and low ambient NH_4^+, spiking with NH_4^+ will significantly reduce the *f*-ratio, mainly as a result of the inhibition of NO_3^- uptake. This interaction was studied in 2002 during the SOFeX mesoscale open ocean Fe experiment (Cochlan *et al.,* 2004; Cochlan and Kudela, 2006). Ammonium inhibition experiments were conducted with phytoplankton assemblages collected 13 and 22 days after the initial Fe infusion, and compared with samples collected on day 22 from the outside (control) waters of the southern Fe patch. Greater half-inhibition (K_I) concentrations (1.1 and 1.5 μM) estimated for the Fe-patch relative to the K_I of 0.26 μM in the control waters indicate that higher concentrations of NH_4^+ would be needed to reduce the NO_3^- uptake rate by 50% in the Fe–replete assemblages. Despite the increase in ambient NH_4^+ concentrations observed within the blooming Fe patch, the realized NO_3^- uptake rates ranged from 84 to 96% of the potential NO_3^- uptake rates (theoretical maximum rate at zero NH_4^+ concentration) in contrast to the NO_3^- uptake rate in the outside waters, which was only 63% of the potential rate. These results, together with the enhancement in specific NO_3^- rates (up to 10-fold) following Fe enrichment strongly suggest that the alleviation of Fe deficiency resulted in decreased sensitivity to NH_4^+ inhibition, not increased sensitivity as predicted by the models. A similar reduction in the inhibitory effects of NH_4^+ on NO_3^- uptake rate was determined during the second Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study (SEEDS-II) conducted in July–August 2004 in the western subarctic Pacific Ocean (Cochlan *et al.,* 2005), despite a relatively modest increase in phytoplankton biomass following Fe enrichment.

Interactions between urea and other N-forms have been studied even less, and primarily with cultured, non–polar phytoplankton; however, it is generally assumed that urea suppresses the uptake of NO_3^- to a lesser extent than NH_4^+ (Cochlan and Harrison, 1991b; Molloy and Syrett, 1988) or not at all (Lund, 1987). No actual perturbation or correlative studies of the effects of urea on NO_3^- or NH_4^+ uptake have been published to date for the Southern Ocean, although conversely, Elskens

et al., (2002) found that NH_4^+ additions had no significant effect on urea uptake in natural assemblages. However, urea appears to be particularly important in communities under the ice, where elevated ambient concentrations are present, and urea is the dominant N source (Bury *et al.,* 1995).

In most open ocean regions of the Southern Ocean, ambient NH_4^+ concentrations are generally low (<0.5 µM), but exceptions exist (e.g., Glibert *et al.,* 1982; Sambrotto and Mace, 2000), whereas elevated concentrations are routinely found in association with the productive waters of receding ice-edges (e.g., Goeyens *et al.,* 1995; Tréguer and Jacques, 1992), pack ice (Ross Ice Shelf: Biggs *et al.,* 1985), continental ice (Jacobs *et al.,* 1979), and in the vicinity of land masses (e.g., Bransfield Strait; Owens *et al.,* 1991). Near the islands of South Georgia, it has been shown that mesozooplankton (including krill) are important contributors to the elevated (>1 µM) mixed layer concentrations of NH_4^+ (Atkinson and Whitehouse, 2000, 2001), are responsible for diurnal changes in the mixed layer NH_4^+ concentrations (Priddle *et al.,* 1997), and high NH_4^+ concentrations within and adjacent to intense aggregations of these populations (Johnson *et al.,* 1984). Based on these observations, one might expect ephemeral micropatches of concentrated NH_4^+ (e.g., McCarthy and Goldman, 1979), not measurable by traditional analytical methods, although the existence of such micropatches has been criticized on theoretical grounds (Jackson, 1980). However, as the above studies suggest, ambient NH_4^+ concentrations need not be elevated to have a crippling inhibitory effect on NO_3^- uptake by phytoplankton in the Southern Ocean, particularly where dissolved Fe concentrations are considered limiting to phytoplankton growth.

2.4. Temperature effects

Given the known influence of temperature on phytoplankton growth (e.g., Eppley, 1972; Raven and Geider, 1988) and photosynthesis (e.g., review by Davidson, 1991) it is not surprising that temperature affects cell N composition (e.g., Thompson *et al.,* 1992a, b), maximum N uptake rates (e.g., Lomas and Glibert, 1999a; Underhill, 1977), NO_3^- assimilatory enzymes (e.g., Berges *et al.,* 2002; Lomas and Glibert, 1999b), NH_4^+ inhibition of NO_3^- uptake (Lomas and Glibert, 1999a; Reay *et al.,* 2001), and even short-term NH_4^+ uptake (Raimbault, 1984) in cultured phytoplankton and field assemblages. While these effects may be subtler than those on growth and photosynthesis, it is likely that they all contribute somewhat to species succession and dominance, and thus should be considered when examining the phytoplankton and N dynamics of the Southern Ocean. The water temperature of the Southern Ocean (south of the Antarctic Polar Front) ranges from $-1.8°C$ (the freezing point of seawater) to ~5°C in the summer (e.g., Neori and Holm-Hansen, 1982), but most of our knowledge on the effects of temperature on phytoplankton N uptake have been conducted at temperatures far in excess of this range and may not be directly applicable to polar assemblages; few studies on temperature effects have been conducted using natural assemblages in the Southern Ocean.

During the first ^{15}N uptake study in the Southern Ocean, specific NO_3^- uptake rates were positively related to temperature over large temporal (2–16°C) and spatial

scales (40–62°S latitude) during a north–south transect (Slawyk, 1979). However, during this study, not only were there gradients in temperature, but also in ambient NH_4^+ concentrations, and likely species composition, which renders the interpretation of N uptake solely as a function of temperature difficult; see also Harrison *et al.* (1996) and Kanda *et al.* (1985) for examples of similar relationships in non-polar environments. During short-term (6 h) $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake experiments conducted in the Scotia Sea at three different temperatures, Olson (1980) found that phytoplankton from two stations were not adapted for optimal N uptake at their *in situ* temperature (0°C), and that they increased uptake rates with 5–10°C increases in temperature. More recently, phytoplankton from the southwest Atlantic sector of the Southern Ocean were incubated at ambient and elevated temperatures (ambient plus 3°C and ambient plus 6°C), and both long-term (72 h) N-depletion and short-term (24 h) NH_4^+ and NO_3^- uptake rates were determined (Reay *et al.*, 2001). Although the relationship between temperature elevation and the specific depletion rates of NH_4^+ and NO_3^- was highly variable, they observed a positive relationship between temperature and specific $^{15}NO_3^-$ uptake rates at 8 of 9 stations, even when NH_4^+ concentrations were experimentally elevated by 1 μM; there was no clear temperature dependence of specific NH_4^+ uptake observed. These NO_3^- uptake results support the temperature dependence seen previously in Antarctic sea-ice microalgae for NO_3^- uptake and nitrate reductase activity, but differ in that the sea-ice microalgae NH_4^+ uptake rates were also temperature dependent (Priscu *et al.*, 1989). The microalgae study also suggests that at *in situ* temperatures (−1.9°C), utilization of N would be limited by transport into the cell, not intracellular assimilation because of the differential temperature characteristics between N transport and assimilation. Overall, the results of Southern Ocean studies are similar to those conducted in the Arctic Ocean where Q_{10} values for NH_4^+ and NO_3^- uptake of ~2–3 have been determined (Smith and Harrison, 1991); the Q_{10} values are very similar to the photosynthesis–temperature responses of Arctic (Li *et al.*, 1984) and Antarctic (Neori and Holm-Hansen, 1982; Tilzer *et al.*, 1986) assemblages. It appears that natural assemblages of polar phytoplankton do not have any special adaptive strategies to cope with the cold water temperatures of their environment, but high rates of primary productivity and NO_3^--based new production are observed frequently in the Arctic, including areas such as the western Bering Strait (e.g., Sambrotto *et al.*, 1984) and during *Phaeocystis* blooms in the Greenland Sea (Codispoti *et al.* 1991; Smith *et al.*, 1991).

Temperature effects on N utilization have also been examined in unialgal cultures isolated from the Southern Ocean (Reay *et al.*, 1999). During growth kinetic experiments for a *Chaetoceros* sp., the maximum growth rate (μ_{max}) decreased for both NO_3^- and NH_4^+-chemostat grown cultures with reduced temperature, whereas slightly greater reduction in affinity indices (K_s, half-saturation constant and α, initial slope of the Michaelis–Menten hyperbola) were seen for NO_3^- with lower temperatures. The reduction in affinity for NO_3^- observed for this Antarctic isolate was shared by a series of six bacterial isolates and two other phytoplankton isolates and interpreted by the authors as evidence of a bias away from NO_3^- in low-temperature planktonic systems; note that they were generally

grown at temperatures not seen in polar systems. However, such an interpretation ignores the fact that the ambient concentrations of NO_3^- in the Southern Ocean would be considered generally saturating for uptake, and affinity measures are of little value in assessing the potential NO_3^- uptake capabilities in high NO_3^- systems.

2.5. Irradiance effects

The irradiance experienced by phytoplankton in the Southern Ocean is controlled primarily by three factors: (1) the degree of ice cover (including both thickness and overlying snow cover), (2) the depth of vertical mixing, and (3) the time of year (and hence photoperiod and solar angle); factors that are discussed in detail by Smith and Sakshaug (1990). Phytoplankton in both the Arctic and the Antarctic adapt to their *in situ* irradiances by modifying their maximal light-saturated photosynthetic rates (P_{max}^B), and their photosynthetic efficiency (the light-limited rate or initial slope, α) such that low light assemblages generally have similar or slightly lower values of P_{max}^B and α than assemblages adapted to higher irradiances (Harrison and Platt, 1986). However, the N uptake response to irradiance is unclear since numerous factors can affect the relationship between light and the substrate being utilized, and the exact mechanism(s) by which light regulates N metabolism is unresolved (e.g., see review by Syrett, 1981). The dependence of N uptake by phytoplankton upon irradiance has been described by a rectangular hyperbola parallel to that of the Michaelis–Menten equation for N uptake as a function of concentration (MacIsaac and Dugdale, 1972), modified to account for dark uptake (e.g., Cochlan *et al.,* 1991) or characterized by a 3-parameter, exponential (*P* vs. *E*) model to account for photoinhibition (Kudela and Cochlan, 2000; Priscu, 1989). Culture and field studies have demonstrated that N-deprived phytoplankton have greater dark N uptake rates than N-replete phytoplankton (see review by Cochlan *et al.,* 1991); however, given the N-rich environment of the Southern Ocean it is highly unlikely that dark uptake is attributable to this stress response, and instead has been suggested as indicative of heterotrophic N uptake (see below).

The relationship between irradiance and N uptake in the Southern Ocean is unclear and often contradictory, and most of our earlier understanding is based on comparison of specific N uptake rates measured at various depths throughout the euphotic zone using either *in situ* or simulated *in situ* irradiance fields. Although maximal rates for both NO_3^- and NH_4^+ (and during a few studies—urea) are routinely observed at either the 100 or 50% isolumes, maximal specific N uptake rates are also found as low as the 0.1–1.0% isolume (e.g., Collos and Slawyk, 1986; Glibert *et al.,* 1982). Only a few N uptake versus irradiance experiments actually have been conducted in the Southern Ocean.

In the Indian sector, Slawyk (1979) demonstrated that both NH_4^+ and NO_3^- uptake could be described by a hyperbolic relationship, NH_4^+ uptake required less light than NO_3^- to support the maximal uptake rates realized (the K_{LT} for NH_4^+ was less than the K_{LT} for NO_3^-), and irradiances in excess of 25% of the surface irradiance did not enhance specific rates of NH_4^+ and NO_3^- uptake. During a series of NO_3^- uptake versus irradiance experiments conducted in the Ross Sea during austral spring and summer, Hu and Smith (1998) also showed that relatively low irradiances

were needed to saturate uptake during the spring and summer cruises (means of 37% and 32% of surface irradiance, respectively), and that dark uptake (defined as uptake of NO_3^- at the 0.1% isolume) averaged 9.4% and 16.9% that of the surface rate. Previously, Nelson and Smith (1986) observed a similar saturation response of NO_3^- uptake to irradiance, but found that dark uptake was a much greater proportion (~40%) of the uptake rate realized at the surface. Hu and Smith (1998) explain these differences as a consequence of a difference in either the sampling time during the season, the species composition of the assemblages (diatom versus haptophyte blooms), or the stage of bloom development.

Such differences may indeed limit the comparison of N uptake response to irradiance, and may partially explain the negative relation observed between integrated NO_3^- uptake rates (normalized to chlorophyll) and irradiance along a 66.5°E transect in the Indian sector (Collos and Slawyk, 1986). In contrast, Sambrotto and Mace (2000) demonstrated that using the ratio of critical depth (Z_c; Sverdrup, 1953) to the mixed layer depth (Z_m), the integrated rates of NO_3^- uptake are positively related to the average light conditions experienced in the euphotic zone, and that this index explains over 50% of the variance in the measured rates of NO_3^- uptake along a 170°W transect spanning the Antarctic Polar Front in the Pacific sector.

2.6. Bacterial uptake of nitrogen

Distinguishing bacterial N uptake from phytoplankton N uptake is difficult in field studies. Both size fractionation techniques and metabolic inhibitors have been used, but with limited success due to a lack of specificity between autotrophic and heterotrophic processes, and the overlap in size range between the two groups of marine microorganisms. Despite these methodological limitations, it is widely regarded that heterotrophic bacteria are responsible for a significant, but highly variable (<5 to >90%) fraction of the total dissolved N utilized in estuarine and marine systems (e.g., review by Hoch and Kirchman, 1995; Kirchman *et al.,* 1994; Kirchman, 1994; Middelburg and Nieuwenhuize, 2000). In general, heterotrophic bacteria preferentially utilize NH_4^+ before NO_3^- or urea, but likely gain most of their N requirement from dissolved free amino acids in coastal environments (e.g., Billen and Fontigny, 1987; Hoch and Kirchman, 1995; Kirchman, 1994), although Middelburg and Nieuwenhuize (2000) found that bacteria have a strong preference for NH_4^+ and NO_3^- in the turbid estuaries of western Europe.

In the Southern Ocean, bacterial uptake of NO_3^- was first hypothesized by Glibert *et al.* (1982) based on observations of anomalously high rates of specific NO_3^- uptake at low light levels in the Marginal Ice Zone of the Scotia Sea, and 24-h dark NO_3^- uptake rates, which approximated rates determined under normal light/dark cycles. The importance of heterotrophic N uptake in the Scotia Sea was also suggested by Rönner *et al.* (1983) based on similar 24-h experiments where the percentage of total uptake independent of light increased with depth such that dark NO_3^- uptake equaled light uptake at the 1% isolume. More recently, during 3-day incubation experiments of samples collected near South Georgia Island, Reay *et al.* (2001) interpreted the minimal depletion of NH_4^+ and NO_3^- within dark controls as

indicative of insignificant heterotrophic inorganic N utilization. Others, however, have reported that nighttime NO_3^- uptake either ceased (Olson, 1980) or was very low (10–30%) compared to daytime uptake rates (Koike $et\ al.$, 1986), or argue that phytoplankton are adapted to utilize NO_3^- at low light depths (Nelson and Smith, 1986).

Direct measurements of N uptake by heterotrophic bacteria in the Southern Ocean have only been conducted in the coastal waters of the northern Gerlache Strait region of the Antarctic Peninsula (Tupas $et\ al.$, 1990, 1994). They determined that during a rich phytoplankton bloom, bacteria (<0.80 μm filtrates incubated in the dark) were responsible for 8–25% (mean = 17%) of the total community $^{15}NH_4^+$ uptake. They also found that heterotrophic bacteria were taking up and regenerating NH_4^+ simultaneously, in contrast to the concept presented earlier by Goldman $et\ al.$ (1987) where active heterotrophic NH_4^+ utilization implies an insufficiency of suitable organic substrates in the environment, and consequently no regeneration of NH_4^+.

Bacterial N demand for a region can also be estimated from bacterial C production rates (determined using ^{3}H-thymidine or –leucine incorporation rates), assuming a constant bacterial C:N ratio, and correcting for the efficiency of the filters used in standard ^{15}N uptake experiments (generally combusted glass-fiber filters) to retain bacteria (e.g., Fuhrman $et\ al.$, 1988). Using these assumptions in the western Ross Sea, the potential bacterial contribution to ''tracer-level'' community NH_4^+ uptake rates averaged 13%, (range: <1–26%), and was only high (35%) when bacterial abundance and biomass were maximal during a bloom of $Phaeocystis\ antarctica$ (Cochlan and Bronk, 2001). Similarly, if we assume that all bacterial N demands are met by NO_3^-, rather than NH_4^+, the average bacterial portion of total NO_3^- uptake is 10% (range: <1–36%), substantially lower than the 5–60% (mean = 32%) reported for the HNLC waters of the subarctic Pacific Ocean (Kirchman and Wheeler, 1998).

The extent to which bacteria compete with phytoplankton for inorganic and organic N in the Southern Ocean is still uncertain, but if results from temperate coastal environments (Long Island Sound, New York, USA) are applicable, ambient N concentration may influence the partitioning of N into bacteria and phytoplankton. Using ^{13}N, Suttle $et\ al.$ (1990) showed that by increasing the NH_4^+ concentration from undetectable (<0.08 μM) to just 0.5 μM, relatively more of the NH_4^+ goes into the larger plankton; uptake into the >1 μm assemblage increased from 50 to almost 80%. In the only study that has actually quantified the N uptake kinetics of a natural heterotrophic bacterial assemblage, Fuhrman $et\ al.$ (1988) report that in Long Island Sound, the maximum specific uptake rate of $^{13}NH_4^+$ by bacteria is less than for most marine phytoplankton, but the half-saturation constant (K_s) for uptake is extremely low (50 nM) resulting in an NH_4^+ affinity (estimated as α, the initial slope of the uptake vs. concentration hyperbola) that is 6-fold greater than the estimate for similarly-sized, eukaryotic picoplankton ($Micromonas\ pusilla$, Cochlan and Harrison, 1991a), and 1–2 orders of magnitude greater than N affinity estimates derived from natural assemblages and cultured phytoplankton (e.g., Goldman and Glibert, 1983; Riegman $et\ al.$, 2000), including those from the Southern Ocean (Cochlan and Bronk, 2001). These results suggest that at the low NH_4^+ concentrations normally experienced in the open waters of the Southern

Ocean, the high surface area to volume ratio of bacteria may provide them with a competitive advantage over phytoplankton. Superior competitive ability of bacteria to remove NH_4^+ from the water would force phytoplankton to utilize NO_3^- to satisfy their N demands, resulting in higher f-ratios, while factors which suppress bacterial uptake of NH_4^+ could have a negative feedback on autotrophic uptake, resulting in decreased f-ratios (Probyn, 1992). Thus, the relationship between the availability of N and the uptake capacities of bacteria and phytoplankton may be important in controlling their relative biomass levels in natural systems, including the Southern Ocean (Azam and Smith, 1991).

3. SUMMARY

A number of environmental factors exert a strong influence on the nitrogenous nutrition of Antarctic phytoplankton, and the evidence to date cannot demonstrate the dominance of one factor over another for the whole of the Southern Ocean. Observational and experimental studies conducted over the past three decades demonstrate that NO_3^- is not limiting primary production since surface concentrations of NO_3^- are in excess of those generally assumed saturating for its uptake. Other limiting factors must be considered in order to develop a mechanistic understanding of phytoplankton N utilization, and ultimately primary productivity, in the waters surrounding Antarctica. Von Liebig's Law of the Minimum (see review by de Baar, 1994) states that co-limitation, where phytoplankton growth and/or nutrient uptake are simultaneously limited by more than one factor, does not occur in natural phytoplankton populations. However, in the Southern Ocean, the largest of the HNLC regions, co-limitation by a micro-nutrient (Fe) and a macro-nutrient (NO_3^-) appears to occur, in that Fe regulates the capability of phytoplankton to utilize NO_3^- that is normally found in excess. Others have suggested similar co-limitation (or simultaneous limitation) of phytoplankton processes in the Southern Ocean by Fe and Si, or Fe and irradiance (e.g., Boyd, 2002 and references therein).

With the development of trace metal clean techniques, it has become apparent that the availability of Fe is a major factor limiting phytoplankton growth in the deep, open ocean regions of the Southern Ocean; regions minimally affected by potential sources of Fe from upwelled waters associated with shallow sea bottoms, or adjacent to land and/or glacial masses. Based on the Fe requirements for N assimilation it also would be expected that Fe would be more important in the regulation of NO_3^- uptake, and have a lesser effect on the uptake of reduced N substrates (urea and NH_4^+), although only a few studies have concurrently measured all three major substrates as a function of Fe. Given the greater Fe requirements for phytoplankton growth at low light, the regulatory role of Fe is likely accentuated in open ocean areas dominated by deep, wind–mixed layers, and less so in marginal ice zones where low density meltwater stabilizes the water column. The effect(s) of one N source on the utilization of another, namely NH_4^+ inhibition of NO_3^- uptake, cannot be ignored, particularly in regions of elevated surface concentrations

of NH_4^+. However, the few studies of concentration-dependent experiments demonstrate that even the relatively low concentrations of NH_4^+ (0.1–0.2 μM) present throughout the Southern Ocean can potentially reduce NO_3^- uptake by 50% or more. Of particular interest is the possibility of a common source of both Fe and NH_4^+ in melting sea-ice, and their opposing effects on NO_3^- uptake rates. Finally, although the regulatory role of temperature has not been investigated in detail, low ambient temperature undoubtedly factors into the subdued rates of nitrogenous nutrient uptake and primary productivity realized by most pelagic phytoplankton assemblages in the Southern Ocean.

Although much progress has been made toward solving the Antarctic Paradox in the last 25–30 years, temperature may ultimately prove to be the limiting environmental factor controlling the absolute magnitude of both new and regenerated productivity, whereas the availability of trace metals (notably, Fe), regenerated N sources, and light, appear crucial in dictating their relative importance in the open ocean and seasonal ice zones of the Southern Ocean.

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Nitrogen in the Atlantic Ocean

Dennis A. Hansell *and* Michael J. Follows

Contents

1. Introduction

The location of the North Atlantic (between Europe and North America) has made it the focus of considerable study; some of the most important advances in marine nitrogen dynamics have been developed in the region (Lipschultz *et al*, 2002). The South Atlantic, in contrast, has undergone expeditionary surveys, but fewer process studies have been specific to that basin.

The Atlantic is fed by the northward flow of upper ocean waters that are depleted in nitrate and phosphate, while North Atlantic deep waters, formed in the northern high latitudes, move southwards through the basin gathering remineralized nutrients. The proximity of the vigorous source of iron from Saharan dust suggests that productivity in the basin is limited by the availability of these macro-nutrients. The North Atlantic is unique among ocean basins with a nitrate:phosphate ratio (N:P) in thermocline waters in excess of the requirements of "average" Redfieldian organisms (Fanning, 1992), a situation commonly interpreted as a signature of N input by nitrogen fixation.
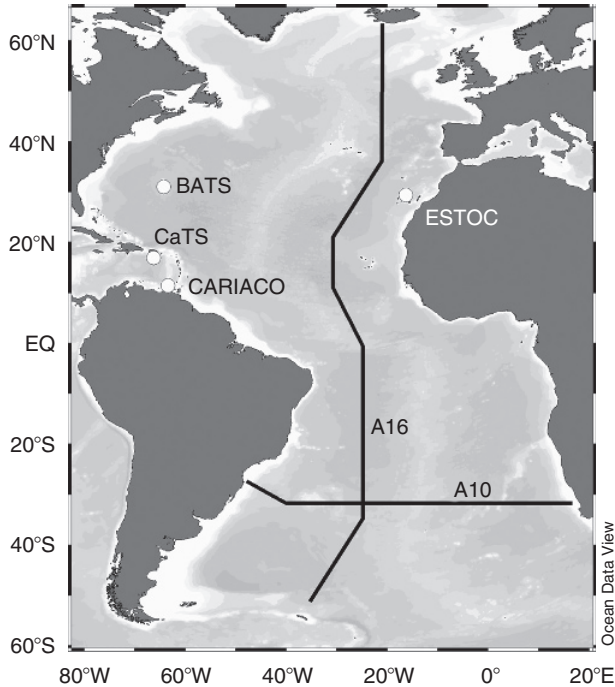
Figure 13.1 Map of the Atlantic Ocean depicting locations of hydrographic sections contoured for this chapter, as well as the locations of the time series study sites referenced. Map (as well as several figures in this chapter) prepared using Ocean DataView (Schlitzer, 2005).

This feature suggests that the Atlantic Ocean may be a source of fixed nitrogen to the global ocean, thus balancing the nitrogen removal due to denitrification occurring elsewhere.

In this chapter we focus on the factors regulating the nitrogen cycle of the Atlantic Ocean, with special attention on the North Atlantic, where data are plentiful. We begin the chapter with a description of the distributions of the major forms of N. A map of the Atlantic depicting locations of data collection for ocean sections and time-series discussed here is given in Fig. 13.1. We then consider the processes that regulate the nitrogen budget in the region, including physical transports, N_2 fixation, atmospheric deposition, and denitrification. We close with a quantitative evaluation of the major reservoirs and fluxes of nitrate in the North Atlantic.

2. DISTRIBUTION OF NITROGEN IN THE ATLANTIC

2.1. Nitrate

The meridional distribution of nitrate in the Atlantic generally follows the major water masses, with a clear and dominant signature of North Atlantic Deep Water (NADW) formed from northern high latitude, nutrient depleted waters (Fig. 13.2A).
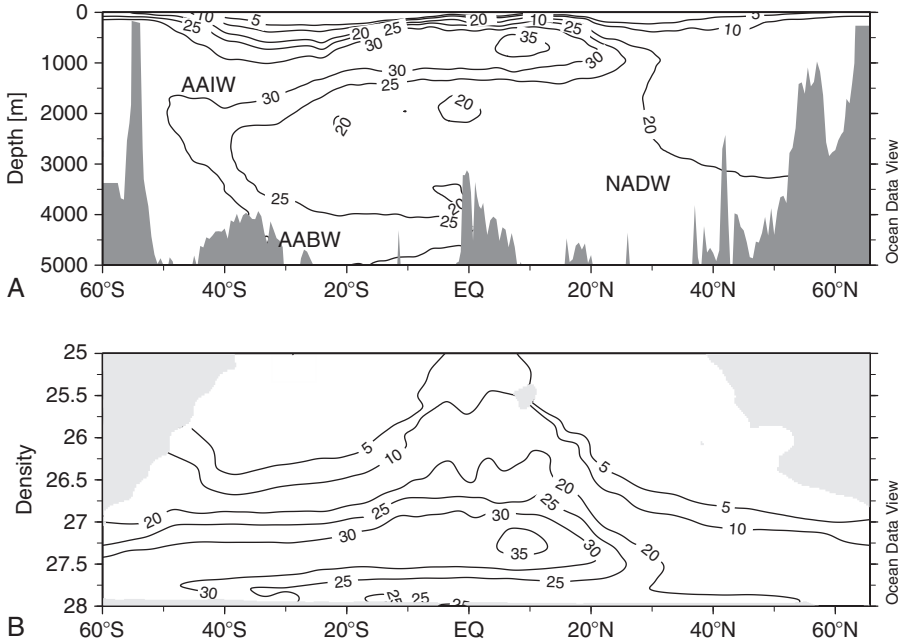
Figure 13.2 Section of (A) nitrate versus depth and (B) nitrate versus potential density (sigma theta) using combined data from the 2003 (North Atlantic) and 2005 (South Atlantic) occupations of the A16 line. North Atlantic Deep Water (NADW), Antarctic Bottom Water (AABW) and Antarctic Intermediate Water (AAIW) are indicated. Units μmol kg^{-1}.

Nitrate-rich signatures of Antarctic Bottom Water (AABW) and Antarctic Intermediate Water (AAIW) extend northward, reflecting the high surface concentrations where these water masses are formed. These water masses are also evident in the zonal structure of the North and South Atlantic, and nitrate-rich (>30 μM) AAIW is evident in the South Atlantic (Fig. 13.3) at mid-depth, along with a hint of AABW with elevated nitrate concentrations in the deep western part of the basin. There is
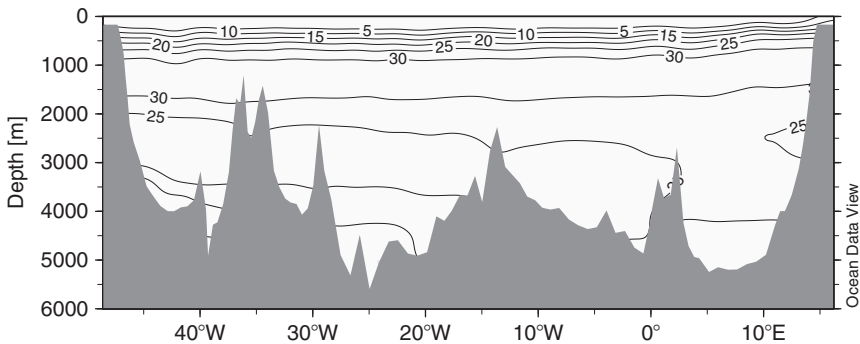
Figure 13.3 Section of nitrate versus depth at 30°S (WOCE A10) in 1993. AAIW present in the waters with nitrate >30 μmol kg^{-1}. Units μmol kg^{-1}.

only weak horizontal variation in the east–west direction; nonetheless, the gradient can be significant when coupled with the east–west variations in volume transport (Rintoul and Wunsch, 1991; see Section 3.1).

The large scale pattern of wind stress over the oceans drives the Ekman suction (upwelling) over the cyclonic subpolar gyres and in the tropics, advecting nitrate upwards in those regions and providing a source of nutrients to the euphotic zone. Strong seasonality in mixed-layer depths at northern mid- and high-latitudes is reflected in the nutrient supply (Glover and Brewer, 1988), which fuels the subsequent spring bloom following stratification (Sverdrup, 1953) in the North Atlantic, where the aeolian iron supply is strong.

The high southern latitudes are dominated by zonal features associated with the Antarctic Circumpolar Current. To the south of the Polar Front, surface nitrate concentrations are high year-round, owing to the upwelling of nitrate-rich, iron-starved circumpolar deep waters (e.g., Boyd *et al.*, 2000) by the residual mean overturning circulation (e.g., Marshall, 1997). During northward transit across the circumpolar current region, nitrate is stripped from the surface waters, aided by the aeolian iron source, though surface concentrations are still elevated in the regions of Antarctic Intermediate Water (AAIW) and Sub-Antarctic Mode Water (SAMW) formation, providing these water masses with pre-formed nitrate (Fig. 13.2A).

In the subtropical gyres, Ekman pumping (downwelling) drives nitrate-rich waters away from the surface. In the oligotrophic surface waters, nitrate concentrations are generally at low nanomolar levels (Cavender-Bares *et al.*, 2001; Eppley *et al.*, 1990; Garside, 1985; Lipschultz, 2001). In the ventilated thermocline, spatial gradients of nitrate along isopycnal surfaces (Fig. 13.2B) are determined by the balance between transport and remineralization of organic matter: On the σ_θ 26 surface, for example, nitrate increases from 5 to 10 μM towards the equator, reflecting the accumulation of regenerated nutrients as the waters of the ventilated thermocline transit away from the subduction region. While the nitrate distribution on σ_θ 26 (Fig. 13.2B) reflects the somewhat symmetrical subtropical overturning cells (Zhang *et al.*, 2003), deeper isopycnals also exhibit an asymmetry due to the northward transit of relatively nitrate-rich AAIW and SAMW.

There is anomalously high nitrate (>35 μM) at low latitudes in the density class of AAIW and SAMW (Oudot *et al.*, 1998), with elevated tongues north and south of the equator (Fig. 13.2A; centered near 8–10°N and 8–10°S, at 400–900 m). These persistent features were present during the earlier GEOSECS occupations (see Fig. 4 in Sharp, 1983) and appear to be transported from regions of strong coastal upwelling off Africa (Fig. 13.4C; see also model study of Williams *et al.*, 2006). Particulate organic nitrogen (PON), formed during primary production in systems enriched by upwelled nutrients, sinks out of the euphotic zone before eventual remineralization to nitrate in the water column or sediments. In coastal upwelling zones this occurs over the shelf and at relatively shallow depths (<200 m in the NW Iberian shelf system of Alvarez-Salgado *et al.*, 1997). Regenerated nutrients are "trapped" on the shelf by the combination of upwelling and biological export. Several studies of upwelling off Africa have reported relatively shallow (<200 m) length scales for regeneration (see Codispoti, 1983; Calvert and Price, 1971; Dittmar and Birkicht, 2001; Minas *et al.*, 1982; Rowe *et al.*, 1977; Treguer and LeCoore,
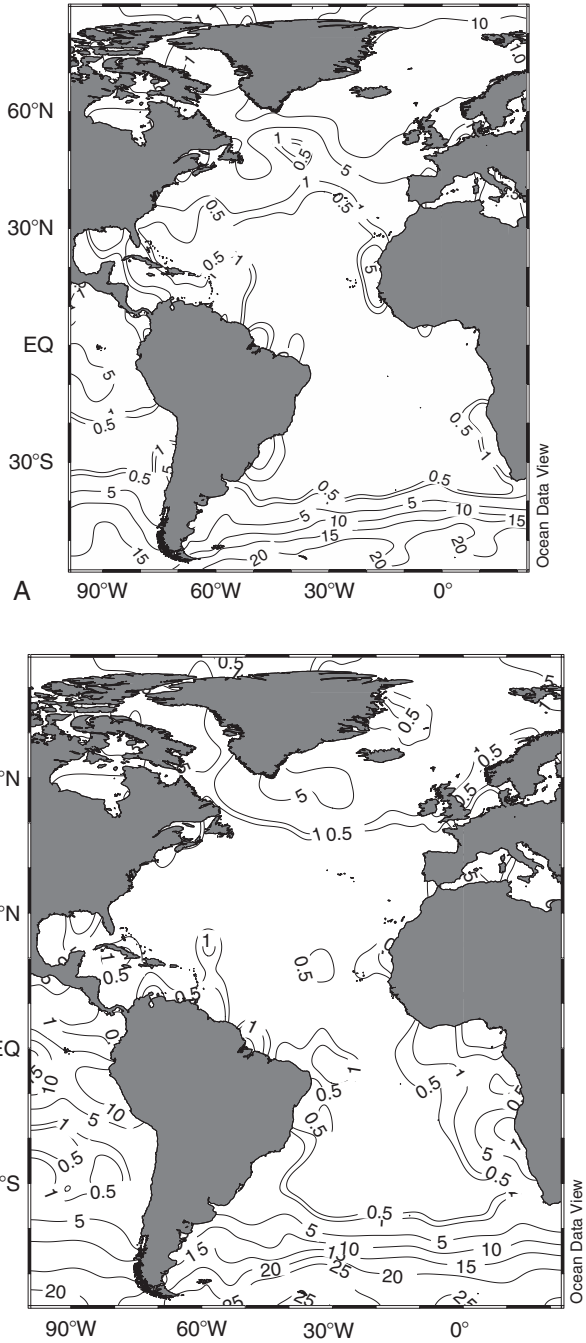
Figure 13.4 Climatological distributions of nitrate at the surface during (A) the January–March period and the (B) July–September period, and

(Continued)
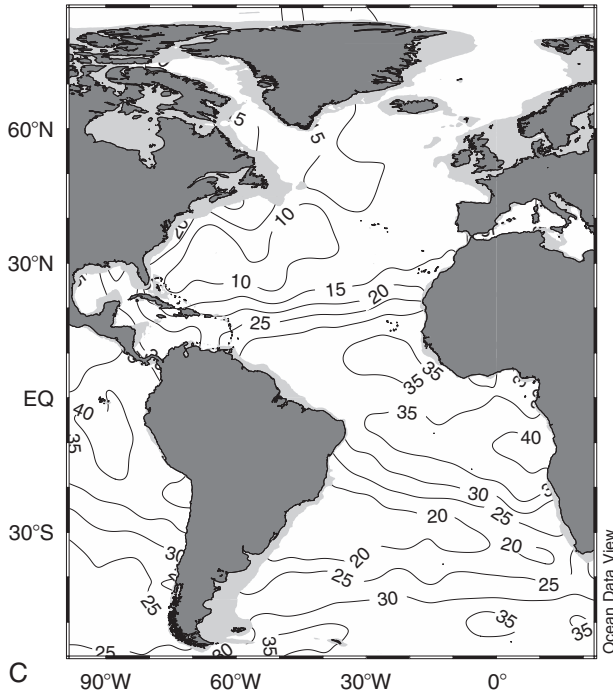
Figure 13.4 cont'd (C) at 500 m (January–March). Data source was the World Ocean Atlas. Units μmol kg^{-1}.

1979) though the high concentrations of nitrate emanating from the coastal zone of West Africa occur at depths of 500 m or more (Fig. 13.4C). The presence of enhanced nitrate at these depths suggests either a significant contribution from deeper remineralization, or significant physical interaction with the shelf waters at these depths. The spreading of this nitrate at intermediate depths eventually feeds into the source waters for the North Atlantic (Williams *et al.*, 2006; see Section 3.1), suggesting a significant role for the African upwelling regions in maintaining macro-nutrient abundance in the northern basin.

2.2. Dissolved organic nitrogen

A significant fraction of dissolved nitrogen is in the form of dissolved organic nitrogen (DON; recently reviewed by Bronk, 2002), the prevalent form in oligo-trophic surface waters (Bode *et al.*, 2001; Cavender-Bares *et al.*, 2001; Hansell and Carlson, 2001; Vidal *et al.*, 1999). Measurements of DON concentrations are imprecise because the value is calculated as the difference between total dissolved nitrogen and nitrate, both of which can be relatively large values compared to DON. The propagation of errors causes low precision in DON estimates, particularly in deep waters. Because the DON data are often inadequately precise for resolving small concentration differences or minor changes with time, the DON field can
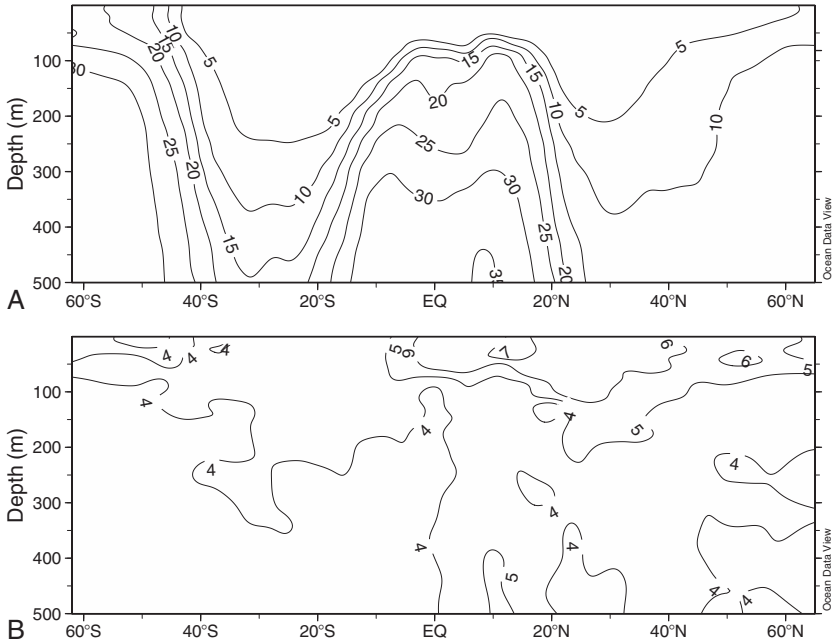
Figure 13.5 Upper 500 m distributions of (A) nitrate (μmol kg^{-1}) and (B) DON (μmol kg^{-1}) along section A16 (North and South Atlantic basins occupied in January 2003 and June 2005, respectively, with data combined from those cruises). Dots indicate sample depths.

appear relatively invariant (e.g., Hansell and Carlson, 2001). In nutrient-replete systems such as the English Channel, however, DON has a strong seasonality and large concentration changes that are anti-correlated with nitrate (Butler *et al.*, 1979).

There is a paucity of information on the large scale spatial distribution of DON in the Atlantic, but DON has been determined on a few sections. Mahaffey *et al.* (2004) presented data from the Atlantic Meridional Transect, evaluating the dynamics of nitrogen and phosphorus, with special consideration for the role of DON. Vidal *et al.* (1999) presented a quasi-meridional section from 22°N to 31°S in the central Atlantic, while Doval *et al.* (2001) reported DON in the temperate, transitional and subtropical waters of the Azores Front region east of Azores. A survey of DON in the upper 500 along Climate Variability and Predictability (CLIVAR) section A16 (a meridional section from 60°S to 60°N) is shown in Fig. 13.5. DON is measured with highest precision where nitrate concentrations are minimal (Hansell, 1993), and thus the most informative DON assessments are in oligotrophic surface waters. On A16, in the North Atlantic, surface waters with <5 μmol kg^{-1}NO$_3^-$ had DON concentrations >5 μmol kg^{-1} in the subtropical and subpolar regions and >7 μmol kg^{-1} in the more stratified tropical waters (<20°N) (Fig. 13.5). This tropical enhancement may be due to the abundance of diazotrophs in tropical waters

(Capone *et al.*, 1994; Glibert and Bronk, 1994), as has been suggested to explain elevated DON in the western equatorial Pacific (Hansell and Feely, 2000) and the central Atlantic (Vidal *et al.*, 1999). But Mahaffey *et al.* (2004) reported that the particulate organic N fraction in the tropics are enriched in ^{15}N, suggesting nitrate as a likely source. Similarly, the ^{15}N content of total organic N in the Sargasso Sea suggests that N$_2$ fixation is a minor contributor to DON (Knapp *et al.*, 2005; Meador *et al.*, 2007). Upwelling of nitrate (whether coastal or equatorial), subsequent enhanced productivity, and horizontal transport likely supports the DON accumulation observed. It is puzzling, though, that the nitracline is similarly shallow in the subpolar region (e.g., 50°N), thus making nitrate available by vertical mixing there, but DON remains less abundant than in the tropics (Fig. 13.5). This finding suggests differing controls on DON concentrations for the various zonal sectors.

In the South Atlantic, with similarly low surface nitrate concentrations, DON is consistently <5 µmol kg^{-1}, or 2 µmol kg^{-1} lower than the North Atlantic. In fact, the western Sargasso Sea (near Bermuda) also has relatively low surface DON values (Hansell and Carlson, 2001), similar to values found in the South Atlantic. The relatively elevated DON concentrations in the low latitude North Atlantic are suggestive of unique inputs there (such as atmospheric deposition, N$_2$ fixation, or upwelled nitrate with horizontal transport) compared to the South and western North Atlantic.

DON concentrations are also elevated in the Atlantic coastal and shelf regions (Bode *et al.*, 2001; Doval *et al.*, 1997) where freshwater inputs are significant (Bates and Hansell, 1999; Lopez-Veneroni and Cifuentes, 1994). This material may be transported off-shelf and photo-oxidized, releasing ammonium to the phytoplankton community (Morell and Corredor, 2001).

2.3. Seasonal variations of surface nitrate

The broad pattern of surface nitrate concentrations is related to the patterns of wind-driven upwelling and downwelling in the upper ocean. The subpolar and tropical surface waters have seasonally elevated nitrate concentrations in contrast to the continuously oligotrophic subtropical gyres (Figs. 13.4A and B). The North Atlantic undergoes a basin-wide seasonal and meridional advance and retreat in the nitrate front, related to a springtime advance of the phytoplankton community (Siegel *et al.*, 1990), while the South Atlantic apparently undergoes greater seasonal nitrate enrichment along the continental margins than in the interior (Figs. 13.4A and B).

Seasonal and longer term variability of upper ocean nitrate has been observed at several sites in the North Atlantic (Table 13.1). These time-series studies span a range of latitudes (Fig. 13.1) and physical forcing regimes. The Bermuda Atlantic Time-series Study (BATS) site is located within the subtropical gyre of the North Atlantic (31.66°N, 64.16°W), remote from continental land masses and coastal influences. There, winter cooling results in vertical mixing that, if strong enough, introduces nitrate to the surface layer (Fig. 13.6). Since insolation is relatively high year-round, this nutrient injection stimulates a late winter bloom (Michaels *et al.*, 1994b; Steinberg *et al.*, 2001).

Table 13.1 Scientific studies of nitrogen dynamics near four time-series study sites in the North Atlantic (BATS, ESTOC, CARIACO, CaTS)

BATS—Bermuda Atlantic Time-series Study	
Steinberg *et al.* (2002)	Investigated role of vertically migrating zooplankton on transport of nitrogen. This active transport of dissolved N (NH_4^+ and DON) averaged 27% of mean monthly sinking PON flux at 300 m.
Lipschultz *et al.* (2002)	Review of the history and current issues associated with the concept of new production in the Sargasso Sea
Lipschultz (2001)	Report on the temporal variability of nitrate and ammonium (nanomolar) concentrations and uptake rates in the oligotrophic upper layer. Nitrate uptake during the oligotrophic periods does not strongly contribute to new production; it is locally regenerated
Steinberg *et al.* (2001)	Overview of BATS time-series data, including nitrogen nutrients and vertical fluxes
Lohrenz *et al.* (1992)	Seasonal variability in sinking flux of PON
Sweeney *et al.* (2003)	Impacts of mesoscale eddies on primary production and particle flux. High export events occurred during passage of mode water eddies, but production was suppressed during anticyclones
Ono *et al.* (2001)	Diagnostic model of annual cycles of O_2, DIC and nitrate below the mixed layer is used to investigate organic matter remineralization.
McGillicuddy *et al.* (1999)	Surveys of mesoscale eddies showed that upper ocean nutrient distributions were controlled by vertical isopycnal displacements associated with the mesoscale field
Doney *et al.* (1996)	1-D, N-based biophysical model applied to the region near Bermuda.
Michaels and Knap (1996)	Overview of BATS time-series data, including nutrients and fluxes
Lipschultz *et al.* (1996)	Seasonal fluctuation of nitrite in the deep ocean
Hansell and Carlson (2001)	Report on the apparent absence of strong seasonal variability in DON at BATS; included C:N ratios of DOM.
Orcutt *et al.* (2001)	Reports rates of N_2 fixation by colonies and single cells of *Trichodesmium* spp
Hood *et al.* (2001)	Modeling analysis indicates significant interannual variability in N_2-fixation at BATS; variability linked to decadal-scale fluctuations in the North Atlantic climate
ESTOC—European Station for Time-series in the Ocean Canary Islands	
Neuer *et al.* (2002b)	Export production and stable N isotope composition reported along a productivity gradient north of the Canary Islands. Study links productive shelf and subtropical gyre provinces
Pätsch *et al.* (2002)	1-D C and N cycling model applied for period of 1987–1996

(Continued)

Table 13.1 Scientific studies of nitrogen dynamics near four time-series study sites in the North Atlantic (BATS, ESTOC, CARIACO, CaTS) *(continued)*

Freudenthal *et al.* (2001)	Particle fluxes and stable nitrogen isotope ratios reported in productivity gradient near the Canary Islands. Region influenced by coastal upwelling
Pérez *et al.* (2001)	Uses nutrients and other hydrographic variables to define water masses found in the region
Llinás *et al.* (1993)	Mean values of nutrients reported to be similar to historical values reported for the region
CARIACO—Carbon Retention in a Colored Ocean Project	
Ho *et al.* (2004)	Temporal and vertical variability of major electron donors and acceptors in the water column, including nitrate
Walsh *et al.* (1999)	Simulation of carbon/nitrogen cycling during spring upwelling in the Cariaco Basin
Walsh (1996)	C:N depletion ratios are reported to be elevated (relative to the canonical Redfield ratio) in the Venezuelan upwelling system. N_2 fixation may provide a Redfield balance
Ward and Kilpatrick (1991)	Nitrification and nitrate reduction are considered in the oxic and oxygen deficient zones of the Cariaco Trench
CaTS—Caribbean Time-series Study	
Corredor *et al.* (2003)	Evaluates N budgets in a persistent front generated by the confluence of western tropical North Atlantic surface waters with the massive freshwater plumes of the Orinoco and Amazon Rivers
Morell and Corredor (2001)	Ammonium release from DOM constitutes a major source of inorganic nitrogen to phytoplankton communities in the Orinoco River plume
Corredor and Morell (2001)	Describes vertical and temporal variation of water mass structure, dissolved nutrients, phytoplankton chlorophyll a, and dissolved organic matter with particular emphasis on variability of Caribbean Surface Water features
Morel *et al.* (2001)	Present data on the vertical and latitudinal distribution of N_2O and estn. of fluxes across the sea–air and thermocline interfaces in tropical ocean waters north and south of Puerto Rico. Observations provide evidence for active N_2O production in near-surface tropical waters
Corredor *et al.* (1999)	A review of mechanisms leading to nutrient limitation in tropical marine systems, emphasizing N cycling in Caribbean ecosystems

There exist time-series observations at two locations in the Caribbean: South of Puerto Rico at the Caribbean Time-series Study site (CaTS; 17.6°N, 67°W), and north of Venezuela at the CARIACO site (Carbon Retention in a Colored Ocean Project; 10.5°N, 64.67°W). At the former, the Caribbean Surface Water (upper 75 m) experiences a yearlong influence of continental river input, as evidenced by lowered
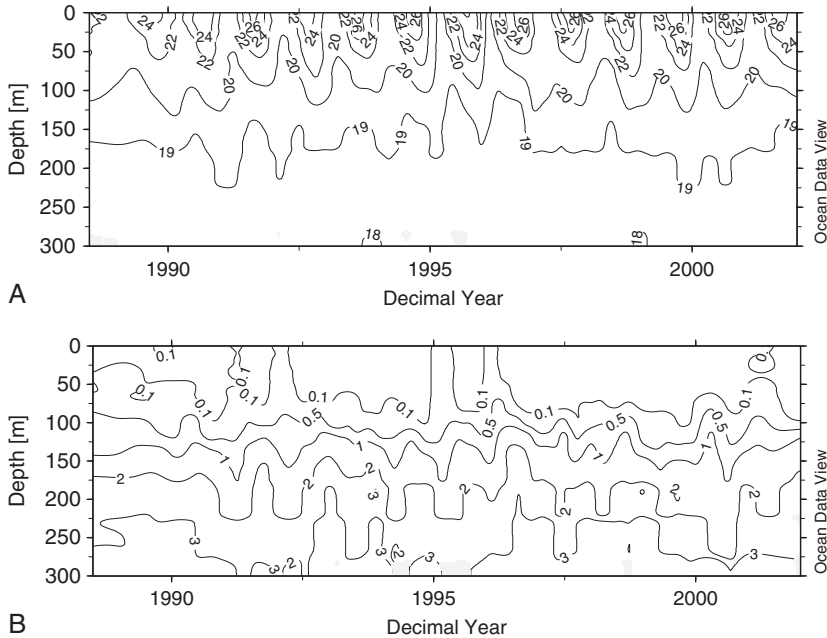
Figure 13.6 Temporal variability of (A) temperature ($^\circ$C) and (B) nitrate (μmol kg^{-1}) at the BATS site. Sampling was conducted at approximately monthly intervals.

salinity and elevated silica (Corredor and Morell, 2001). Marine nutrients, elevated at depths greater than 100 m, do not break through to the surface due to the stratification imposed by the low density surface layer (Fig. 13.7). At the CARIACO site, elevated nitrate waters are close to the surface layer year-round, with water of >1 μmol kg^{-1}NO$_3^-$ mixing to the surface on occasion (Fig. 13.8). Upwelling of Subtropical Underwater (SUW) starts around October and lasts through May each year, fuelling a winter maximum in chlorophyll *a* (Muller-Karger *et al.*, 2001).

The European Station for Time-Series in the Ocean, Canary Islands (ESTOC), located north of the Canary Islands at 29.16°N, 15.5°W, was established in 1994 in the eastern boundary system of the subtropical North Atlantic Gyre. The seasonality of phytoplankton biomass and productivity is very similar to that found at BATS (Neuer *et al.*, 2002a). Nitrate is usually below detection in the surface layer (<0.1 μmol kg^{-1}) but convective overturning in late fall and winter introduces nitrate into the euphotic zone, initiating a phytoplankton maximum coincident with the period of deepest mixing (Neuer *et al.*, 2007). In contrast to BATS, N:P is near the Redfield ratio (Neuer *et al.*, 2002a). Intermittent increases in mixed layer nitrate in summer might be related to the passage of mesoscale features or the mixing of the water column due to the onset of the strong trade winds.

No dedicated biogeochemical observation sites currently exist in the subpolar North Atlantic, but studies such as the North Atlantic Bloom Experiment (Ducklow and Harris, 1993) and weather ship observations (Stramska *et al.*, 1995) provide
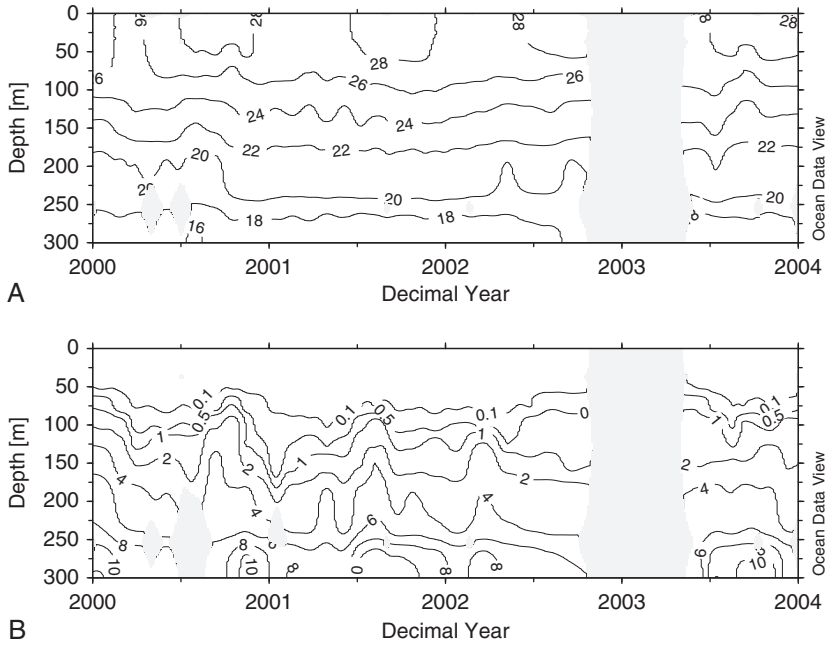
Figure 13.7 Temporal variability of (A) temperature (°C) and (B) nitrate (μmol kg^{-1}) at the CaTS site. Sampling was conducted at approximately monthly intervals. Note gap in data at end of 2002 and early 2003.
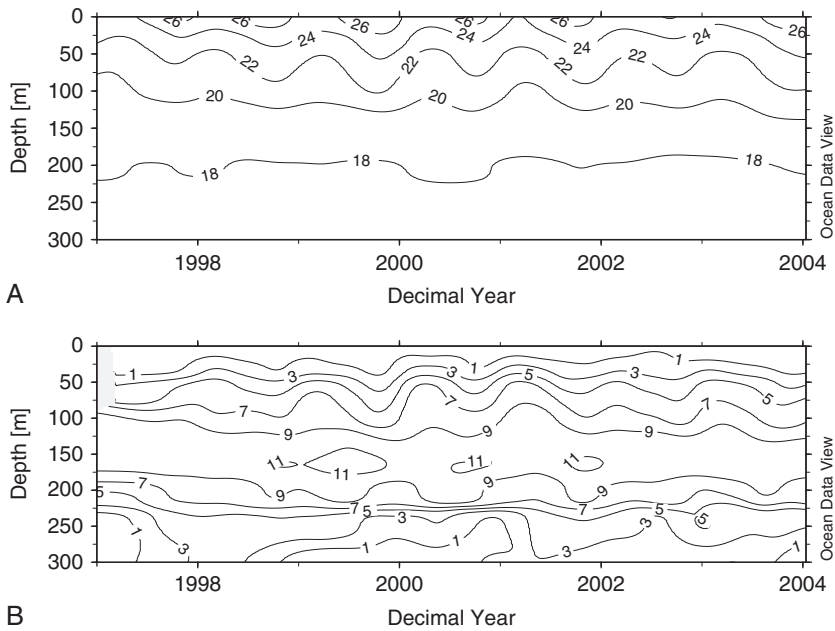
Figure 13.8 Temporal variability of (A) temperature (°C) and (B) nitrate (μmol kg^{-1}) at the CARIACO site. Sampling was conducted at approximately monthly intervals.

insights into the seasonal cycle at higher latitudes. There is a strong seasonal amplitude both in winter deepening of the mixed-layer and insolation, leading to elevated surface nutrient concentrations in the winter that are drawn down by the onset of the spring bloom as insolation increases and the water column stratifies again (Sverdrup, 1953).

In the equatorial Atlantic, surface nitrate concentrations vary seasonally with the rate of upwelling. Measurable nitrate is present in the east during the boreal summer but apparently absent year-round in the west (Oudot and Morin, 1987).

3. Sources of Nitrogen to the Euphotic Zone

The availability of nitrogen in the euphotic zone is an important, potentially limiting factor for productivity and the biological sequestration of carbon in the ocean. There are three principal routes by which new nitrogen makes its way into the euphotic zone of the Atlantic Ocean: Physical transport of nitrate, nitrogen fixation by diazotrophic organisms, and aeolian transport and deposition.

3.1. Physical supply of nitrate

The subpolar North Atlantic is a site of significant deepwater formation and export (Fig. 13.2) balanced by the northward flow of warm, nutrient depleted surface waters into the basin. Biological activity further strips the macro-nutrients from the surface waters, while newly formed deep waters make their way southward, accumulating regenerated nutrients. This contrasts with the deep Indo-Pacific basins where the deep inflow brings nutrient-rich bottom waters into the basin where they are upwelled. This simple, two-dimensional view of the Atlantic, with inflow of low nutrient surface waters and outflow of high nutrient deep waters, suggests a significant net transport of macro-nutrients out of the basin. However, the horizontal circulation in the thermocline provides a compensatory northward transport of nitrate (Rintoul and Wunsch, 1991; Williams and Follows, 2003; Williams *et al.*, 2006). The northward flowing Gulf Stream swiftly transports nutrient rich waters in the thermocline, termed the ''nutrient stream'' (Pelegri and Csanady, 1991; Pelegri *et al.*, 1996). The nutrient stream in the North Atlantic includes contributions from the Southern Hemisphere (Fig. 13.9B) associated with the basin scale meridional overturning circulation, but also from waters recirculating within the subtropical gyre. The stream is strongest on isopycnals associated with the northward transport of SAMW (Fig. 13.9A). The relative distributions of nitrate and silica in the global ocean suggest that this water mass is the major source of macro-nutrients to the surface of the Atlantic basin north of the Antarctic Circumpolar Current (Sarmiento *et al.*, 2004). Nutrient trapping in the Equatorial Upwelling region may increase the nitrate concentration on this density surface and further enhance the nutrient stream (Williams *et al.*, 2006).

In the subpolar gyre and in the northern sector of the subtropical gyre, the nutrient stream encounters the deep winter mixed-layers and outcrops (Fig. 13.9A), thus sustaining the annual convective supply of nitrate to the surface (Williams and
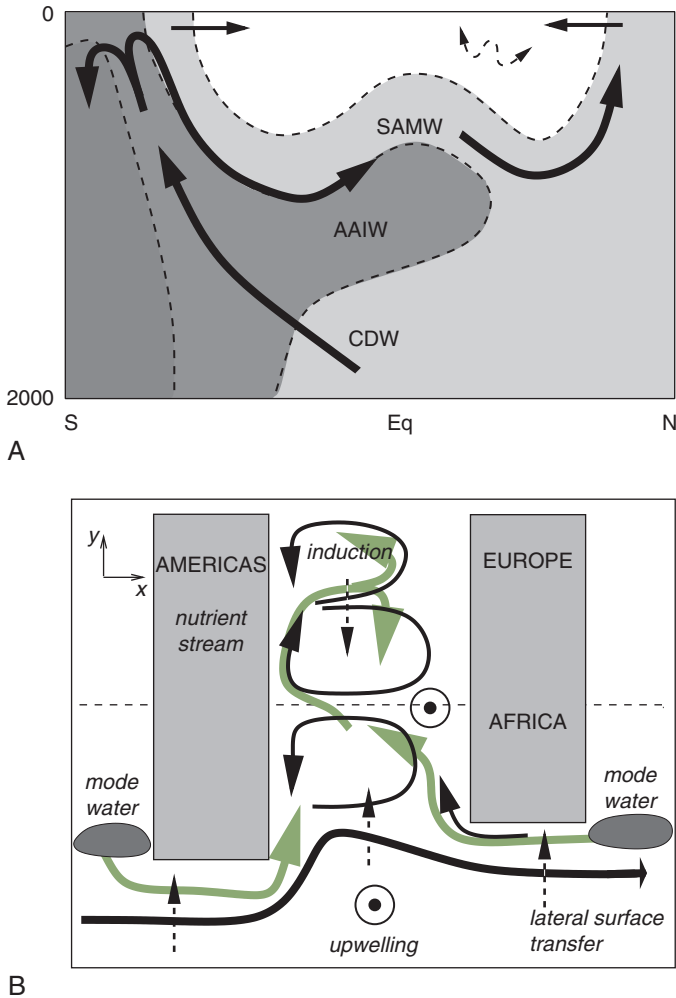
Figure 13.9 Schematic views of (A) meridional and vertical transport of nitrate in the upper Atlantic basin and (B) associated horizontal transport pathways. Nitrate-rich Circumpolar Deep Water (CDW) upwells in the Southern Hemisphere and the residual mean flow transports some of this upwelled water across the polar front into the regions of intermediate and mode water formation. Nitrate-rich Sub-Antarctic Mode Water (SAMW) and Antarctic Intermediate Water move northward in the thermocline, ultimately outcropping in the subpolar North Atlantic. Ekman transfer provides a source of nitrate to the subtropical gyre along with lateral and vertical eddy transport processes.

Follows, 2003). There, seasonal variations of surface ocean mixing drive a wintertime supply of nitrate to the euphotic zone (Glover and Brewer, 1988), fuelling most of the annual export production.

While the North Atlantic subtropical gyre shows the classical signatures of oligotrophy, with low surface nutrient concentrations and little surface chlorophyll,

there is a significant rate of export production (Jenkins, 1982; Jenkins and Doney, 2003) and there has been considerable effort to understand the source of nitrogen (and phosphorus) to support this biological activity. Interpretations of oxygen and tritium–helium data (Jenkins, 1982; Jenkins and Goldman, 1985) suggest regional export production to be as much as 0.5 mol N m^{-2} year^{-1} in the North Atlantic subtropical gyre; greater than could be sustained by the upwards diapycnal flux assuming mixing rates observed in the interior thermocline (Ledwell et al., 1993). Recent estimates for the flux of nitrate into the euphotic zone near Bermuda are higher yet, at 0.8 mol N m^{-2} year^{-1} (Jenkins and Doney, 2003).

Wintertime convection supports a significant fraction of annual productivity in the subtropics (Michaels et al., 1994b) but it cannot sustain production over longer time-scales (Williams and Follows, 1998), though other physical transport processes may be important. The heaving of nutrient-rich isopycnals into the euphotic zone associated with ocean eddies ("eddy pumping") is suggested to be a significant, adiabatic source of nitrate to the euphotic zone of the subtropical oceans (Falkowski et al., 1991; McGillicuddy and Robinson, 1997; McGillicuddy et al., 2007; Oschlies and Garcon, 1998) though some studies suggest it is not a major contributor (Martin and Pondaven, 2003). In fact, particle export within a cyclonic eddy occupied in the Sargasso Sea near Bermuda was indistinguishable from export outside the eddy (McGillicuddy et al., 2007; Pointer et al., 2006). The biogeochemical imprint of export was evident in the eddy core (McGillicuddy et al., 2007), suggesting that the export event (and upward pumping of nitrate to the euphotic zone) had occurred and was indeed strong, but the process may have occurred nearer the time of eddy formation, and less so during transit in the Sargasso Sea. Numerical models suggest that smaller, frontal-scale features may be more significant sources of nitrate (Levy et al., 2001; Mahadevan and Archer, 2000).

These vertical motions can locally transfer nitrate to the surface from modest depths, but the three-dimensional circulation of the gyre must be invoked to maintain supply from the main thermocline and below. The nitrate-rich nutrient stream feeds nitrate to the deep winter mixed layers of the subpolar gyre and northern subtropical gyre (Fig. 13.9). The high surface concentrations of nitrate in the subpolar latitudes can be transferred into the subtropical gyre at the surface, along with dissolved organic nitrogen, by the ageostrophic Ekman flow and lateral eddy transfers (Abell et al., 2000; Lee et al., 1997; Mahaffey et al., 2004; Rintoul and Wunsch, 1991; Williams and Follows, 1998). Using climatological observations, Williams and Follows (1998) estimated that the Ekman transport of nitrate provides a modest contribution to the maintenance of new production locally in the North Atlantic subtropical gyre (equivalent to $\sim$0.2 mol C m^{-2} year^{-1}). However, this "trickle" of nutrients into the subtropics may be significant in maintaining the integrated nutrient load of the bowl of the gyre, balancing the continual flux of sinking organic matter to depths below the thermocline (Williams et al., 2006). Likewise, a substantial lateral transfer of surface properties from one side of the current to the other might occur in association with baroclinic eddies and their subsequent interactions with the Gulf Stream (Dutkiewicz and Paldor, 1994; Dutkiewicz et al., 2001). Such diapycnal, inter-gyre transports can be supported in the surface ocean by significant buoyancy forcing due to air-sea heat fluxes in combination with the strong inter-gyre surface nutrient gradients.

While some of the nutrient stream nitrate originates in the southern hemisphere with SAMW, a fraction has recirculated within the subtropical gyre (Fig. 13.9B). Jenkins and Doney (2003) hypothesize a "nutrient spiral" in which enhanced diapycnal mixing associated with the eddy-rich, western boundary current also leads to a significant vertical transfer of nutrients (and other properties including helium) into the mixed-layer of the subtropical gyre.

3.2. Geochemical investigations of nitrogen fixation

An alternative source of nitrogen to the euphotic zone is the fixation of N_2 by diazotrophic organisms (Karl *et al.*, 2002; Mahaffey *et al.*, 2005). This process has received considerable attention using direct biological observations (Capone *et al.*, 2005; Voss *et al.*, 2004), modeling (most recently for the Atlantic; Coles *et al.*, 2004a and Hood *et al.*, 2004), remote sensing (Coles *et al.*, 2004b; Hood *et al.*, 2002) and indirect geochemical approaches. Fanning (1987, 1992) first invoked nitrogen fixation to explain high N:P ratios (or excess nitrate) in the thermocline of the Sargasso Sea. Michaels *et al.* (1994a) noted decreases in carbon stocks from the spring to autumn in the upper 150 m of the Sargasso Sea near Bermuda not attributable to nitrate consumption, thus suggesting a role for N_2 fixation (see also the models of Anderson and Pondaven, 2003; Bissett *et al.*, 1999; Marchal *et al.*, 1996).

The relative abundances of nitrate and phosphate are typically discussed in terms of the diagnostic parameters $N\star$ ($N\star = NO_3^- - 16PO_4^{3-} + 2.90$) (Deutsch *et al.*, 2001; Gruber and Sarmiento, 1997; Michaels *et al.*, 1996) or DIN_{xs} ($DIN_{xs} = NO_3^- - 16PO_4^{3-}$) (Bates and Hansell, 2004; Hansell *et al.*, 2004). These indices measure the departure from classical Redfield ratios of the dissolved inorganic forms of nitrogen and phosphorus (the $N\star$ and DIN_{xs} indices differ only in the offset of 2.90 μM, a value that was intended to fix the global mean $N\star$ to zero). Negative values of DIN_{xs} (or $N\star$ values < 2.9 μmol kg^{-1}) indicate a deficit in N relative to P with respect to the requirements for Redfieldian production of organic matter; positive values of DIN_{xs} (or $N\star$ values >2.9 μmol kg^{-1}) indicate excess N relative to P. In the Atlantic (Fig. 13.10), there is negative DIN_{xs} throughout the upper layer ($\sigma_\theta < 26$), in the deep subpolar waters (>40°N), and in the South Atlantic (south of 10°N). The region of positive values is largely confined to the North Atlantic subtropical thermocline, between σ_θ surfaces 26 and 27.5 (to depths of ≈1100 m). This distribution implies net addition of excess nitrate (or the removal of P relative to N) in that region.

A number of recent studies have used the observed nitrate and phosphate distributions to infer local and basin scale rates of allochthonous nitrogen inputs to the North Atlantic (presumably due to nitrogen fixation, but other processes likely contribute; Hansell *et al.*, 2007). Due to the relative scarcity of appropriate data (required to provide high resolution spatial coverage), unresolved temporal variability, and uncertainties in estimated ventilation timescales, the inferred N_2 fixation rates have a large uncertainty, and the published basin integrals span an order of magnitude (0.15–6.4×10^{12} mol N year^{-1}; Table 13.2). Michaels *et al.* (1996) assessed the $N\star$ gradients on isopycnal surfaces in the upper thermocline between the BATS site and sites of ventilation for those surfaces. Using concurrent age (time since ventilation)
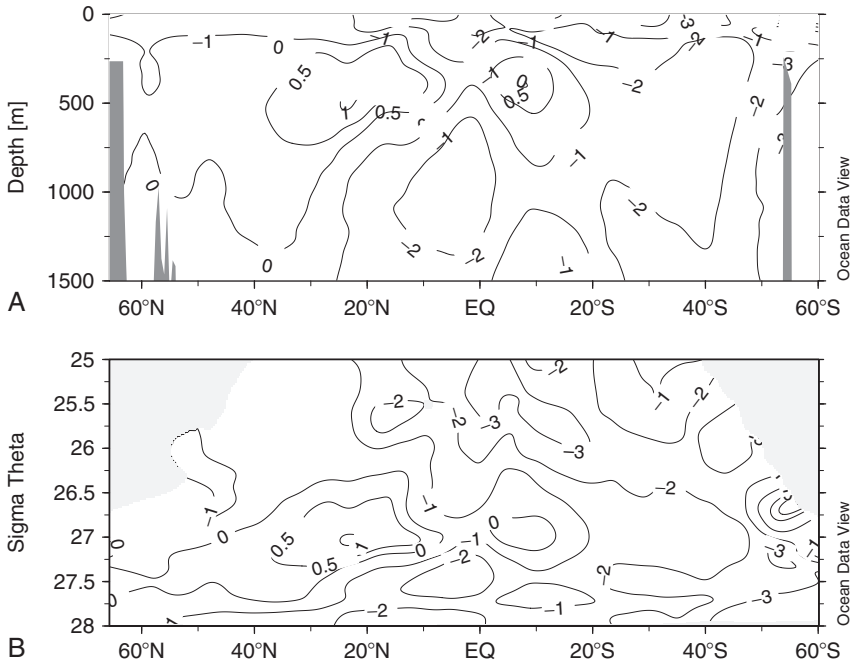
Figure 13.10 Section of (A) DINxs vs depth and (B) DINxs versus density (sigma theta) using data combined from the 2003 (North Atlantic) and 2005 (South Atlantic) occupations of the A16 line. Units $\mu mol\,kg^{-1}$.

estimates from transient tracers they inferred the rate of excess nitrate accumulation that is ascribed to N_2 fixation in the overlying waters. Their inferred N_2 fixation rates are the highest reported (up to 6.4×10^{12} mol N year^{-1} over the North Atlantic), but this was an extrapolation from a small region to the entire northern basin. Gruber and Sarmiento (1997) presented a more complete derivation of N* and an assessment of N* gradients in the global ocean from which they determined a lower, but still substantial, rate of N_2 fixation (2×10^{12} mol N year^{-1}) in the North Atlantic. Lee *et al.* (2002) estimated the net summer-time drawdown of inorganic carbon in nitrate-depleted tropical and subtropical waters and hypothesized that it must be balanced by N_2 fixation, totaling 2.3×10^{12} mol N year^{-1} for the whole basin with most occurring in the North Atlantic.

Ganachaud and Wunsch (2002) evaluated volume fluxes, nutrient transports, and net nutrient sources using a global set of hydrographic sections in the context of an inverse model. Their analysis inferred a significant net source of nitrogen (i.e., a high rate of N_2 fixation) in the South Atlantic (0.4 ± 0.17 mol N m^{-2} year^{-1}) and a net sink of nitrate (i.e., denitrification) in the subtropical North Atlantic, contrary to expectations. Note that their results do not imply that nitrogen fixation is not significant in the North Atlantic, but rather that the total N balance may be dominated by denitrification or the influence of lateral transport of dissolved organic nitrogen (Lee and Williams, 2000; Rintoul and Wunsch, 1991).

Table 13.2 Summary of indirect (geochemical) estimates of N_2 fixation in the Atlantic Ocean

	Fixation rate		Period of data collection	Comments
	$mol\,N\,m^{-2}\,year^{-1}$	$mol\,N\,year^{-1}$		
Michaels *et al.* (1996)	0.13–0.23★	$3.7–6.4 \times 10^{12}$	1989–1992	North Atlantic. Rate determined from N★ gradient between two sites in North Atlantic subtropical gyre, then extrapolated to entire gyre. ★Areal rate calculated using tropical and subtropical area of 27.8×10^{12} m^2
Gruber and Sarmiento (1997)	0.072	2×10^{12}	1977–1982	North Atlantic rate. Basin wide survey of N★
Lee *et al.* (2002)	–	2.3×10^{12}	1981–1998	40°S to 40°N. Estimated from summertime drawdown of inorganic carbon in nitrate depleted waters (data selection criteria: SST >20°C; NO_3^- <0.1 µmol kg^{-1}), converted to N using C:N molar ratio of 7
Ganachaud and Wunsch (2002)	0.4	–	1991–1994	Subtropical South Atlantic only; inverse methods using WOCE data
Hansell *et al.* (2004)	0.045	$0.15–0.46 \times 10^{12}$	1992–1998	North Atlantic rate. Basin wide survey of DIN_{xs} gradients using WOCE data.
Bates and Hansell (2004)	–	$0.22–0.61 \times 10^{12}$	1988–2001	North Atlantic rate; largely BATS and WOCE data.
Hansell *et al.* (2007)		$\sim 0.4 \times 10^{12}$	2003	Calculated by mass balance of total excess N accumulation rates and estimated inputs by atmospheric deposition and DOM export

Hansell *et al.* (2004) followed the analytical approach of Gruber and Sarmiento (1997), but using data collected during the World Ocean Circulation Experiment (WOCE; mostly 1990s) while Gruber and Sarmiento (1997) used data from earlier programs. Both analyses of the North Atlantic resulted in comparable areal rates for N_2 fixation (0.045 and 0.072 mol N m^{-2} year^{-1}, respectively), yet their extrapolated rates for the basin differed by more than 6-fold (Table 13.2). Gruber and Sarmiento (1997) applied their volumetric N accumulation rates for each isopycnal surface to the entire area of 10–50°N and 10–90°W. Hansell *et al.* (2004), deeming this area too large to be representative of the zone of N_2 fixation in the North Atlantic, limited their extrapolation to subtropical waters showing positive spatial gradients in the concentrations of DIN_{xs} (thus indicating regions of net excess nitrate additions to the thermocline waters). Without greater spatial coverage of the distribution of excess N accumulation, the basin integral remains very uncertain.

Besides uncertainty in the spatial extent of excess N, it is possible that significant temporal variability, manifested on large spatial scales, may be reflected in the contrasting estimates. Bates and Hansell (2004), in a study of DIN_{xs} and its variation at the BATS (Bermuda Atlantic Time-Series Station) site, found that local variations in excess nitrate in the Sargasso Sea correlated with the North Atlantic Oscillation Index. Positive states of the index correlated with elevated concentrations (and presumably production rates) of excess nitrate, and it is during such phases that the Michaels *et al.* (1996) and Gruber and Sarmiento (1997) data were collected. Negative phases correlated with lower concentrations of excess nitrate, and it was during such a phase that the data employed by Hansell *et al.* (2004) were collected. It may be that the difference in basin integral estimates of nitrogen fixation partly reflects large-scale, temporal variability.

What might give rise to such temporal variability? Atmospheric dust inputs of iron to the subtropical North Atlantic have been implicated (Gruber and Sarmiento, 1997; Mahaffey *et al.*, 2003; Michaels *et al.*, 1996) since diazotrophs have a high iron requirement relative to other plankton (Berman-Frank *et al.*, 2001; Kustka *et al.*, 2003; Raven, 1988). The magnitude and timing of the atmospheric transport of dust to the region is related to the changes in atmospheric circulation characterized as the North Atlantic Oscillation (NAO), which has also been linked to variability in the excess nitrate signal in the western Sargasso Sea (Bates and Hansell, 2004). Hansell *et al.* (2004) found the strongest signature of excess nitrate in the southeastern North Atlantic where there is an input of North African dust. It is not yet clear, though, the extent to which diazotrophs are iron limited in the North Atlantic (Mills *et al.*, 2004; Sanudo-Wilhelmy *et al.*, 2001; Voss *et al.*, 2004).

Geochemical estimates for N_2 fixation rates can be compared to those determined by direct biological measurements (i.e., bottle incubations of *Trichodesmium*). Based on such direct, though sparse, measures Capone *et al.* (1997) estimated the average N_2 fixation rate for the tropical oceans to be 106 μmol N m^{-2} day^{-1} (0.039 mol N m^{-2} year^{-1}) and tabulated measurements from the Atlantic showing a range of 1.4–278 μmol N m^{-2} day^{-1} (0.050–0.10 mol N m^{-2} year^{-1} if extrapolated), with the highest rate located in the Caribbean Sea. Capone *et al.* (2005) conducted an extensive survey of N_2 fixation by *Trichodesmium* in the North Atlantic and conservatively estimated a basin-wide rate of 1.6×10^{12} mol N; a rate in general

agreement with the Gruber and Sarmiento (1997) estimate but larger than that of Hansell *et al.* (2004, 2007). Estimates of N_2 fixation in the western tropical North Atlantic have also been made after combining remote sensing and modeling techniques (Coles *et al.*, 2004b), resulting in a rate of 220 µmol N m^{-2} day^{-1}.

There are intriguing DIN_{xs} features in the eastern tropical South Atlantic (Fig. 13.11) that suggest N_2 fixation, but that are, as yet, unsubstantiated. Coincident with the enrichment of nitrate on the σ_θ 26.8 surface along the western Africa coast (Fig. 13.11A) is an elevation of DIN_{xs}, (Fig. 13.11B) with values of –1 µM near the coast, though the source waters (SE South Atlantic) typically show $DIN_{xs} \sim -3$ µM. This coastal region is known for its strong upwelling and, therefore, nutrient inputs, so it seems an unlikely candidate for strong N_2 fixation. Hood *et al.* (2004) employed a coupled biological/physical model, with explicit representation of *Trichodesmium*, which consistently suggested possible high and persistent *Trichodesmium* spp. concentrations in the Gulf of Guinea, off the west coast of Africa.
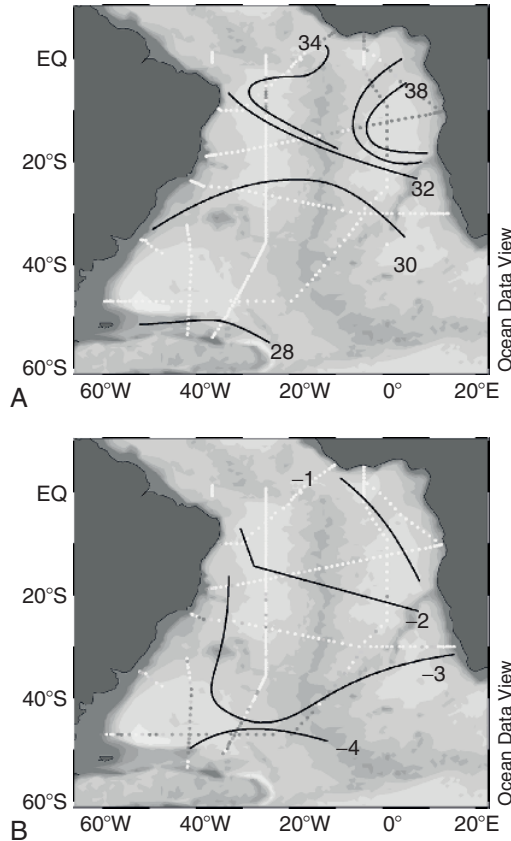
Figure 13.11 South Atlantic distributions of (A) nitrate (µmol kg^{-1}) and (B) DIN_{xs} (µmol kg^{-1}) on σ_θ surface 26.8. Data collected in the South Atlantic Ventilation Experiment (SAVE) project. Dots are station locations.

It is also probable that N and P are not remineralized in Redfield proportion. Some organisms may directly utilize DOP as a source of phosphorus and there may be preferential remineralization by bacteria (Wu *et al.*, 2000). Perhaps processes such as differential remineralization of N and P in sinking particles are significant here and the attribution of DIN_{xs} to N_2 fixation alone should be reconsidered. This highlights the issue that the evaluation of nitrogen fixation rates from geochemical evidence is not simple since a number of processes can affect the DIN_{xs} ($N^\star$) signals (Gruber, 2004) and because of uncertainties in ventilation time scales. Denitrification will also have a direct impact on the N:P ratio (e.g., Deutsch *et al.*, 2001, 2007), though it is expected that denitrification is more significant over the shelves than in the interior of the Atlantic, which is generally well oxygenated.

3.3. Atmospheric deposition and possible contribution to excess nitrate

New nitrogen is also introduced to the Atlantic through wet and dry atmospheric deposition (Baker *et al.*, 2003; Spokes *et al.*, 2000). Nitrogen emitted to the atmosphere in the northeastern United States moves downwind for deposition to the mid-latitudes of the North Atlantic, while material emitted from equatorial Africa is transported to the low latitudes of the Atlantic (Fig. 13.12; Galloway *et al.*, 2004). Duce *et al.* (1991) estimated input of 0.6×10^{12} mol N year^{-1} for the total North Atlantic and 0.3×10^{12} mol N year^{-1} for the subtropical and tropical North Atlantic alone. Similarly, Prospero *et al.* (1996) estimated N added by deposition to
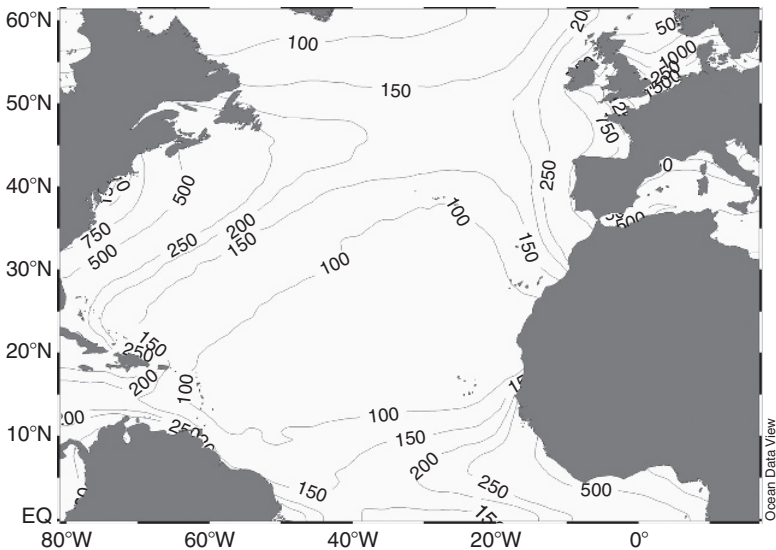
Figure 13.12 Modeled nitrogen deposition (sum of NOy and NHx; mg N m^{-2} year^{-1}) to the North Atlantic in the year 1990. These are net rates as they are corrected for the emissions of NH_3 from the oceans. Data adapted from Dentener *et al.* (2006).

the entire North Atlantic of 0.6×10^{12} mol N year^{-1}. Galloway *et al.* (2004) modeled the deposition of N to the North Atlantic, finding a rate of 0.8×10^{12} mol N year^{-1}. These values lie between the rates of excess N accumulation given by Gruber and Sarmiento (1997) and by Hansell *et al.* (2004) (Table 13.2), suggesting that deposition from the atmosphere has to be considered in the geochemical budget for excess N accumulation in the thermocline. Net input by atmospheric deposition to the South Atlantic is smaller at 0.15×10^{12} mol N year^{-1} (Galloway *et al.*, 2004).

Deposition of both inorganic and organic forms of nitrogen is of interest (Cornell *et al.*, 1995), since both are available as nutrients that can increase new and export production, though far fewer data exist for organic N. Wet deposition (during precipitation events) is readily measurable, but the contribution from dry deposition has been more difficult to quantify. Russell *et al.* (2003) reported that 43% of total N deposition on the US coast is dry, while Rendell *et al.* (1993) found a similar fraction in the North Sea. The greatest deposition rates occur in waters adjacent to the continents, particularly where anthropogenic influences are greatest (Paerl *et al.*, 2002), and decreases into the ocean interior. Much of the DON deposited is biologically labile and thus available to phytoplankton (Scudlark *et al.*, 1998; Seitzinger and Sanders, 1999), but the low fluxes have relatively little direct impact on the open ocean biota (Knap *et al.*, 1986; Michaels *et al.*, 1993).

In considering the characteristics of nutrients deposited to the ocean, it becomes clear that N_2 fixation is not the only process that (1) leads to the accumulation of "excess N" in the upper thermocline (that is, the nutrients regenerated there have high N:P ratios), (2) introduces excess N with low δ^{15}N values, (3) has seasonality such that it would cause a summertime drawdown of surface layer CO_2 in extremely oligotrophic conditions, and (4) occurs in regions of high atmospheric inputs of material such as dust. The existence of high N:P signatures in the North Atlantic thermocline has been ascribed to N_2 fixation, yet the deposition of nutrients from the atmosphere also occurs with elevated N:P ratios (ratios of 50–100 are common, with some ratios >1000 in dry deposition; Baker *et al.*, 2003). The low δ^{15}N values found in organic matter in the North Atlantic have been assigned to N_2 fixation (which produces organic matter with δ^{15}N values <0‰; Carpenter *et al.*, 1997), yet N added by deposition has low δ^{15}N values as well (Hastings *et al.*, 2003; Paerl and Fogel, 1994; exceptions have been reported where values >8‰ have been reported for N of an African source, Swap *et al.*, 1996). Drawdown of CO_2 during oligotrophic periods could be due to N_2 fixation (Lee *et al.*, 2002) driven by Fe inputs, but along with the heavy input of iron is a load of macronutrients that need to be considered (e.g., Herut *et al.*, 2002; Krom *et al.*, 2004).

Since the atmospheric deposition of nutrients could result in the same biogeochemical features as presently ascribed to N_2 fixation, dissecting the contribution each makes to the tracers we now ascribe to N_2 fixation should be a priority (Hansell *et al.*, 2007). Interestingly, where DIN_{xs} grow-out in the thermocline is greatest (15–30°N in the eastern Atlantic, according to the analysis of Hansell *et al.*, 2004), deposition of N is relatively low. But *Trichodesmium* biomass is low there as well (Tyrrell *et al.*, 2003). The highest atmospheric inputs in the mid- to low-latitude North Atlantic are in the tropics (Galloway *et al.*, 2004), where N_2 fixation may be high (Capone *et al.*, 1997; Tyrrell *et al.*, 2003), so separating these inputs may prove difficult.

4. DENITRIFICATION

Denitrification in the Atlantic Ocean is not as well studied as N_2 fixation, but it is the most important N sink in the basin. The shelves are important sites for this sink (Christensen *et al.*, 1987; Laursen and Seitzinger, 2002), though denitrification appears to make a very small contribution to total organic matter oxidation on the continental margin of the NW Atlantic (Martin and Sayles, 2004). Denitrification on the shelves exceeds the combined N inputs by rivers and atmospheric deposition, thus requiring that the deficit be balanced by the influx of nitrate from the open ocean (Seitzinger and Giblin, 1996; see N budget for the North Atlantic below).

Seitzinger and Giblin (1996) used data from a wide range of continental shelf regions to establish the relationship between denitrification and sedimentary oxygen consumption rates, and also to establish the relationship between primary production and sediment oxygen consumption. Combining these relationships resulted in a predictive correlation between shelf denitrification and local primary production rates. They suggested that 13% of N consumed by phytoplankton in shelf waters is eventually lost to denitrification in the sediments. The model predicted the average denitrification rate for the North Atlantic shelves to be 0.69 mmol N m^{-2} day^{-1}, for a total shelf N loss in the basin of 143×10^{10} mol N year^{-1} ($\approx 0.7 \times 10^{12}$ mol N year^{-1}, comparable to the estimates of basin-wide nitrogen fixation). Denitrification rates over the shelves were highest at mid-latitudes and lowest at high latitudes. Twice the N removal occurs in the western shelves than in the eastern shelves.

Studies evaluating denitrification in the South Atlantic are few. Dittmar and Birkicht (2001) evaluated nutrient distributions from on- to off-shore of Namibia, and found decreasing N/P and N/Si ratios with distance away from the upwelling zones. They suggested that these changes indicate considerable N losses in the region, with the low oxygen waters present likely favoring denitrification. As for the deep basin, Bender *et al.* (1977) evaluated pore water distributions of nutrients in pelagic sediments in the eastern equatorial Atlantic, suggesting that these sediments should not be dismissed as important sites of denitrification.

5. THE ATLANTIC AS A SOURCE OF NITROGEN TO THE ATMOSPHERE

Some important atmospheric nitrogen compounds have biogenic sources in the ocean; most significantly N_2O and, to a lesser extent, alkyl nitrates. N_2O has 200–300 times the greenhouse warming effect of CO_2, and it is an intermediate in the destruction of stratospheric ozone and a source of tropospheric ozone (Delwiche, 1981). It is supersaturated in the surface waters of the equatorial Atlantic (Oudot *et al.*, 1990, 2002) and the Caribbean (Morell *et al.*, 2001), and thus a source to the atmosphere. In these waters it is most likely a by product of nitrification (Oudot *et al.*, 1990). Nevison *et al.* (2003) estimated a global release of 0.3×10^{12} mol N year^{-1} as N_2O, a small fraction of the Gruber and Sarmiento (1997) estimate

of global pelagic N_2 fixation (8×10^{12} mol N year^{-1}), an important source to balance this atmospheric sink. Water column N_2O production and loss to the atmosphere is thus more important in terms of atmospheric nitrogen cycling than as a sink for oceanic nitrogen.

Methyl and ethyl nitrates play a role in regulating tropospheric ozone levels in remote marine regions. These alkyl nitrates are reservoir species for NO_x ($= NO_2 +$ NO), while photolysis of NO_2 is the mechanism for producing ozone in the troposphere. Sources of alkyl nitrates, including the ocean, are under investigation. Like N_2O, nitrate-enriched equatorial waters are an important site of formation and export (Chuck *et al.*, 2002). Little work has been done on understanding the mechanisms of production or controls in the ocean, though biological processes may be invoked with methyl nitrate being found to depths of 800 m, with surface enhancement (Moore and Blough, 2002).

6. Is the Atlantic a Source of Nitrogen to the Global Ocean?

Is the Atlantic a net source or sink of nitrogen to the global ocean? This question is difficult to answer, especially in light of the scarcity of relevant studies in the South Atlantic and given the difficulties of accurately determining net meridional transports in the basins. Ganachaud and Wunsch (2002), using hydrographic data, estimated a net southward flux in the South Atlantic (30°S) of about 1×10^{12} mol N year^{-1} to indicate a net loss from the basin, though they did not account for transport of dissolved organic nitrogen. The North Atlantic has been more intensively studied, documented and evaluated (Howarth, 1996). Contributions to the North Atlantic nitrogen budget have been estimated for atmospheric deposition (Prospero *et al.*, 1996), riverine fluxes (Howarth *et al.*, 1996), N in the land–sea margin (Nixon *et al.*, 1996), transformations within the open ocean (Michaels *et al.*, 1996), shelf sediment denitrification (Seitzinger and Giblin, 1996), N_2 fixation (Lipschultz and Owens, 1996; Michaels *et al.*, 1996), and air/sea exchange of ammonia (Quinn *et al.*, 1996). These flux estimates were summarized by Galloway *et al.* (1996), and it is a modification of that budget that we discuss here. Significant uncertainties in the sources, sinks and fluxes prevent an accurate nitrogen budget assessment for the North Atlantic. The exercise is informative nonetheless, providing an indication of the relative contributions from each of these processes and an indication of where additional study is required.

Figure 13.13 illustrates the major fluxes across the boundaries of the basin, as well as sinks (denitrification) and new sources (N_2 fixation) of reactive N. The important physical reservoirs included in the budget are estuaries, continental shelves, and the open ocean, each undergoing additions and removals of N by various mechanisms. The estuaries receive N from rivers (540×10^9 mol year^{-1}), about half of which (250×10^9 mol year^{-1}) is lost from the system via denitrification (Seitzinger, 1988). The bulk of the remainder passes through the system to the shelves, with a small amount lost to the estuarine sediments. Major rivers do not have the conditions
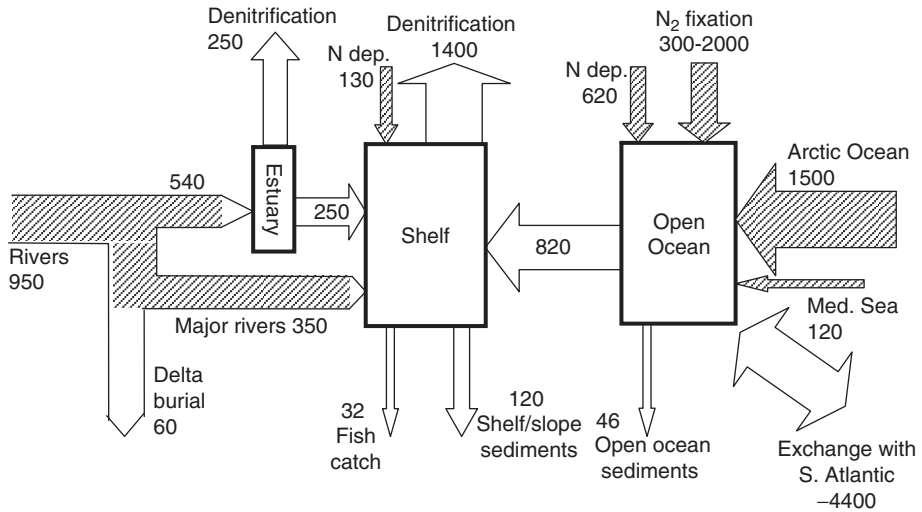
Figure 13.13 Nitrogen budget for the North Atlantic, modified from Galloway *et al.* (1996), demonstrating the major fluxes ($\times 10^9$ mol year^{-1}) across the boundaries of the basin as well as sinks (denitrification) and new sources (nitrogen fixation; nitrogen deposition (N dep.)) of reactive N. Fluxes into the major physical realms of the North Atlantic (open ocean, shelf waters, and estuaries) are highlighted by diagonal-line fills; loss terms and exchange fluxes between realms are indicated by open arrows.

(e.g., long residence time, quiescent environments) necessary to lose much N by denitrification in their estuaries or by delta burial, so most of this fluvial N is passed to the shelf systems.

The continental shelves receive N from the open ocean (820×10^9 mol year^{-1}), from estuaries (250×10^9 mol year^{-1}), from major rivers (350×10^9 mol year^{-1}) and from atmospheric deposition (130×10^9 mol year^{-1}). Some is lost to the sediments (120×10^9 mol year^{-1}) and fish catch (32×10^9 mol year^{-1}), but the majority is removed from the system via sedimentary denitrification (1400×10^9 mol year^{-1}). Nitrogen introduced to the shelves from the open ocean appears to contribute the most to shelf denitrification (Seitzinger and Giblin, 1996).

On balance, the shelves are not a net source of N to the open ocean. Instead, the North Atlantic has major exchanges with the Arctic Ocean and with the South Atlantic. Ganachaud and Wunsch (2002) estimate southerly nitrate fluxes of 2200 ($+/-$ 3800) and 6600 ($+/-$ 4700) $\times 10^9$ mol N year^{-1} at 7.5°N and 4.5°S, respectively. We take their mid point value of 4400 ($+/-$ 4000) $\times 10^9$ mol N year^{-1} as the net transport of nitrate from the North Atlantic to the South Atlantic. A significant uncertainty lies in the net meridional transport of DON in the basin-wide N transport budgets in the Atlantic. Rintoul and Wunsch (1991) speculated that the imbalance they quantified in the poleward nitrate flux across subtropical sections may be compensated by unobserved fluxes of organic nitrogen. DON measurements are still too sparse and too imprecise to test this hypothesis.

The nitrate flux from the Arctic to the Atlantic was estimated by Galloway *et al.* (1996) as 1500×10^9 mol year^{-1}, with most of the nitrate due to Pacific water

passing through Bering Strait, and loss to denitrification in Arctic shelf sediments. This value is also highly uncertain. Ganachaud and Wunsch (2002) estimated a net northward nitrate flux of 300 $(+/- 1050) \times 10^9$ mol year^{-1} at 47°N while Martel and Wunsch (1993) reported a net southward flux of 1800×10^9 mol year^{-1} at 60°N. Hence the direction and magnitude of the flux between the Arctic and the Atlantic remain unknown, so we retain the value from Galloway et al. (1996) in the present budget (Fig. 13.13).

The input from N$_2$ fixation in the North Atlantic was taken to be 5000×10^9 mol year^{-1} by Galloway et al. (1996). More recent work now suggests a lesser value, so that input term in Fig. 13.13 has been reduced to the larger range resulting from Gruber and Sarmiento (1997) and Hansell et al. (2004). Addition of nitrogen by atmospheric deposition, taken from Michaels et al. (1996) and Prospero et al. (1996), remains unchanged from Galloway et al. (1996).

The budget for the open North Atlantic, as presented in Fig. 13.13, is out of balance, with export of nitrogen from the open North Atlantic (to the sediments, to the South Atlantic, and to the shelves; totaling $\approx 5300 \times 10^9$ mol N year^{-1}) exceeding the import of $2500–4200 \times 10^9$ mol year^{-1}. However the two net fluxes are likely not distinguishable given the very large uncertainties associated with the flux estimates summarized here. Too few data exist at present to draw up a similar budget for the South Atlantic.

7. Key Unresolved Issues

The Atlantic basin, particularly the North Atlantic, is the most studied of the global oceans, yet there are several important processes and issues remaining to be addressed. Arguably, the most important issue is the paucity of data from the South Atlantic. There have been excellent expeditionary efforts there (i.e., the WOCE sections and the South Atlantic Ventilation Experiment), so the hydrographic system and the nutrient provinces can now be assessed in greater detail. However there has been little focus on processes of the nitrogen cycle in the basin.

Second, nitrogen fixation remains a poorly quantified process: We need to better understand the relationship of the excess nitrate signals in the thermocline to nitrogen sources and sinks, and the role of physical transport, in order to determine if those signals truly (and primarily) represent N$_2$ fixation in the overlying waters. There are large differences in the published estimates of N$_2$ fixation in the North Atlantic.

Third, temporal variability in nitrogen is poorly quantified. The time series stations are helpful but they are too few. Decadal re-occupation of most ocean sections (such as A16) is too infrequent to resolve the processes forcing change.

Finally, precise estimations of meridional transports provide powerful constraints of the basin scale nitrogen budgets. To provide better constraints, we require zonal sections including appropriately dense observations of the organic forms of dissolved nitrogen (measured at higher precision than now possible) to be used in quantification of the meridional transport of nitrogen.

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THE INDIAN OCEAN

S. Wajih A. Naqvi

Contents

1. INTRODUCTION AND BACKGROUND

The first major scientific expedition to the Indian Ocean (Arabian Sea)—the John Murray Expedition—was undertaken in 1933–1934 using the Egyptian vessel *Mabahiss*. It was on this expedition that the thickest oxygen (O_2) deficient zone (ODZ) in the open-ocean was first discovered (Sewell and Fage, 1948) and nitrogen cycling investigated in considerable detail (Gilson, 1937). Subsequently, during the International Indian Ocean Expedition (IIOE, 1962–1965) extensive measurements of nutrients including nitrate (NO_3^-), and in some cases nitrite (NO_2^-), were made; these data were utilized mainly to interpret variations in primary productivity (PP) (e.g., Ryther *et al.*, 1966) or to prepare large-scale maps and sections of NO_3^- (Wyrtki, 1971). It was not until the 1970s that systematic efforts were made again by Sen Gupta and coworkers (e.g., Sen Gupta *et al.*, 1976) to investigate nitrogen cycle processes in the region. A number of studies have since been conducted, but the

majority of them have focused on the northwestern Indian Ocean simply because the most significant processes are by and large confined to this region. Results of these investigations have been reviewed from time to time (Bange *et al.*, 2005; Naqvi, 2001; Naqvi *et al.*, 1992; Sen Gupta and Naqvi, 1984). Benefiting from the excellent data sets that have been made available more recently by the Joint Global Ocean Flux Study (JGOFS) and the World Ocean Circulation Experiment (WOCE) from various geographical areas, this chapter takes a broader view of the nitrogen cycle in the Indian Ocean, highlighting the newer findings and outstanding issues.

There are two unique aspects of geography of the Indian Ocean that profoundly influence its climate and circulation: (a) The Indian Ocean's northern expanse is curtailed by the Eurasian landmass around the Tropic of Cancer (making it the only ocean that is cut off from temperate and polar areas of the Northern Hemisphere), and (b) The Indian Ocean is connected to the Pacific through the Indonesian Archipelago (the only low latitude inter-ocean connection on the planet). The resultant surface circulation divides this ocean into three parts (Wyrtki, 1973): (a) the monsoon gyre, (b) the southern subtropical gyre, and (c) the Antarctic waters with the Circumpolar Current. Surface circulation patterns in the two southern parts do not show any major deviation from those observed in other oceans with the exception of a seasonally-variable poleward surface flow (the Leeuwin Current) and the associated equatorward undercurrent off western Australia. What really differentiates the Indian Ocean from the Atlantic and the Pacific is the unique circulation of its monsoon gyre.

A discontinuity in physicochemical characteristics around Lat. 10°S, called the Hydrochemical Front, marks the boundary between the monsoon gyre and the southern subtropical gyre. This discontinuity is maintained by the unidirectional, perennial flow of the South Equatorial Current (SEC) from the east to the west. The zonal flow of the SEC and related restriction of cross-equatorial exchange of thermocline waters to the western region play a key role in determining O_2 distribution and therefore biogeochemical cycling of nitrogen in the North Indian Ocean (Swallow, 1984; Warren, 1994). Embedded in the SEC is the Indonesian Throughflow (ITF) which is derived from the Pacific Ocean and is carried eastward all across the Indian Ocean and then to the south by the western boundary (the Mozambique and the Agulhas) currents to be transferred eventually to the Atlantic Ocean. This inter-ocean exchange forms a part of the global conveyor belt circulation (Gordon, 1985), and it affects regional climate and rainfall, particularly across Indonesia and Australia. The ITF is also believed to be responsible for setting up the anomalous circulation off western Australia referred to above (Schott and McCreary, 2001).

The differential heating and cooling of air over land and sea forces semi-annual reversals of winds and surface currents north of the Hydrochemical Front. Surface currents flow generally clockwise during the summer or southwest monsoon (SWM; June-September) and anticlockwise during the winter or northeast monsoon (NEM; December-March) in both the northwestern (the Arabian Sea including the Laccadive Sea) and the northeastern (the Bay of Bengal including the Andaman Sea) basins. However, the two basins are disparately affected by the monsoons.

The SWM winds are stronger in the Arabian Sea leading to the development of a very strong western boundary flow (the Somali Current) and vigorous upwelling off the coasts of Somalia, Yemen and Oman (Schott and McCreary, 2001). A notable feature of circulation in the region during the SWM is the persistence of several meso-scale eddies that account for the bulk of the kinetic energy (Flagg and Kim, 1998). While moderate upwelling also occurs along the west coast of India during this season (Banse, 1959, 1968), this process is much less intense in the Bay of Bengal (Shetye et al., 1991). On the other hand, the Bay of Bengal receives far greater freshwater inputs through both precipitation and river runoff as compared to the Arabian Sea. In fact, about half of the total runoff in the entire Indian Ocean occurs in the northeastern Indian Ocean (Dai and Trenberth, 2002). Consequently, the water balance (precipitation + runoff − evaporation) is highly negative in the northwestern Arabian Sea and highly positive in the northeastern Bay of Bengal. The latter results in a very strong thermohaline stratification that severely inhibits vertical mixing. This is why the Bay of Bengal does not experience convective mixing in spite of substantial sea surface cooling during winter in contrast with the Arabian Sea where winter convection occurs over a very large area extending as far south as Lat. 10°N off the African coast (Naqvi et al., 2006a).

There is yet another geography-related factor that differentiates the hydrographies of the northwestern and northeastern sectors of the Indian Ocean. The Arabian Sea is connected to two Mediterranean-type marginal seas - the Red Sea and the Persian Gulf. Warm, saline waters produced in these seas flow out and spread into the Arabian Sea with their core layers at ∼250 and 500 m (Wyrtki, 1971). The O_2 supply by these waters is quite modest, but they contribute substantially to water mass structure and facilitate the O_2-depleted North Indian Intermediate water to occupy a larger volume in the Arabian Sea as compared to the Bay of Bengal, which does not receive such outflows. As discussed in Sect. 3.2.1, this has important implications for the O_2 balance and nitrogen cycling.

2. General Distribution of Water Column Properties

2.1. Nitrate and oxygen

In order to investigate basin-scale distributions (north-south and east-west variabilities) of NO_3^-, by far the most abundant combined nitrogen species in seawater, and O_2 (a key variable controlling nitrogen biogeochemistry), vertical sections of these properties along WOCE Lines I09N and I08S (Fig. 14.1) and I03 (Fig. 14.2) are examined here (Schlitzer, 2000; additional sections are available at http://www.awi-bremerhaven.de/GEO/eWOCE/). These are supplemented with NO_3^- data from a few other regions (Figs. 14.3–14.5).

Within the main thermocline, relatively low-NO_3^- waters of the southern subtropical gyre are separated from those rich in NO_3^- in the monsoon gyre to the north and the sub-Antarctic zone to the south by the Hydrochemical Front and the Subtropical Convergence (STC), respectively (Fig. 14.1B). The maximum in NO_3^- generally occurs in the 1–2 km depth range north of the STC, and at
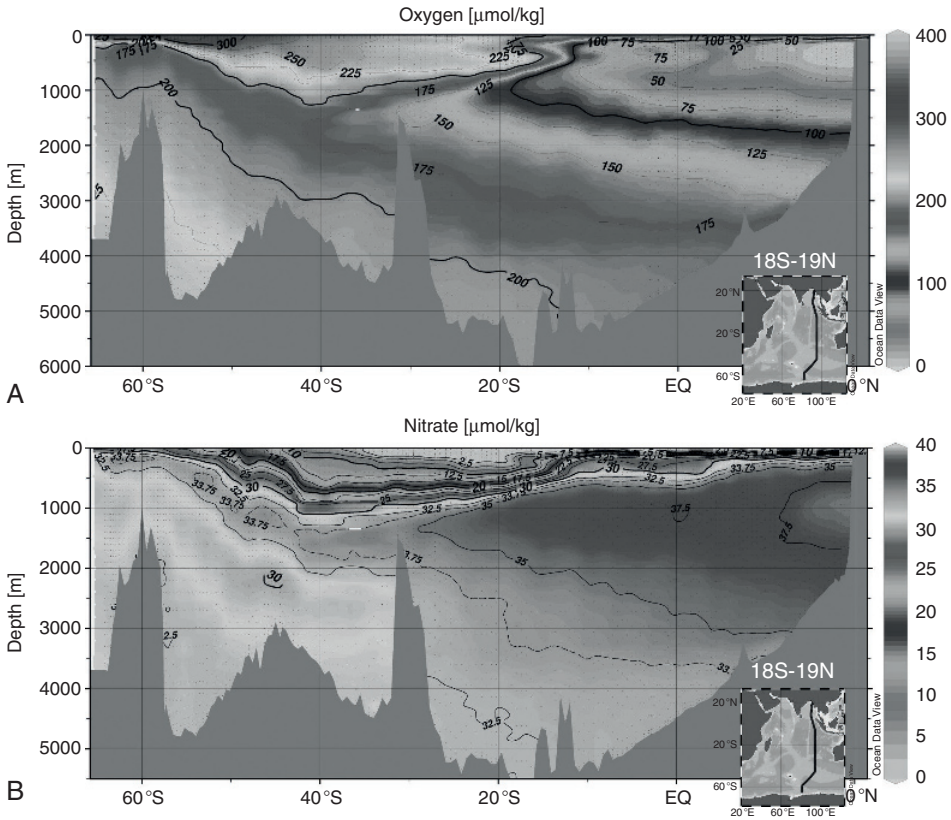
Figure 14.1 Vertical sections of (A) dissolved O_2 (upper panel) and (B) NO_3^- (lower panel), extending from Antarctica to Myanmar, constructed using data from WOCE Legs I08S and I09N. The combined transect is shown in the insets (http://www.awi-bremerhaven.de/GEO/eWOCE/).

progressively shallower depths to the south of this feature. This maximum, which intensifies northward, usually coincides with the maximum in the apparent oxygen utilization (AOU, not shown). South of the Hydrochemical Front, the NO_3^- maximum also coincides with the deep O_2 minimum, but to the north of this discontinuity, as O_2 concentrations fall rapidly and the two O_2 minima merge to form a broad, intense ODZ with its core at $\sim$200–300 m (Fig. 14.1A), the AOU maximum and O_2 minimum are vertically separated. Distributions of O_2 and NO_3^- in the upper few hundred meters in the northwestern Indian Ocean (Fig. 14.3; also see WOCE I07 sections; Schlitzer, 2000) show some important differences from the pattern described above in that in the former region, O_2 deficiency is more severe and widespread, and a pronounced minimum in NO_3^-, dealt with in detail in Sect. 3.2.1, occurs within the core of the ODZ (at $\sim$200–300 m).

At the sea surface, NO_3^- concentrations are below the limit of detection (<0.1 μM) in most areas including the equatorial Indian Ocean, which does not experience
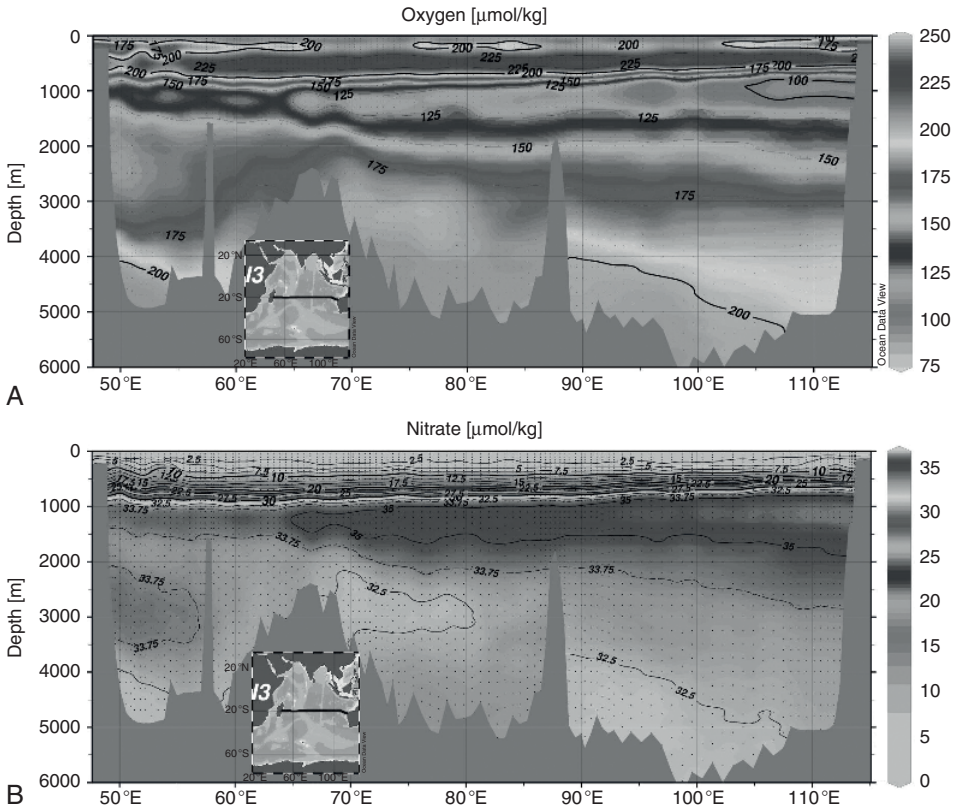
Figure 14.2 Vertical sections of (A) dissolved O_2 (upper panel) and (B) NO_3^- (lower panel), extending from Madagascar to Australia, along the WOCE Leg I03 (shown in the insets) (http://www.awi-bremerhaven.de/GEO/eWOCE/).

much upwelling. Surface waters are, as usual, NO_3^-–replete south of the STC (Fig. 14.1B), and also in the upwelling zones (e.g. Fig. 14.3F), most of which are found in the Arabian Sea. NO_3^- concentrations in the freshly-upwelled waters frequently exceed 15 μM off Somalia (Smith and Codispoti, 1980), Yemen (van Weering et al., 1997) and Oman (Fig. 14.3f; Morrison et al., 1998; Woodward et al., 1999). After upwelling these waters spread several hundreds of kilometers offshore as filaments and plumes, enriching the euphotic zone with essential nutrients over a very large area (Fig. 14.3F) thereby supporting widespread phytoplankton blooms as revealed by satellite-derived ocean color images (Banse and English, 2000; Wiggert et al., 2005, and references therein). Fertilization of comparable magnitude also occurs along the west coast of India, albeit within a much narrower coastal belt (Fig. 14.3F; Jayakumar et al., 2001; Naqvi et al., 1998a; 2000; 2006a). Moreover, as mentioned earlier, the Arabian Sea experiences convective mixing during winter that raises the surface dissolved inorganic nitrogen (DIN = NO_3^- + NO_2^- + ammonium (NH_4^+)) level to 6 μM (Fig. 14.3; Morrison et al., 1998; Naqvi et al.,
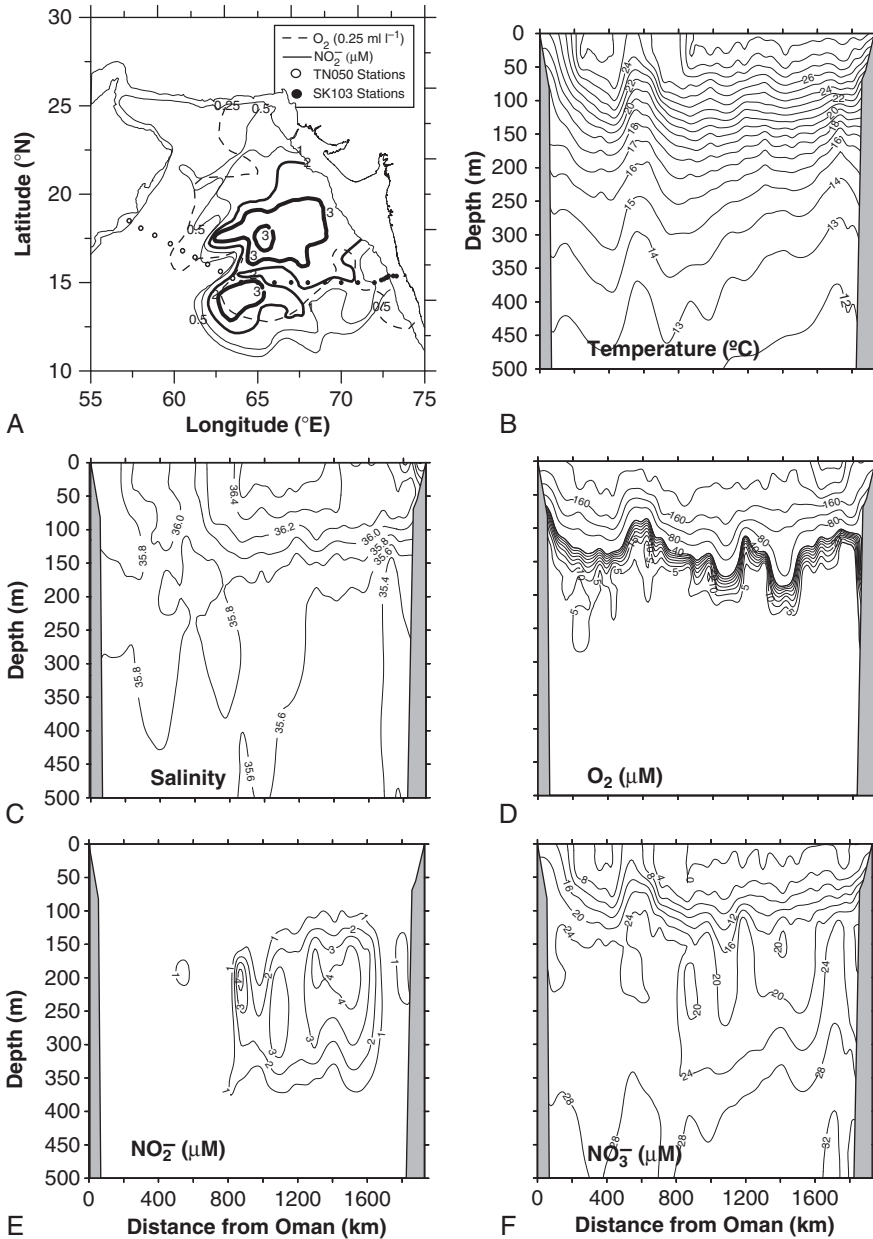
Figure 14.3 Vertical sections of (B) temperature, (C) salinity, (D) O_2, (E) NO_2^- and (F) NO_3^-, extending from Oman to India, constructed using data collected by the Research Vessels *Sagar Kanya* and *Thomas G. Thompson* during June–September, 1995. Locations of stations comprising the section are shown in (A) with reference to the secondary nitrite maximum zone (after Naqvi, 1991) and the zone of minimum (Winkler) O_2 at 300 m as demarcated by the 0.25 ml L^{-1} contour (dotted curve) (Wyrtki, 1971).
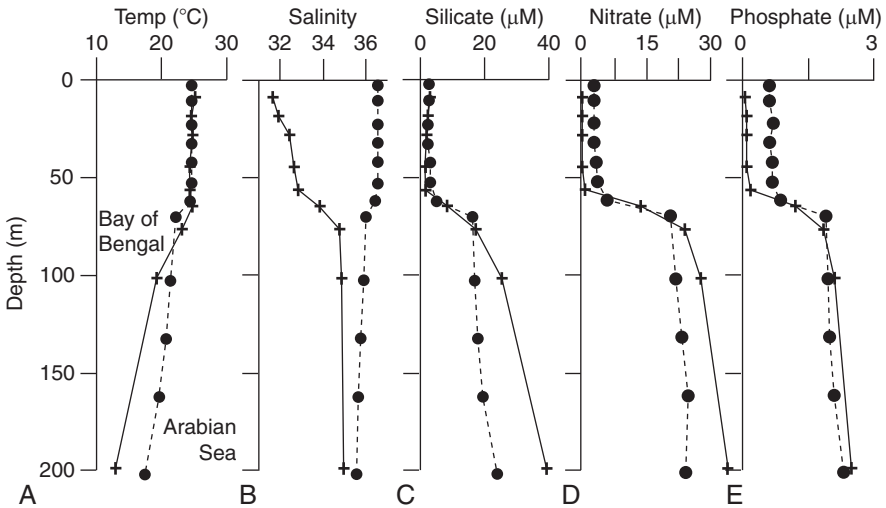
Figure 14.4 Comparison of vertical profiles of (A) temperature, (B) salinity, (C) silicate, (D) nitrate and (E) phosphate in the upper 200 m at one site each in the Arabian Sea (19.17°N, 67.17°E; US JGOFS Cruise TN043; sampling date 13/01/1995) and the Bay of Bengal (19.11°N, 92.65°E; WOCE Leg I09N; sampling date 24/02/1995).

2002). Together these processes maintain high rates of PP over much of the Arabian Sea during a large part of the year (Barber *et al.*, 2001).

Nutrient reflux to surface waters from the thermocline in the Bay of Bengal is limited by the previously mentioned weak upwelling and subdued vertical mixing. Also, the riverine flux of dissolved nutrients (except silicate) into the northeast Indian Ocean is quite modest (Rao *et al.*, 1994; Naqvi *et al.*, in press). This is reflected by an overall nutrient depletion in the surface layer (Fig. 14.4) and accordingly lower biological productivity of the Bay of Bengal relative to the Arabian Sea as revealed by field measurements (Gomes *et al.*, 2000; Qasim, 1977) as well as satellite–based observations (Gomes *et al.*, 2000; Naqvi *et al.*, 2006a). The latter show striking differences in surface chlorophyll concentrations between the two regions including the lack of winter-spring phytoplankton blooms in the Bay of Bengal, except in its southwestern sector (Vinayachandran and Mathew, 2003), unlike the dense blooms occurring in the northern Arabian Sea (Banse and McClain, 1986; Naqvi *et al.*, 2006a; Wiggert *et al.*, 2002; 2005).

Zonal sections through the southern subtropical gyre at about Lat. 20°S show a trend of eastward intensification of the O_2 minimum and its associated NO_3^- maximum (Fig. 14.2). However, the east-west gradients in both properties are much weaker than the north-south gradients across the Hydrochemical Front; for example, the lowest O_2 concentration along I03 is >90 μM as compared to <5 μM along I07 (not shown; but see Fig. 14.6). Even the modest intensification of the O_2 minimum in the eastern tropical South Indian Ocean is most likely related to relatively poor ventilation rather than an enhanced O_2 demand, a major factor for the acute O_2 depletion in the North Indian Ocean (Sen Gupta and Naqvi, 1984).
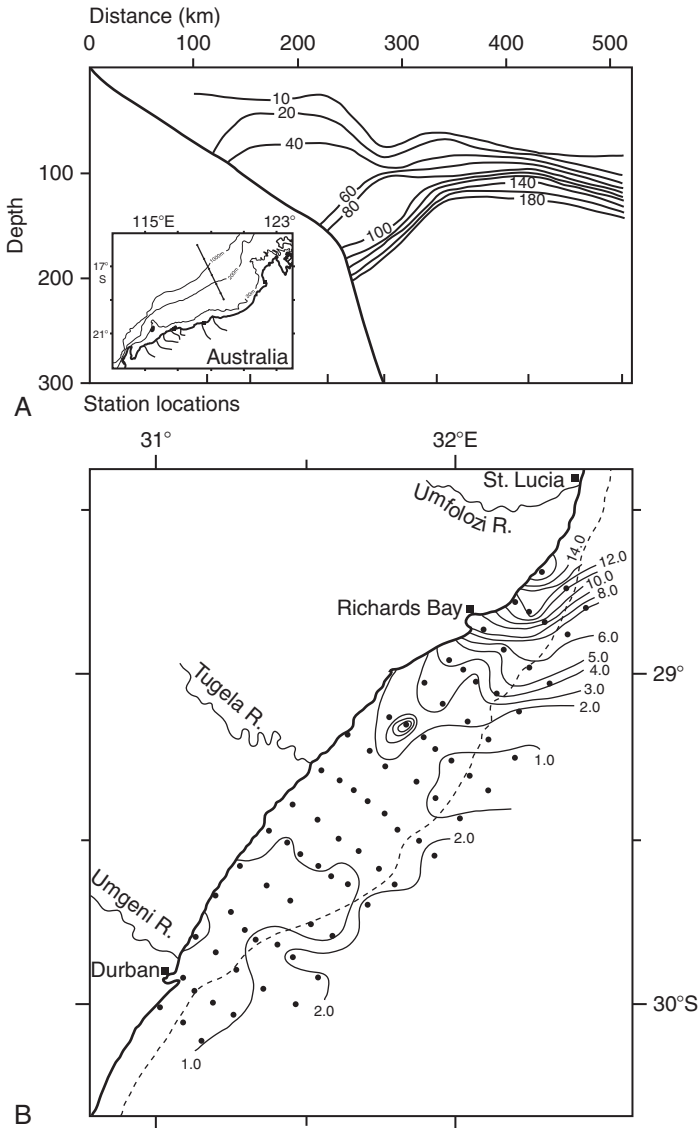
Figure 14.5 (A) Vertical section of NO_3^- (in $\mu g\,L^{-1}$; $10\,\mu g\,L^{-1} = 0.714\,\mu M$) off Northwest Australia based on observations in August 1976 (from Holloway *et al.*, 1985). (B) Distribution of NO_3^- (μM) at 10 m over the Natal Bight, east coast of South Africa during July 1989 (from Lutjeharms, 2006).

This is because unlike the North Indian Ocean the eastern tropical South Indian Ocean is not very productive due to its atypical, upwelling–unfavorable circulation. If there were no deep passages through the Indonesian islands linking the Pacific and the Indian Oceans, the west coast of Australia would probably host an eastern-boundary upwelling environment with associated severe O_2 deficiency and vigorous nitrogen transformations similar to what one finds in the ocean off Peru–Chile today.
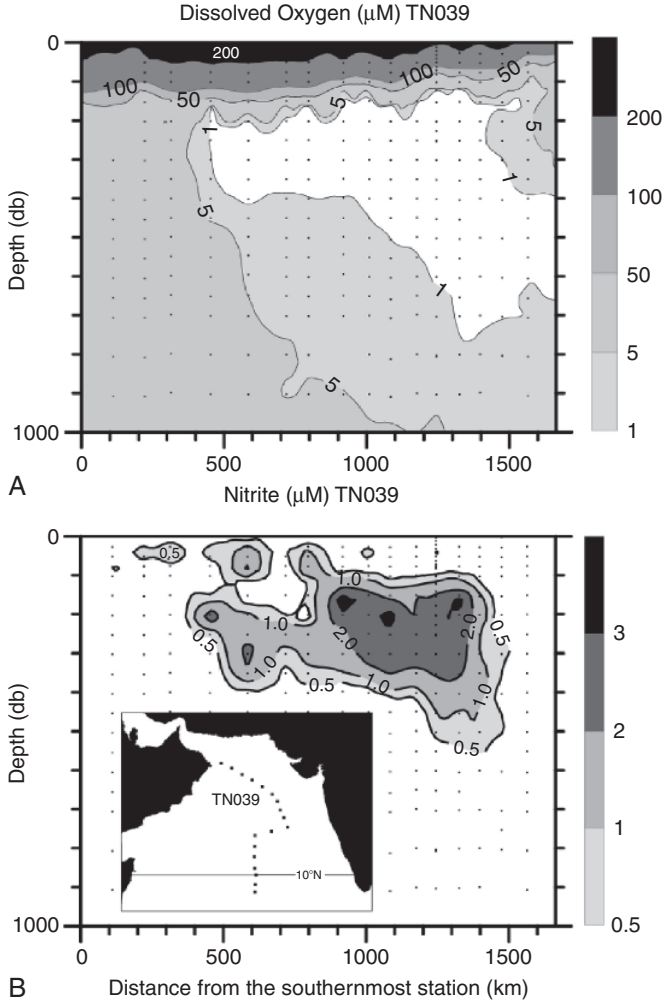
Figure 14.6 Vertical sections of (A) dissolved O_2 (µM) and (B) NO_2^- (µM) in the Arabian Sea based on observations in October, 1994. Station locations are shown in the inset (from Codispoti *et al.*, 2001).

Notwithstanding some localized upwelling during summer due to weakening of the Leeuwin Current, coastal waters off western Australia are among the most oligotrophic found along the oceans' eastern boundaries (Fig. 14.5a; also see Condie and Harris, 2006). Feng and Wild-Allen (in press) proposed a nitrogen budget for the continental shelf off the west coast of Australia according to which DIN inputs from terrestrial sources are quite small ($\sim 9 \times 10^9$ g year^{-1}) as is the annually-integrated rate of N_2-fixation ($\sim 7 \times 10^9$ g). DIN (largely NO_3^-) fluxes from the open ocean through upwelling (1.2×10^{11} g year^{-1}) and advection by the Leeuwin Current (1.4×10^{11} g year^{-1}) are an order of magnitude higher, but still

lower than the estimated supply from the seafloor (6.2×10^{11} g year^{-1}). Thus, the internal cycling seems to dominate nitrogen budget and supports an estimated mean primary production of $\sim$110 gC m^{-2} year^{-1} over this shelf. Episodic events (tropical cyclones) probably play an important role in supplying new nitrogen to the water column largely through entrainment of DIN from the sediments (Feng and Wild-Allen, in press; Holloway et al., 1985).

Farther north, moderate upwelling has been known to occur seasonally (during the southeast monsoon) along the southern coasts of Sumatra and Java (Susanto et al., 2001; Wyrtki, 1962), but still on a far smaller scale than in the Arabian Sea. Unfortunately, little is known about the biogeochemical processes along this coastal segment.

In the southeastern Indian Ocean, containing the only typical boundary environment in the Indian Ocean, topographically-induced upwelling occurs at several places over the continental shelf landward of the Agulhas Current which flows along the shelf break. In two such cells of intense, persistent upwelling found north of the Richards Bay in the Natal Bight (Fig. 14.5B) and off Port Alfred near the northeastern corner of the Agulhas Bank, surface NO$_3^-$ concentrations reach up to 15–18 μM (Baird, in press; Lutjeharms, 2006). In comparison, surface waters are NO$_3^-$ depleted outside these upwelling zones with the exception of the Sofala Bank off Mozambique that receives substantial runoff from the Zambezi River (Lutjeharms, 2006).

2.2. Nitrite

NO$_2^-$, an intermediate of both nitrification and denitrification (see Devol, this volume; Ward, this volume), is found in measurable concentrations either just below the surface mixed layer/euphotic zone or within the ODZ. These features are called the primary nitrite maximum (PNM) and the secondary nitrite maximum (SNM), respectively. While the SNM in the Indian Ocean is restricted only to the Arabian Sea, as will be discussed in Section 3.2.1, the PNM occurs everywhere. The latter is believed to arise from assimilatory reduction of NO$_3^-$ by phytoplankton (Vaccaro and Ryther, 1960; Wada and Hattori, 1971) and/or due to differential photo-inhibition of NH$_4^+$ and NO$_2^-$ oxidation by nitrifying bacteria (Olson, 1981). NO$_2^-$ concentrations at this maximum rarely exceed 0.5 μM, but higher concentrations (up to $\sim$2.5 μM) sometimes extend over the entire euphotic zone in the Arabian Sea following NO$_3^-$ enrichment through upwelling during the SWM and vertical mixing during the NEM, most likely due to incomplete assimilation by phytoplankton (Morrison et al., 1998; Naqvi et al., 2002). Significant amounts (>0.2 μM) of NO$_2^-$ are also found in surface waters of the Southern Ocean where PP is limited by low concentrations of iron (Fe). The assimilatory reduction of NO$_3^-$ by phytoplankton involves the enzymes nitrate reductase (NR) and nitrite reductase (NiR) (see Berges and Mulholland, this volume; Mulholland and Lomas, this volume). Experimental work has demonstrated that due to its much higher requirement of Fe, the activity of NiR is inhibited to a larger extent than that of NR under Fe-limited conditions (Milligan and Harrison, 2000), and this might account for the hitherto unexplained buildup of NO$_2^-$ in the Antarctic surface layer as indeed in all surface waters that contain NO$_3^-$ at micromolar levels.

2.3. Ammonium and organic nitrogen

NH_4^+, like NO_2^-, is also usually undetectable in the water column except within or just below the surface mixed layer. Observations by Woodward *et al.* (1999) in the Arabian Sea during late SWM revealed peak concentrations exceeding 1 μM, a few meters shallower than the PNM. On a subsequent cruise during the following autumn intermonsoon season, when the measurements were made using a more sensitive fluorescence system, maximal concentrations were lower by a factor of three, but the surface waters were found to contain 100–200 nM NH_4^+. The latter observations are perhaps typical of the North Indian Ocean, and even lower levels are expected for the oligotrophic southern gyre. In contrast, coastal waters are often characterized by substantially higher NH_4^+ concentrations. Over the Indian shelf, for example, near-bottom concentrations frequently exceed 1 μM, indicating NH_4^+ flux from the sediments (Naik, 2003), and even higher values (up to ∼20 M) are found in the water column affected by sulphate reduction (see Section 3.2.1).

Apart from an extensive data set on total organic nitrogen (TON) – most of which is expected to be present as the dissolved organic nitrogen (DON) – generated by D. Hansell as a part of US JGOFS in the Arabian Sea (http://usjgofs.whoi.edu/jg/dir/jgofs/), there has not been any recent study focusing on organic nitrogen in the Indian Ocean, the earlier works (Fraga, 1966; Sen Gupta *et al.*, 1977) being largely of historical significance. Hansell's measurements (e.g., Fig. 14.7) reveal TON concentrations exceeding 5 μM in the surface layer, decreasing rapidly across the thermocline to rather constant values between 1 and 2 μM in deep waters. As expected, coastal waters have relatively higher TON content, and with the exception of waters affected by upwelling and convective mixing, organic nitrogen dominates the combined nitrogen pool in both coastal and offshore areas. It is not clear what fraction of this TON pool is bioavailable, though (see Aluwihare and Meador, this volume). The much higher surface levels relative to the nearly uniform deep-water concentrations of TON (expected to be largely refractory) imply that that a substantial portion of the TON in surface waters is labile. However, the limited information available from the Arabian Sea on the distribution of urea, a major labile DON compound (see Mulholland and Lomas, this volume), indicates that its concentrations are generally below the limit of detection in the open ocean (Woodward *et al.*, 1999). Variability of suspended particulate organic nitrogen (PON) will be dealt with together with its isotopic composition in Section 4.2.

3. Nitrogen Cycle Processes

3.1. Remineralization ratios

Ratios between O_2 consumption and inorganic carbon and nutrients regeneration, called the remineralization ratios, provide useful insights into biogeochemical cycles in the oceans (e.g., export of material to the deep sea and depth-dependent preferential regeneration, if any, of one constituent over the others). For the Indian Ocean, these ratios have been derived from observed variations in concentrations of O_2, nutrients and inorganic carbon. Utilizing linear relationships between NO_3^- and
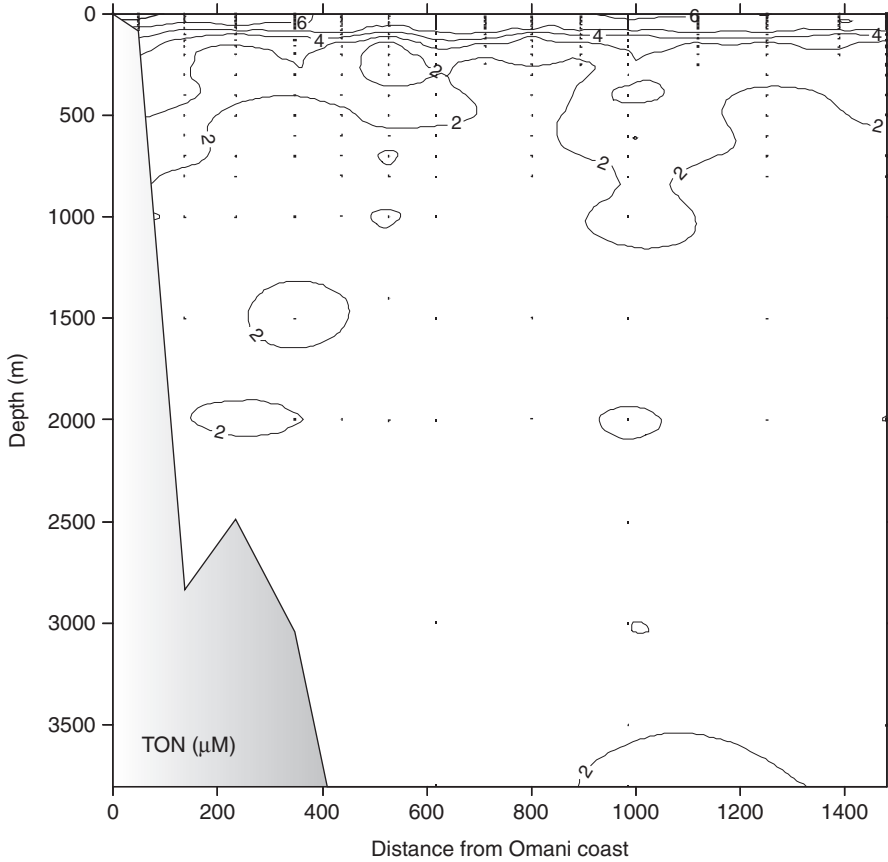
Figure 14.7 Vertical section of total organic nitrogen (TON) constructed using hitherto unpublished data collected by D. Hansell along US JGOFS southern leg (Cruise TN043 of *Thomas G. Thompson*; http://usjgofs.whoi.edu/jg/dir/jgofs/).

phosphate (PO_4^{3-}) with the AOU in subsurface waters above the AOU maximum, and assuming the organic matter to have a composition of $(CH_2O)_n(NH_3)_{15}H_3PO_4$, Sen Gupta and Naqvi (1984) computed average $-\Delta O_2 : \Delta C : \Delta N : \Delta P$ to be 126:96:15:1 in the North Indian Ocean, as compared to widely used values of 138:106:16:1 proposed by Redfield *et al.* (1963). The N:P ratio was based on measured nitrogen and phosphorus contents of several cultured and natural plankton from the Arabian Sea (Sen Gupta *et al.*, 1976), and the value of *n* was obtained by averaging published slope values for the AOU-PO_4^{3-} regression.

A binary mixing analysis along two isopycnal surfaces ($\sigma_\theta = 27$ and 27.2) in the Atlantic and Indian Oceans by Takahashi *et al.* (1985) yielded relatively constant mineralization ratios implying nearly uniform degradation of organic matter, with the Indian Ocean GEOSECS (Geochemical Ocean Section Study) data giving an average $-\Delta O_2 : \Delta C : \Delta N : \Delta P$ estimate of 172:119:14.9:1. The slightly lower $\Delta N : \Delta P$ for the Indian Ocean as compared to the Redfield value, as well as the corresponding

average for the Atlantic Ocean (17), was ascribed to the loss of NO_3^- through denitrification in the Arabian Sea. Several researchers have since challenged the notion of constancy of remineralization ratios in the oceans. But their findings have not been very coherent with some of the disagreement apparently stemming from methodological differences. For example, reprocessing the GEOSECS data, Minster and Boulahdid (1987) reported that while $\Delta O_2:\Delta N$ was indeed invariable with depth and geographical location, $-\Delta O_2:\Delta P$ decreased to 115 in the sub-pycnocline waters. In contrast, examining property distributions upon 20 neutral surfaces between 400 and 4000 m with a non linear inverse method, Anderson and Sarmiento (1994) found that while $-\Delta O_2:\Delta P$ was more or less constant, averaging around 170 throughout the oceans, $\Delta N:\Delta P$ varied with depth, exhibiting a minimum value of 12 at $\sim$2000 m that was attributed to sedimentary denitrification. The overall averages for the depth range 1000–3000 m and for the rest of the water column examined were $-\Delta O_2:\Delta C:\Delta N:\Delta P$ = 170:117:12:1 and 170:117:16:1, respectively.

The high-quality data generated during the JGOFS and WOCE led to a number of studies focusing on the Indian Ocean. Utilizing the US JGOFS data, Millero *et al.* (1998) derived vastly different $-\Delta O_2:\Delta C:\Delta N:\Delta P$ ratios for waters above (118:125:14.2:1) and below (145:42:6.3:1) the ODZ ($O_2 < 10$ μM) in the Arabian Sea. For the shallower layer, the decomposition of each ''mole'' of organic matter $[(CH_2O)_{125}(NH_3)_{14}H_3PO_4]$ was computed to require 153 moles of O_2, against the observed $-\Delta O_2:\Delta P$ of 118, indicating that degradation with O_2 accounts for 77% (118 $\times$ 100/153) of total respiration, with denitrification contributing the rest. However, the effects of denitrification and anthropogenic CO_2 penetration as well as of mixing of waters with different preformed concentrations were not considered in computation of the ratios themselves. These were taken into account by Hupe and Karstensen (2000) who used a linear inverse mixing model within the depth range 550–4500 m assuming mixing between three predefined water masses: Red Sea/ Persian Gulf Water, Indian Ocean Deep Water and modified Antarctic Intermediate Water. The ratios were again found to vary with depth: $-\Delta O_2:\Delta C:\Delta N:\Delta P$ = 139:90:14.4:1 within 550–1200 m, 152:107:14.9:1 within 1200–2000 m, and 158:127:15.3:1 below 2000 m. The decrease in $\Delta N:\Delta P$ at $\sim$2000 m reported by Anderson and Sarmiento (1994) could not be reproduced in this study. These results implied considerable vertical fractionation between nutrients and carbon during downward transport and decomposition of organic matter, consistent with the results of Shaffer *et al.* (1999). Specifically, it was proposed that phosphorus- and nitrogen-rich compounds such as phospholipids and nucleic acids as well as proteins could be preferentially degraded in the upper 100 m of the water column. Rixen and Ittekkot (2005) calculated $-\Delta O_2:\Delta P$ for depths exceeding 150 m in the Arabian Sea using the US JGOFS data and a mixing model involving three end members: Indian Ocean Deep Water, Indian Ocean Central Water and a mixture of Persian Gulf Water, Red Sea Water and Arabian Sea Surface Water. Their results are in broad agreement with those of Hupe and Karstensen (2000). However, in the upper water column that was not examined by Hupe and Karstensen (2000), Rixen and Ittekkot (2005) found a maximum in $-\Delta O_2:\Delta P$ located near the core of the ODZ, with values similar to those in deep waters. This is counter intuitional since ΔO_2 should reach a plateau value within the ODZ and any further oxidation of organic matter with NO_3^- would

only release PO_4^{3-}, thereby lowering $-\Delta O_2:\Delta P$. This anomaly was attributed to decomposition of organic matter synthesized by cyanobacteria including nitrogen fixers, which is believed to have higher than Redfieldian C:P and N:P ratios (the C: N ratio is, however, not too different from the Redfield value; see Mulholland, 2007). Despite a lack of indication of degradation of organic matter with a non-Redfieldian stoichiometry in the AOU-nutrients relationships in the upper water column (Millero et al., 1998; Naqvi et al., 1982; Sen Gupta et al., 1976), this hypothesis is, nevertheless, supported by N_2:Ar data, as we will see in Sect. 3.2.1.

The studies cited above, with the exception of Anderson and Sarmiento (1994), involved a prior knowledge of the composition of source water masses (end members). In order to eliminate uncertainties associated with subjective choice of end-member characteristics, Li and Peng (2002) introduced a three-end-member mixing model that was applied to selected WOCE and GEOSECS data. The results revealed that remineralization ratios in deep waters ($\theta \leq 12°C$) change more or less systematically along the circulation route of deep waters (from mean $-\Delta O_2:\Delta C:\Delta N:\Delta P$ of 137:73:16.1:1 in the North Atlantic to 162:124:13.3:1 in the North Pacific). Estimates for the deep equatorial Indian Ocean (130:94:10.3:1) are quite different from those for the South Indian Ocean (134:83:14.8:1). The abnormally low $\Delta N:\Delta P$ in the former case was hypothesized to result from partial conversion of organic nitrogen to N_2 and/or N_2O during nitrification by some hitherto unknown microorganisms or alternatively/additionally by heterotrophic denitrification and recently-discovered anaerobic ammonium oxidation (anammox; Strous et al., 1999; see Devol, this volume) in O_2-depleted microenvironments (Li and Peng, 2002).

The "partial nitrification hypothesis" has since been examined in a greater detail by Li et al. (2006) using additional WOCE data from the Indian Ocean. The newly calculated $\Delta N:\Delta P$ also decreases from ~15:1 in the Southern Ocean to 10:1 in the deep equatorial Indian Ocean. In contrast to this, the mean $-\Delta O_2:\Delta C:\Delta N:\Delta P$ ratios (159:110:15.6:1) for the shallower warm water masses ($\theta \geq 10°$) are comparable to the traditional Redfield values. Thus, while NO_3^- is linearly related to PO_4^{3-} in shallow, warm waters with a slope of approximately 16.5, the data points for the Antarctic Bottom Water, the Circumpolar Deep Water, and the Antarctic Intermediate Water (or the Equatorial Indian Phosphate Maximum Water) in the equatorial and subtropical South Indian Ocean fall below the straight line defining this linear trend. The $NO_3^- : PO_4^{3-}$ relationship resulting from in situ oxidation of organic matter and the mixing of the Antarctic Intermediate Water (or the Equatorial Indian Phosphate Maximum Water) with the Sub-Antarctic Oxygen Maximum Water (or the eastern Equatorial Indian Central Water) was derived by fitting the data to an empirical cubic equation, and for the data points lying between these curves and regression lines for the shallow, warm waters, NO_3^- deficits were proposed to be caused by partial nitrification. Any further departures from the values predicted by the cubic functions, conspicuous in data from the northwestern Indian Ocean, were attributed to denitrification (Li et al., 2006). NO_3^- deficits attributed to partial nitrification exhibit a maximum that intensifies from ~2–3 μM around the Hydrochemical Front to ~8 μM in the northern Arabian Sea, coinciding with O_2 minimum in the south and oxycline below denitrification zone in the north. In comparison, deficits resulting from denitrification are spatially less widespread. Peak values (~10 μM)

expectedly occur within the SNM zone of the Arabian Sea, decreasing southward and becoming insignificant in the equatorial region. Irrespective of the formative mechanism(s), if such large NO_3^- deficits, unrecognized by previous studies except for Anderson and Sarmiento (1994), do occur in deep waters, they have important implications for the regional and global nitrogen budgets.

3.2. Denitrification and nitrous oxide

3.2.1. Denitrification

Although almost the entire North Indian Ocean experiences severe O_2 depletion at mid-depth, vigorous denitrification, as indicated by the accumulation of the intermediate NO_2^-, is confined only to a relatively small, well-defined part of the Arabian Sea (Figs. 14.3, 14.6, and Fig. 14.8). Interestingly, while this zone includes comparatively oligotrophic central Arabian Sea, the most productive upwelling centres off the northeast African and Arabian coasts are outside its borders (Naqvi, 1991a). The boundaries of denitrification zone have been fairly stable with no discernable shift in the intensity of the process occurring over the past four decades for which data are available (Banse *et al.*, in preparation). Nevertheless, significant changes in chemical composition of suboxic waters do occur on the seasonal as well as inter-annual time
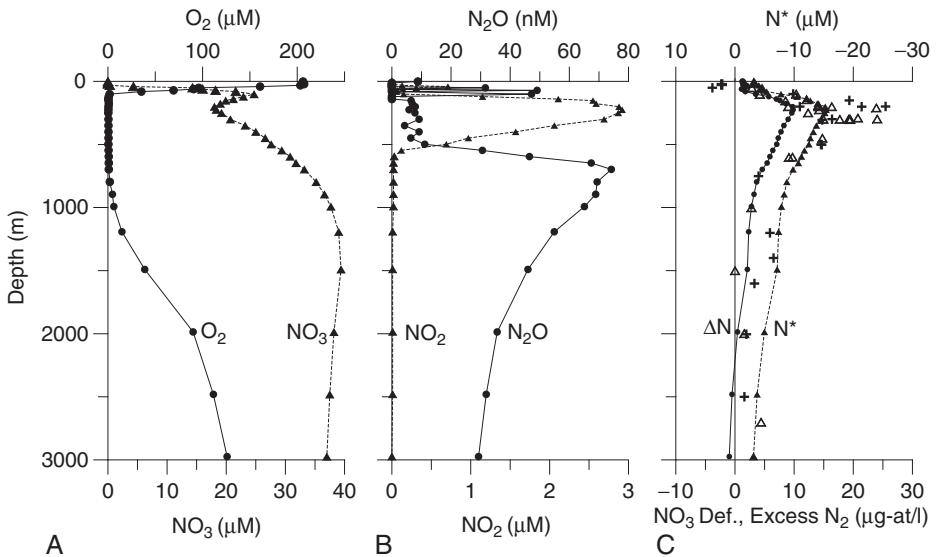
Figure 14.8 Vertical profiles of properties at 19°N, 67°E in the Arabian Sea (all data except for N_2 collected on TN039 cruise of US JGOFS on 1–2/10/1994). (A) O_2 (circles) and NO_3^- (triangles); (B) N_2O (circles) and NO_2^- (triangles); (C) NO_3^- deficit according to Codispoti *et al.* (2001) (dots connected by the solid line), N⋆ according to Gruber and Sarmiento (2002) (small filled triangles connected by the dashed line), and "excess N_2" calculated from the N_2/Ar ratio (larger unconnected symbols – crosses for data collected on two different cruises from this site and triangles for those from other stations also located within the denitrification zone).

scales (Bange *et al.*, 2001a; Morrison *et al.*, 1999; Naqvi, 1987; Naqvi *et al.*, 1990) indicating quick renewal of the ODZ (Howell *et al.*, 1997; Naqvi and Shailaja, 1993; Somasundar and Naqvi, 1988).

For vigorous denitrification to take place, ambient O_2 concentration should fall below a certain threshold value, which is ~0.7 μM or 0.015 mL L^{-1} in the Arabian Sea (Figs. 14.6 and 14.8; Morrison *et al.*, 1999; Naqvi *et al.*, 2003), consistent with observations in the eastern tropical Pacific Ocean (e.g. Cline and Richards, 1972). Outflows from the Red Sea and the Persian Gulf and advection of waters from the south are probably responsible for keeping the minimum O_2 levels marginally above this threshold in the western Arabian Sea, and a seasonal undercurrent (indicated by property distributions in Fig. 14.3), which brings somewhat more oxygenated waters from the south during the SWM, appears to suppress the process off the Indian continental margin up to about 17°N latitude (Naqvi *et al.*, 2006b,c). It has been proposed that ballast provided by copious lithogenic material received by the Bay of Bengal causes rapid sinking of particulate organic matter to deep sea (Ittekkot *et al.*, 1992). The O_2 supply needed to meet the resultant low remineralization rates (Naqvi *et al.*, 1996) could be just met by renewal, especially from the deeper O_2-rich water column given the absence of high-salinity outflows (because of which the transition from intermediate to deep waters occurs at shallower depths; Rao *et al.*, 1994). This is perhaps what prevents the Bay of Bengal from becoming denitrifying (Fig. 14.9). Support for this hypothesis comes from the sediment trap data (Table 14.1), which
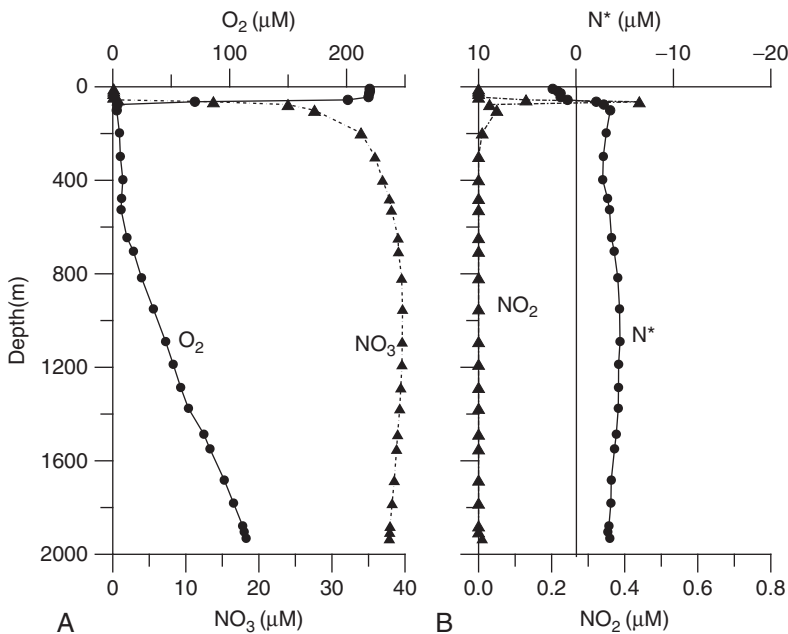
Figure 14.9 Vertical profiles of properties at 19.11°N, 92.65°E in the Bay of Bengal (WOCE Leg I09N; sampling date 24/02/1995). (A) O_2 (circles) and NO_3^- (triangles); (B) N⋆ according to Gruber and Sarmiento (2002) (circles) and NO_2^- (triangles).

Table 14.1 $^{15}N/^{14}N$ ratio in sinking organic matter (from Schäfer and Ittekkot, 1995)

Region	Depth (m)	$\delta^{15}N$ (‰ vs. air)		Nitrogen flux (mg m^{-2} day^{-1})	
		Range	Mean	Range	Mean
Bay of Bengal					
Northern	1700–2100	3.0–4.6	3.5	0.43–1.77	0.83
Central	2300	2.2–6.2	4.2	0.29–2.65	0.97
Southern	3000	3.8–5.9	4.6	0.35–1.48	0.66
Arabian Sea					
Western	3000	5.3–8.8	7.1	0.006–3.97	1.10
Central	2900–3100	6.3–8.7	7.4	0.004–1.48	0.63
Eastern	2800–2900	4.7–8.0	5.9	0.004–1.66	0.68
Lofoten Basin	3000	2.7–8.8	4.2	0.02–2.32	0.62
Sargasso Sea	3200	0.1–2.8	1.2	0.01–0.46	0.15

show that despite the much lower primary production, sinking PON fluxes to the deep Bay of Bengal are comparable to those in the Arabian Sea (Schäfer and Ittekkot, 1995).

The very large volume of nearly suboxic waters in the North Indian Ocean makes it extremely vulnerable to changes in either or both of the two forcing processes – surface organic production and subsurface ventilation – that may result from human activities both on local or regional (eutrophication) and global (greenhouse warming and changes in subsurface circulation) scales. In fact, such alterations are already beginning to be noticed in coastal waters. Along the west coast of India, for example, seasonal upwelling (from May to November) brings low-O_2 water over the shelf where its O_2 content is further depleted due to respiration. This water is generally prevented from surfacing by a low-salinity lens that is formed due to intense SWM rainfall in the coastal zone (Fig. 14.10), which contributes to sustenance of severe O_2 depletion at very shallow depths and maintenance of distinct zonations where various electron acceptors are sequentially utilized: hypoxia over the outer shelf, suboxia (denitrification) over the mid shelf, and anoxia (sulphate reduction) over the inner shelf north of about 12°N latitude (Fig. 14.10). This shallower ODZ, the largest of its kind in the world, has been known for almost half a century (Banse, 1959; Carruthers *et al.*, 1959), although until recently it had not been investigated in detail for nitrogen cycling. An examination of the available data going back to the 1970s suggests that the waters over the inner shelf used to experience seasonal denitrification but no sulphate reduction until the 1980s (supplementary information in Naqvi *et al.*, 2000; Naqvi *et al.*, 2006b). The latter process, recurring every year since systematic monitoring began in 1997, is thus most likely of recent origin, representing an ecosystem shift caused by increased nutrient loading through runoff and atmospheric deposition (Naqvi *et al.*, 2000, 2006b).

In the open-ocean suboxic zone denitrification does not reach completion, but a minimum in NO_3^- profile invariably occurs within the SNM (Figs. 14.3 and 14.8).
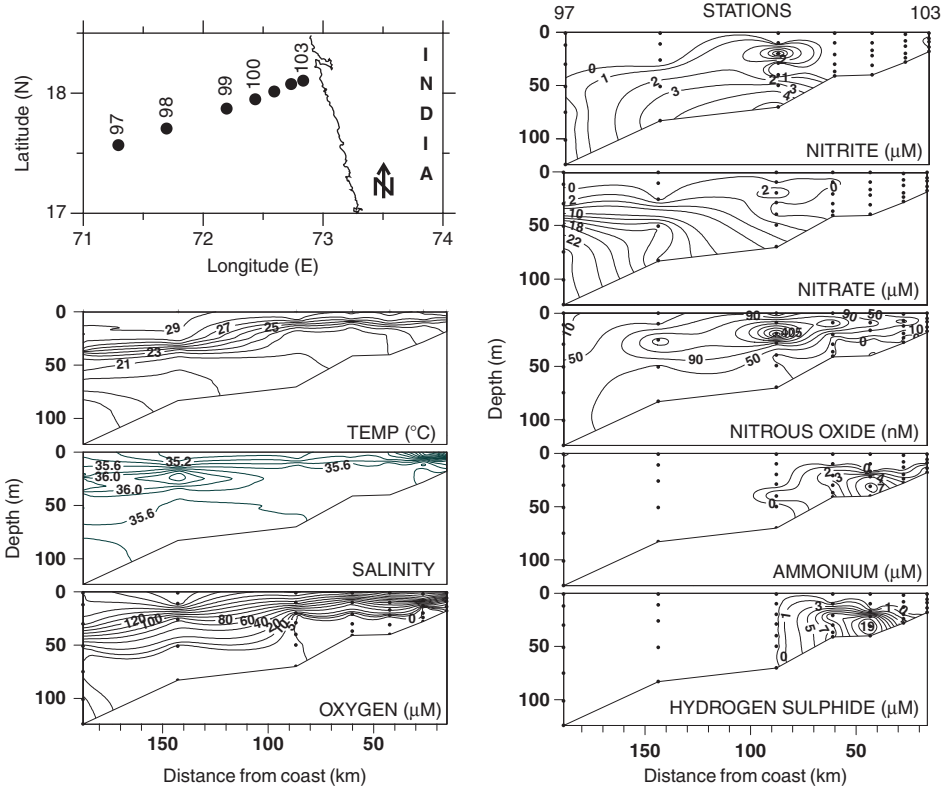
Figure 14.10 Vertical sections of temperature, salinity, O_2, inorganic nitrogen species and hydrogen sulphide off Mumbai based on observations during 6–7 October 1999 (from Naqvi *et al.*, 2000).

The magnitude of depression of $NO_3^- + NO_2^-$ concentration below the expected value (NO_3^- deficits) provides a quantitative measure of denitrification. The expected NO_3^- concentrations have been computed, except in once case (Deuser *et al.*, 1978), using remineralization ratios (Bange *et al.*, 2000; Codispoti *et al.*, 2001; Howell *et al.*, 1997; Mantoura *et al.*, 1993; Naqvi, 1987; Naqvi and Sen Gupta, 1985; Naqvi *et al.*, 1982; 1990; Sen Gupta *et al.*, 1976). The procedure that has been followed in the majority of recent studies utilizes the relationships between the geochemical tracer "NO," defined by Broecker (1974) as $O_2 + NO_3^- \times |\Delta O_2/\Delta N|$ and temperature for estimating expected NO_3^- (Naqvi and Sen Gupta, 1985). The regional denitrification rate estimate of $\sim$30 Tg N year^{-1} is based on NO_3^- deficits derived from this approach (Bange *et al.*, 2000; Naqvi, 1987). However, as demonstrated by Codispoti *et al.* (2001), this method yields systematically low deficits presumably because the data used to derive the "NO"- temperature relationship were affected by mixing with denitrification-affected waters having low NO_3^-. Defining the NO_3^- deficit as -N* (*cf.* Gruber and Sarmiento, 1997) and using a locally derived relationship between NO_3^- and PO_4^{3-} [NO_3^- deficit $= NO_3^- + NO_2^- - 0.86 \times 14.89$ ($PO_4^{3-} - 0.28$)], these authors found significant deficits to extend to $\sim$2500 m depth.

As exemplified in Fig. 14.8 for a station located close to the heart of the denitrification zone, this equation still yields negative deficits for deep waters, and generally lower values at all depths, as compared to the deficits ($-N\star$) obtained from the global equation of Gruber and Sarmiento (2002), *viz.* $N\star = NO_3^- - 16 \times (PO_4^{3-}) + 2.9$. Note that the factor (0.86) used by Codispoti *et al.* (2001) corrects for the PO_4^{3-} regenerated during denitrification. This factor will be closer to unity if some of the deficits are produced by incomplete nitrification as suggested by Li *et al.* (2006). The removal of this factor from the equation of Codispoti *et al.* (2001) or its inclusion in that of Gruber and Sarmiento (2002) would bring the estimated deficits slightly closer. Moreover, the $N\star$ procedure gives cumulative nitrate deficits (resulting from both local and far-field denitrification) as against only the locally produced deficits quantified by the method of Codispoti *et al.* (2001). The latter values are, therefore, expected to be somewhat lower. Anyhow, the two methods provide the lower and the upper limits of NO_3^- deficits in the Arabian Sea, as confirmed by the N_2:Ar data for deep waters (see below).

The equation of Gruber and Sarmiento (2002) yields relatively constant but significantly negative $N\star$ values below the surface layer in the Bay of Bengal, a region without a SNM (Fig. 14.9; Howell *et al.*, 1997; Rao *et al.*, 1994). While it is possible that these values reflect some loss of NO_3^- through denitrification in microenvironments or in sediments, it must be borne in mind that the actual ΔN:ΔP ratio in the North Indian Ocean is probably less than 16, as discussed above, and also that the absolute value of $N\star$ is dependent on the constant used for its computation. These factors may systematically offset $-N\star$ from the actual, locally-generated deficits.

With N_2 being its end product, denitrification should cause an increase in the ratio between concentrations of N_2 and argon (Ar) in water (Richards and Benson, 1960). Recently-acquired data on N_2:Ar from several sites in the Indian Ocean, presented in Fig. 14.11 as composite depth profiles, conform to this expectation. Data from four stations located outside the denitrification zone (crosses in Fig. 14.11) between Lats. 29°S and 8.5°N (along WOCE Leg I07) do not exhibit much geographical variability, and are also quite similar to data from other oxygenated parts of the ocean (Devol *et al.*, 2006a,b). The commonly observed increase in N_2:Ar with depth is believed to be an artifact of bubble injection and the gases' solubilities. In contrast, N_2:Ar profiles within the denitrifying zone are characterized by a pronounced peak within the SNM where the solubility-normalized ratio climbs up to 1.030–1.035 (circles in Fig. 14.11). One can calculate the amount of "excess" nitrogen by subtracting from each observed ratio within the denitrification zone the corresponding value at the same density outside this zone (derived from the best fit equation N_2:$Ar = -1.5976 + 0.3263\sigma_t - 0.013654\sigma_t^2 + 0.000190526\sigma_t^3$), and then multiplying the "excess" N_2:Ar by the equilibrium saturation of N_2 gas for the given temperature and salinity (Hamme and Emerson, 2004). Surprisingly, the "excess" N_2 (maximum value at 19°N, 67°E = 25.5 µg-at L^{-1}) far exceeds peak NO_3^- deficits (9.8–15.4 µM computed from the two approaches described above) at the core of the SNM (Fig. 14.8). Below the SNM, in contrast, "excess" N_2 concentrations generally fall between those expected from the two profiles for NO_3^- deficits with some indication of a less-pronounced secondary N_2 peak occurring between 1000 and 1500 m. This is about the same depth range where Li *et al.* (2006) found
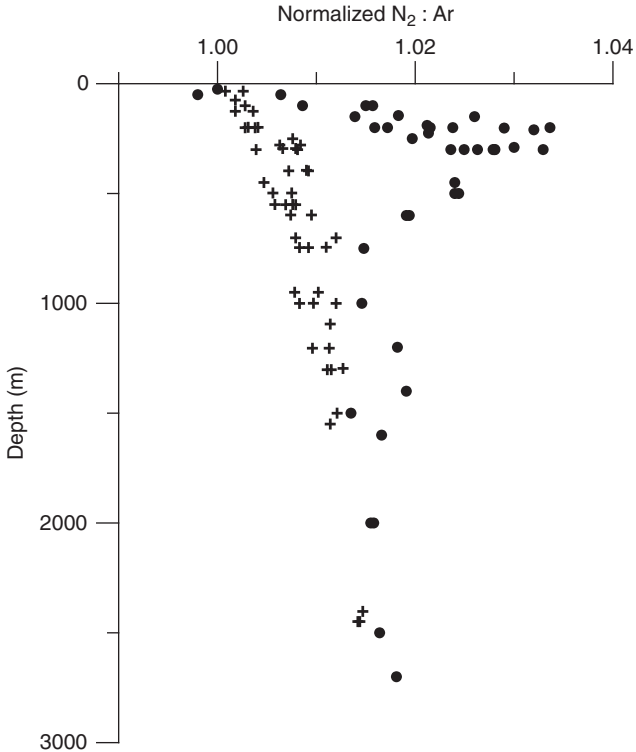
Figure 14.11 N_2/Ar ratio normalized by equilibrium saturation values within (circles) and outside (crosses) the denitrification zone. Note that stations outside the denitrification zone were located within a broad latitudinal band (29°S–8.5°N) (from Devol *et al.*, 2006b).

peak "NO_3^- deficit due to nitrification," but several hundred meters deeper than the secondary peak in N_2O (Fig. 14.8), indicating that some nitrogen may indeed be lost at this level mostly as N_2, even though the mode of loss cannot be established. Incidentally, the lower suboxic/oxic interface located just above this depth range has been found to be a zone of high biological activity, POC concentration as well as particle aggregation (Morrison *et al.*, 1999; Wishner *et al.*, 1998), and the secondary N_2 peak is possibly related to this phenomenon, most likely through denitrification within particle aggregates.

Potential processes that may be called upon to explain the large discrepancy between "excess" N_2 and NO_3^- deficits within the SNM are: (1) sedimentary denitrification, (2) anammox, (3) metal (Fe, Mn)-catalyzed denitrification, and (4) non-Redfieldian organic matter mineralization (Codispoti *et al.*, 2001; Devol *et al.*, 2006a,b). The first of these possibilities requires a decoupling between nitrogen and phosphorus cycles (e.g. possible greater burial of phosphorus in continental margin sediments). The less likely additional requirement is that the maximal inputs from the sediments should occur at the same depth as the water column peak of NO_2^-. In other words, the exact coincidence of the extrema in NO_3^-, NO_2^-, NO_3^-

deficit and excess N_2 strongly favors an in situ production mechanism of the N_2 anomaly. One such mechanism is anammox, a process that, in fact, has been reported to be responsible for the bulk of the N_2 production in the Black Sea (Kuypers et al., 2003) and over the Namibian shelf (Kuypers et al., 2005). However, in the open ocean anammox is expected to be constrained by the availability of NH_4^+, and its inclusion would only enhance N_2 production by ~17% (Devol et al., 2006a,b). The importance of metal-catalyzed reactions (e.g. oxidation of NH_4^+ and reduction of NO_3^- by the reduced and oxidized Mn species, respectively; Luther et al., 1997) is probably also not very significant in the open ocean (Codispoti et al., 2001). That leaves the last explanation (d) as the most plausible.

It has been suggested that the organic matter degraded during denitrification may be primarily proteins (Van Mooy et al., 2002), which may lead to a greater production of N_2 relative to mineralization of phosphorus in suboxic environments (Codispoti et al, 2001; Devol et al., 2006a,b). In addition to this, as will be discussed in Section 3.3, the Arabian Sea is a site of substantial N_2-fixation, and since organic matter produced by N_2-fixers is characterized by high N:P ratios (40–125; Gruber, 2004; Gruber and Sarmiento, 1997; Karl et al., 1992), its degradation would enhance the yield of N_2 during denitrification. As an example, Devol et al. (2006a,b) showed that this enhancement would be as much as about 50% if 20–30% of the primary production is carried out by the N_2-fixing organisms.

The higher NO_3^- deficits derived from their procedure (relative to the "NO"-temperature approach) and still higher "excess" N_2 led Codispoti et al. (2001) to propose an upward revision of the Arabian Sea denitrification estimate to ~60 Tg N year^{-1}. This is substantially above the rates obtained from the ETS (electron transport system) assay (24–33 Tg N year^{-1}; Naqvi and Shailaja, 1993) and ^{15}N incubations (41 Tg N year^{-1}; Devol et al., 2006b). Codispoti et al. (2001) argued that even though these approaches do not employ NO_3^- deficits, they still do not take into account the higher yield of N_2 production to NO_3^- consumption indicated by the N_2:Ar data. With the earlier, arguably conservative estimates, the Arabian Sea would account for about one-third of global water-column denitrification. This share may go up to about half if the estimate for the region is pushed up as proposed while those for the other oceanic sites remain unchanged. However, it is not clear to what extent, if any, the latter estimates also need to be revised as processes causing excess N_2 production are probably not confined to the Arabian Sea alone.

3.2.2. Intermediate nepheloid layer and quantitative significance of denitrification in remineralization

As in the suboxic zones of the eastern tropical Pacific Ocean (Garfield et al., 1983; Spinrad et al., 1989), a particle maximum [also called the intermediate nepheloid layer (INL)] occurs within the SNM in the Arabian Sea as well (Fig. 14.12; Gundersen et al., 1998; Morrison et al., 1999; Naqvi et al., 1993). The confinement of the INL only to the denitrifying zone, and its offshore intensification and shoaling preclude the advection of particle-rich layers from the continental margin; instead its association with the maxima in ETS activity and particulate protein point to an in situ biological origin (Naqvi et al., 1993). Further support for this view comes from a several-fold increase in total bacterial counts within the SNM from a minimum
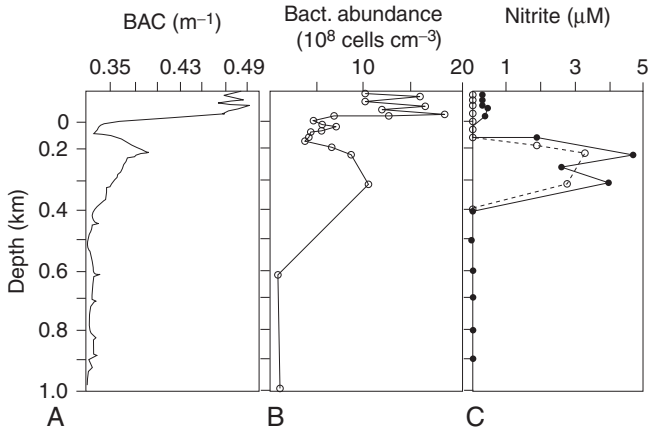
Figure 14.12 Typical profiles of (A) beam attenuation coefficient (BAC, m^{-1}, from Naqvi *et al.*, 1993) and (B) bacterial abundance (10^8 cells L^{-1}, from Ducklow, 1993) within the denitrifying zone. The sampling sites, located close to each other (around 15°N, 67°E) had similar NO$_2^-$ distributions (μM) (C, open circles corresponding to microbial data). Subsequent measurements have confirmed the co-occurrence of these features (Naqvi *et al.*, in preparation).

located just above this feature (Fig. 14.12; note that the bacterial biomass maximum is also ubiquitous to all denitrifying zones – Spinrad *et al.*, 1989), with a substantial fraction of these bacteria capable of reducing NO$_2^-$ (B.B. Ward, unpublished data). While a lack of grazing by microzooplankton could also result in a higher bacterial biomass within the SNM, this would require the critical O$_2$ concentration for the exclusion of micrograzers to be the same as the threshold value for NO$_3^-$ reduction, which is possible but highly unlikely. A more reasonable explanation is that bacterial growth is limited by low O$_2$ concentrations just above the SNM, but once the O$_2$ concentration falls below the threshold for NO$_3^-$ reduction to occur, the environment becomes conducive for anaerobes to proliferate. Besides denitrifiers, these anaerobes probably also include anammox bacteria that are dependent on the availability of NO$_2^-$ arising from NO$_3^-$ reduction.

In addition to the higher respiration rates suggested by the ETS maximum, another interesting issue concerns the mode of supply of labile carbon to the SNM. The traditional paradigm of the mesopelagic respiration being supported predominantly by the sinking flux of particulate organic carbon (POC) is probably not valid for this environment since such POC supply appears to be inadequate to sustain respiration rates derived from the ETS activity (Naqvi and Shailaja, 1993; Naqvi *et al.*, 1993) as well as bacterial production (Ducklow, 1993). This paradox is illustrated by the following calculation. If we convert the conservative denitrification rate estimate of 30 Tg N year^{-1} to the equivalent carbon demand using Richards (1965) stoichiometry, the organic carbon flux consumed by denitrifiers would on average be ∼21 g m^{-2} year^{-1} (29 Tg C year^{-1} divided by 1.4 × 10^{12} m^2, the area of the denitrification zone according to Naqvi, 1994). This is more than three-fourths of the annual mean POC export of 26.8 gCm^{-2} year^{-1} through the 100 m level quantified from the sediment trap data (Rixen *et al.*, 2005). The

[234]Th-based export production rate estimate is of a similar magnitude (28.5 gCm^{-2} year^{-1}; Lee *et al.*, 1998), making it unlikely that the sinking POC flux has been underestimated. On the other hand, it also seems highly improbable that the share of oxic metabolism in subsurface remineralization is so small. Note that the contribution of denitrification to total respiration would be over three times the above-mentioned estimate by Millero *et al.* (1998). Also, given the occurrence of the most intense denitrification in the least productive central region, carbon demand for denitrification and POC export in this region will be respectively much above and much below the average values considered here. This calls for additional supply of organic matter to the ODZ either quasi-horizontally from the continental margins (Somayajulu *et al.*, 1996), or vertically through diurnal migrations of organisms particularly myctophids (Banse, 1994; Morrison *et al.*, 1999) and episodic injections of transparent exopolymer particles (Kumar *et al.*, 1998). Alternatively, denitrifiers may efficiently utilize dissolved organic carbon introduced to subsurface waters through convective mixing (Naqvi *et al.*, 2002) or by the Persian Gulf outflow (Hansell and Peltzer, 1998). Of these possibilities, the one involving the myctophids is the most appealing because of the large biomass involved (>100 million tonnes according to Gjøsaeter (1984); however, more recent data call for a downward revision of this estimate – Sharon Smith, personal communication, 2006). Adapted to tolerate suboxic conditions, these fishes feed in the near-surface layer at night but migrate en-masse to the core of the ODZ during the day to escape from their predators (Morrison *et al.*, 1999).

3.2.3. Nitrous oxide

Nitrous oxide is produced as a by-product during nitrification and both produced and consumed during denitrification as an intermediate of the reduction sequence from NO_3^- to N_2 (see Bange, this volume). Denitrification is, of course, an anaerobic process, but the low ambient O_2 concentration also affects nitrification by elevating the yield of N_2O relative to NO_3^- (Goreau *et al.*, 1980). Therefore, O_2 deficient environments, such as those found in the North Indian Ocean, are conducive for vigorous production as well as consumption of N_2O, but serve as strong net sources of this important greenhouse gas (Codispoti and Christensen, 1985; Cohen and Gordon, 1978; Elkins *et al.*, 1978; Nevison *et al.*, 2003). In contrast, N_2O cycling in well-oxygenated water columns is less interesting with the surface waters being slightly supersaturated with respect to atmospheric N_2O, driving a small flux to the atmosphere (Butler *et al.*, 1989; Weiss, 1978), and the depth profile of N_2O being a mirror image of that of O_2 (Yoshinari, 1976). As a consequence, studies on N_2O in the Indian Ocean have been largely restricted to the Arabian Sea and the Bay of Bengal.

Surface waters of the Arabian Sea exhibit unusually high supersaturation, especially during periods and within zones of upwelling [up to 330% off Somalia (de Wilde and Helder, 1997), 230% off Oman (Bange *et al.*, 1996) and 8250% off India (Naqvi *et al.*, 2005, 2006b,c)]. This is because N_2O concentrations always rise steeply to several tens of nanomolar just below the mixed layer. Within the SNM, the concentration falls below <10 nM, resulting in a two-peaks structure (Figs. 14.8 and 14.13) common to all open ocean denitrification zones (Codispoti and Christensen, 1985; Cohen and Gordon, 1978). The close proximity of intense
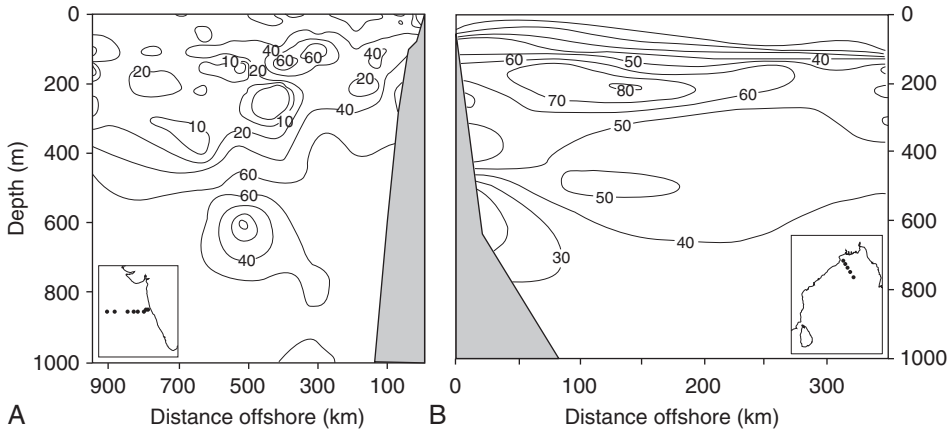
Figure 14.13 Vertical sections of N_2O (nM) in (A) the Arabian Sea and (B) the Bay of Bengal based on observations during 26 April–4 May, 1996, and 17–19 March, 1991, respectively. Station locations are shown in the insets (from Naqvi *et al.*, 2006a).

sources and sinks results in a very rapid turnover of N_2O (Naqvi and Noronha, 1991). Below the deeper maximum, N_2O concentration declines steadily with depth but remains well above saturation at least down to 2500 m (Fig. 14.8: Naqvi and Noronha, 1991; Naqvi *et al.*, 1998a,b). Outside the SNM zone in the southern and western Arabian Sea, vertical N_2O distribution is characterized by only one broad maximum within the O_2 minimum layer (Bange *et al.*, 2001a; Law and Owens, 1990; Naqvi and Noronha, 1991). A similar trend is also seen in the Bay of Bengal (Fig. 14.13), which, as pointed out previously, does not contain a SNM (Naqvi *et al.*, 1994). Results not conforming to this pattern have been published by Hashimoto *et al.* (1998) for the central Bay of Bengal; however, the lack of accumulation of N_2O within the ODZ reported in this study was not observed during subsequent surveys conducted by the author's group (unpublished data), and is probably an artifact of N_2O loss during sample storage. Also, Butler *et al.* (1989) reported substantially higher N_2O concentrations in the southern part of the Bay of Bengal than those observed by Law and Owens (1990) from the southern Arabian Sea for comparable O_2 levels. Nevison *et al.* (2003) ascribed this difference to dilution by low N_2O water from the SNM in the Arabian Sea. However, there is no indication of such a dilution in the data set of Naqvi and Noronha (1991).

The cause of N_2O depletion within the SNM is obvious (reduction to N_2 by denitrifiers), but there persists considerable disagreement in the literature as to what brings about N_2O accumulation in low O_2 waters. In the well-oxygenated waters, ΔN_2O (excess concentration over the saturation value) is positively correlated with AOU, albeit with separate relationships in the upper and deeper water columns in both the Arabian Sea (Law and Owens, 1990; Naqvi and Noronha, 1991) and the Bay of Bengal (Naqvi *et al.*, 1994). Although such linear relationships have been interpreted to reflect N_2O production through nitrification (e.g. Butler *et al.*, 1989; Elkins *et al.*, 1978; Oudot *et al.*, 1990), Nevison *et al.* (2003) pointed out that their

slopes do not provide accurate estimates of N_2O yield per mole of O_2 consumed because of the effect of mixing between various water masses. Still the observed increases in ΔN_2O:AOU with decreases in O_2 concentration in various areas of the ocean (including the Indian Ocean where this ratio increases northward; Fig. 14.14B) could be explained by the nitrification source (Nevison *et al.*, 2003). At O_2 levels slightly above the denitrification threshold, however, production through nitrification and/or nitrification–denitrification coupling (Naqvi, 1991b; Naqvi and Noronha, 1991; Yoshida *et al.*, 1989) was not ruled out. In conflict with the conclusion of Nevison *et al.* (2003), Yamagishi *et al.* (2005) obtained low yield of N_2O per mole of O_2 consumed for organic matter mineralization in the western
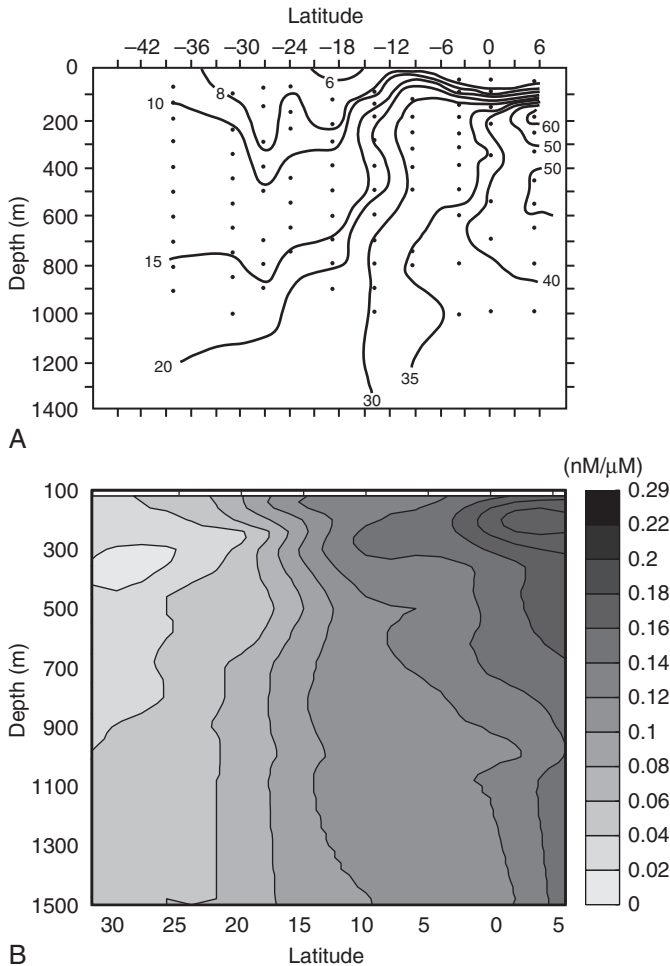
Figure 14.14 Vertical sections of (A) N_2O (nM) and (B) ΔN_2O/AOU ($\times 10^{-3}$) along the 90°E transect in the eastern Indian Ocean based on observations in May–July 1987 (from Butler *et al*, 1989 and Nevison *et al.*, 2003).

North Pacific and asserted that nitrification in aerobic deep waters is a minor source of oceanic N_2O. As will be shown in Section 4.1, isotopic measurements reveal different compositions of N_2O at the shallower and deeper maxima, suggesting the involvement of various formative mechanisms.

Data from the suboxic zone over the Indian continental shelf show distinct departures from the trends observed in the open-ocean suboxic zone. Not only are the peak concentrations of N_2O much higher (up to $\sim$0.8 μM), they are often associated with very high NO_2^- (up to 16 μM) and low NO_3^- (near exhaustion) levels (Fig. 14.10) indicating production through denitrification, in stark contrast to observations within the open-ocean SNM (Fig. 14.8). Incubations of water samples collected from the Indian shelf, that were O_2-poor but not denitrifying to begin with, revealed transient N_2O accumulation at micromolar levels once the system turned reducing. It was hypothesized that frequent aeration due to turbulence in shallow, rapidly-denitrifying systems may suppress the activity of N_2O-reductase leading to a large fraction of nitrogen undergoing bacterial reduction ending up as N_2O (Naqvi et al., 2000). Because such production occurs within a few meters of the sea surface, surface N_2O concentrations (5–436 nM, mean 37.3 nM) during the upwelling season over the western Indian shelf are among the highest recorded in the world oceans, as is the emission rate of N_2O to the atmosphere (0.05–0.38 Tg N_2O year^{-1}). Considered together with the efflux of 0.33–0.70 Tg N_2O year^{-1} estimated by Bange et al. (2001b) for the Arabian Sea as a whole, it is obvious that the Arabian Sea is a globally important source of atmospheric N_2O, accounting for 7–23% of the oceanic source (4.7 Tg N_2O year^{-1}; Prather et al., 2001).

Nitrous oxide saturation in surface waters of the Bay of Bengal is relatively smaller, averaging 125% (range: 89–214%). The modest supersaturation, presumably caused by the strong thermohaline stratification, supports a lower flux to the atmosphere (0.04–0.12 Tg N_2O year^{-1}) from the Bay of Bengal than that from the Arabian Sea (Naqvi et al., 1994).

Apart from some limited surface measurements of N_2O along the GEOSECS cruises (Weiss, 1978), there is only one other report on N_2O from the Indian Ocean outside the Arabian Sea and the Bay of Bengal – by Butler et al. (1989) who carried out extensive measurements of N_2O in water and air in the western Pacific and eastern Indian Oceans during an El Nino event (May–July 1987). The Indian Ocean data, largely coming from a meridional (90°E) transect, show sharp northward increases in the N_2O concentration at all depths (Fig. 14.14A) across the Hydrochemical Front. While the concentrations within the O_2 minimum north of the discontinuity are only slightly below those in the Bay of Bengal, the much lower concentrations in the South Indian Ocean are typical of the oxygenated water columns well removed from the ODZs. A similar trend is exhibited by the surface saturation as well: surface waters are close to saturation south of Lat. 15°S, but north of this latitude saturation increases rapidly to a maximum of $\sim$137% at Lat. 8°S. The computed atmospheric emission rate (2.6–5.2 μmol m^{-2} day^{-1}) for this region is comparable to that in the Arabian Sea (Naqvi and Noronha, 1991). It is not clear whether these results are typical of this region or related to the SST anomalies associated with El Nino. During a similar but more severe event (the Indian Ocean Dipole of 1997), extensive cooling of surface waters was recorded in the eastern

equatorial Indian Ocean due to large-scale upwelling off Sumatra (Murtugudde et al., 1999). It is very probable that such events drive large episodic emissions of N_2O from this region.

3.3. Nitrogen fixation

Distribution of $N^\star$ suggests that the Atlantic Ocean serves as a net source of fixed nitrogen to the oceans whereas the Indian and the Pacific Oceans act as net sinks (Gruber, 2004; Gruber and Sarmiento, 1997). This observation sometimes creates an erroneous impression that N_2-fixation and denitrification occur in geographically separated regions (e.g. Gruber, 2004). On the contrary, the two processes should be coupled because denitrification creates an excess of PO_4^{3-} in surface waters that should stimulate the growth of diazotrophs. Results of a recent study by Deutsch et al. (2005) supports this view. These authors estimated N_2-fixation by combining an Ocean Biogeochemistry/General Circulation Model with observed global nutrient distributions in the upper water column. Of the total estimated N_2-fixation rate ($\sim$130 Tg N year^{-1}), over half (80 Tg N year^{-1}) was computed for the Pacific with the Indian and the Atlantic Oceans contributing roughly equally ($\sim$25 Tg year^{-1}) to the remainder (Curtis Deutsch, personal communication, 2005). The potential for denitrification in sustaining N_2-fixation may be quantified by using the N:P ratio for diazotrophs and the $-\Delta N{:}\Delta P$ ratio for denitrification. The ranges of values for the two are 40–125 (Gruber, 2004) and 60–94 (Devol et al., 2006b). Assuming that PO_4^{3-} regenerated during denitrification is eventually utilized by diazotrophs, for each atomic unit of NO_3^- lost through denitrification, 0.4–2 atomic units of N_2 will be fixed. Considering only the NO_3^- deficits-based water column denitrification estimate for the Arabian (note that anammox does not lead to production of PO_4^{3-}), the PO_4^{3-} excess created by denitrification can potentially sustain fixation of $\sim$12–60 Tg of N_2 annually, which brackets well the above-mentioned estimate of Deutsch et al. The same computation could be not extended to sedimentary denitrification because the sedimentary denitrification rate for the entire Indian Ocean is not available; moreover, it is not known what fraction of PO_4^{3-} regenerated from the organic matter diffuses out to the water column relative to the amount that is utilized for phosphatization. In any case, it would be reasonable to conclude that on a per area basis N_2-fixation rate in the Indian Ocean is higher than in the Atlantic.

Within the Indian Ocean, the model of Deutsch et al. (2005) identifies the Arabian Sea as the most important N_2-fixation site. But here, in contrast to the North Atlantic, geochemical signatures of N_2-fixation (positive $N^\star$ and lighter nitrogen isotopic composition of NO_3^- in subsurface waters; Gruber, 2004) are completely obliterated by the opposing effects resulting from denitrification. Although a strong increase in $N^\star$ occurs upward of the subsurface minimum, the values remain negative even in surface water due to its high PO_4^{3-} content. In contrast, surface waters in the Bay of Bengal are distinguished by positive $N^\star$ values arising from the depletion of PO_4^{3-} (Figs. 14.4 and 14.9). Thus, N_2-fixation in the Bay is most probably PO_4^{3-} limited. Low N_2-fixation in the Bay of Bengal is also reflected by the persistence of significantly negative $N^\star$ in subsurface waters in spite of the fact that the Bay of Bengal water column is not denitrifying; that is, if sizable

N_2-fixation were to occur in this region it would have led to much larger increases in $N^\star$ relative to the surface than is observed (Fig. 14.9).

Biological data also testify to widespread N_2-fixation in the Arabian Sea (Devassy *et al.*, 1978). Blooms of *Trichodesmium* have long been known to occur in this region during the intermonsoon seasons – especially in the weeks preceding the onset of SWM – when the environmental conditions, *viz.* high sea surface temperature, calm weather and low DIN concentration in surface waters, favor its growth (Devassy *et al.*, 1978; Qasim, 1970). The *Trichodesmium* blooms have been detected by satellite-borne ocean colour sensors both in the open ocean (Capone *et al.*, 1998) and coastal zone (Desa *et al.*, 2005). The bloom investigated by Capone *et al.* during 22–27 May, 1995, covered an area of $\sim 2 \times 10^6$ km^2 in the central Arabian Sea. It extended into the denitrification zone but was most intense just south of this region. Field measurements carried out at the same time suggested a regional N_2-fixation rate of around 1 Tg N year^{-1} (Capone *et al.*, 1998). As will be shown in Section 4.1, the natural isotope abundance data yield a higher rate ($\sim$6 Tg N year^{-1}; Brandes *et al.*, 1998). Since autotrophs other than the easily noticeable, colonial *Trichodesmium*, such as heterocystous embosymbiont *Richelia* and the recently discovered unicellular diazo-cyanobacteria (Montoya *et al.*, 2004; Zehr *et al.*, 2001; see Carpenter and Capone, this volume), also contribute to N_2-fixation, and also because the isotope data provide a time-integrated signal, the latter estimate is probably more accurate.

In the Bay of Bengal, not only are the *Trichodesmium* blooms less frequently encountered, they are also mostly confined to coastal areas (Adhikary and Sahu, 1992; Madhupratap *et al.*, 1980). This is perhaps also true for the rest of the Indian Ocean, in general, even though potential for substantial N_2-fixation is indicated by the positive $N^\star$ values extending over the upper 500 m or so in the region between the equator and Lat. 45°S (Gruber, 2004). There are several reports of the occurrence of *Trichodesmium* in waters along the Indian Ocean's eastern boundary – especially off the west coast of Australia (Carpenter and Capone, 1992; Creagh, 1985 and references therein) – as well as the western boundary (e.g., Kowalski, 2001; Kromkamp *et al.*, 1997).

4. Natural Isotope Abundance

4.1. Dissolved inorganic nitrogen

Water column distributions of $^{15}N/^{14}N$ in N_2, NO_3^- and N_2O and $^{18}O/^{16}O$ in N_2O (expressed as $\delta^{15}N$ and $\delta^{18}O$, relative to atmospheric N_2 and $O_2\star$) in the Arabian Sea show large changes bought about by microbial consumption/production of these dissolved nitrogen species (Altabet *et al.*, 1999; Brandes *et al.*, 1998; Naqvi *et al.*, 1998a,b; Yoshinari *et al.*, 1997). The most pronounced mass-dependent fractionations appear to occur through water column denitrification. Preferential

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$\star$ The $^{18}O/^{16}O$ ratio in N_2O is also expressed as the per mil deviation from the standard mean ocean water (SMOW) instead of atmospheric O_2 with the two scales related by the equation (Kim and Craig, 1990):
$\delta^{18}O_{AIR} = -23.0 + \delta^{18}O_{SMOW} /1.0235.$

reduction of $^{14}NO_3^-$ in this process causes enrichment of ^{15}N in NO_3^- and its depletion in N_2: $\delta^{15}N$ of NO_3^- increases from ~6‰ in deep waters (2500–3000 m), comparable with values from other areas (Sigman *et al.*, 1997), to 15–18‰ within the core of the denitrifying layer; the $\delta^{15}N$ of N_2 concurrently decreases from ~0.6‰ to ~0.2‰ (Figs.14.15 and 14.16 Brandes *et al.*, 1998; Altabet *et al.*, 1999). Brandes *et al.* (1998) combined the isotopic data with NO_3^- deficits derived from
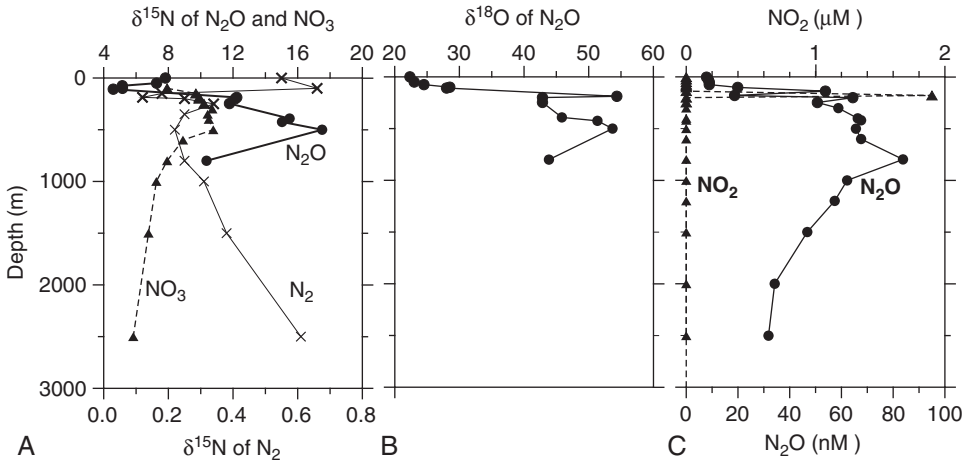
Figure 14.15 Vertical profiles of (A) $\delta^{15}N$ of N_2, NO_3^- and N_2O (‰ vs. air), (B) $\delta^{18}O$ of N_2O (‰ vs. air) and (C) concentrations of NO_2^- (μM, triangles) and N_2O (nM, circles) at 19°45'N, 64°37'E (from Naqvi *et al.*, 1998b).
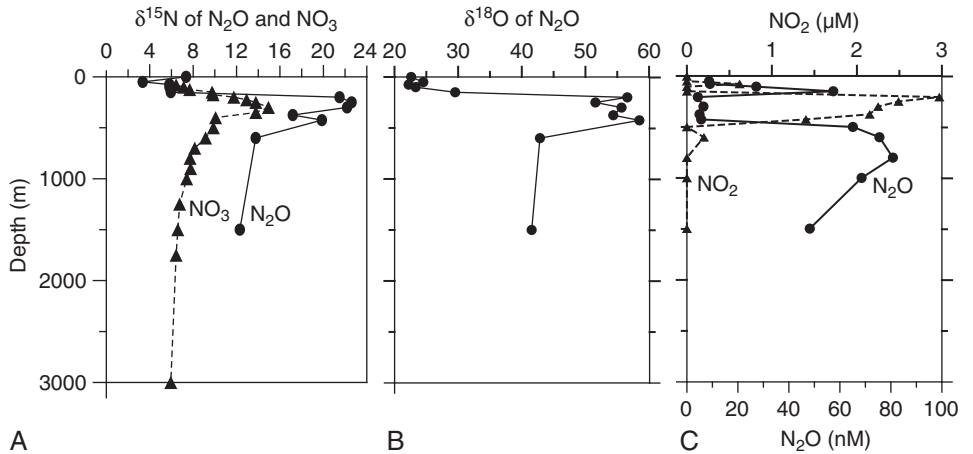
Figure 14.16 Vertical profiles of (A) $\delta^{15}N$ of NO_3^- and N_2O (‰ vs. air), (B) $\delta^{18}O$ of N_2O (‰ vs. air) and (C) concentrations of NO_2^- (μM, triangles) and N_2O (nM, circles) at 17°N, 68°E (from Naqvi *et al.*, 1998b).

the NO-temperature relationship, and used an advection-reaction model (Rayleigh fractionation) and a diffusion-reaction model to compute the fractionation factor (ϵ) of 22‰ and 25‰, respectively. Altabet *et al.* (1999) obtained a somewhat higher value ($\sim$30‰) taking $-N^\star$ as the NO_3^- deficit and assuming Ralyleigh fractionation. These estimates of ϵ for the Arabian Sea are similar to those for the eastern tropical North Pacific (Brandes *et al.*, 1998; Cline and Kaplan, 1975), implying relative constancy of isotopic fractionation associated with open-ocean denitrification.

Isotopic discrimination during uptake by phytoplankton results in an increase in $\delta^{15}N$ of NO_3^- in near-surface waters in most areas including the eastern Indian sector of the Southern Ocean (Sigman *et al.*, 1999). Going against this trend, observations in the Arabian Sea show a strong decrease from the suboxic-layer maximum to values that are not much above the oceanic average at the top of the nitracline (Figs. 14.15 and Fig. 14.16). This requires inputs of isotopically lighter NO_3^- either through advection from the south or, more plausibly, from a local source as the water at this depth (the Arabian Sea high-salinity surface water) is of local origin. This decrease in $\delta^{15}N$ of NO_3^- is accompanied by an increase in $N^\star$, strongly pointing to additions of new nitrogen through N_2-fixation by diazotrophs, a process that involves little or no isotopic fractionation (Wada and Hattori, 1990). Brandes *et al.* (1998) estimated the amount of N_2-fixation required to produce the observed NO_3^- isotope distribution. Taking the $\delta^{15}N$ of NO_3^- of water originating from the suboxic zone to be 11‰, simple mass balance requires that 40% of NO_3^- at the top of the nitracline should come from N_2-fixation. Using data on primary productivity, the rate of N_2-fixation was worked out to be $\sim$6 Tg N year^{-1}.

Isotopic data on N_2O, again coming only from the Arabian Sea (Figs. 14.15 and Fig. 14.16), reveal large variability in the composition of both the elements with depth as well as geographical location (Naqvi *et al.*, 1998a,b; Yoshinari *et al.*, 1997). The most prominent feature is the enrichment of heavier isotopes within the SNM at levels ($\delta^{15}N$ and $\delta^{18}O$, versus air, at times exceeding 20‰ and 60‰, respectively), which are the highest reported from any natural environment. These are associated with low N_2O concentrations (Figs. 14.15 and 14.16), and are obviously caused by the preferential reduction of isotopically lighter N_2O. However, a puzzling aspect of these data is that the $\delta^{15}N$ of N_2O always exceeds that of NO_3^-, opposite to the trend exhibited by cultures of *Paracoccus denitrificans* grown under steady-state conditions (Barford *et al.*, 1999): the culture experiments suggested greater fractionation during the reduction of NO_3^- to N_2O ($\sim$16‰) than of N_2O to N_2 ($\sim$13‰), with the overall fractionation (NO_3^- to N_2, $\sim$29‰) well within the range of the above-mentioned values derived from the field data. The higher $\delta^{15}N$ of N_2O implies that the natural bacterial populations should bring about a larger fractionation for the final reduction sequence. Moreover, as this trend persists also in N_2O-rich waters below the SNM (Figs. 14.15 and Fig. 14.16), it indicates possible involvement of denitrification in N_2O production well outside the SNM. Based on the analysis of N_2O isotopomer ratios (site preference of ^{15}N in the linear N_2O molecule), Yamagishi *et al.* (2005) also identified denitrification to be the dominant process responsible for N_2O production in the O_2 minimum zone of the North Pacific.

The isotopic data suggest a more important role for nitrification in N_2O production in the upper water column. The shallow, sharp peak of N_2O is characterized by

the lowest values of $\delta^{15}N$ and $\delta^{18}O$. The isotopic minima, more pronounced in case of nitrogen as compared to oxygen, are similar to those reported from the Pacific Ocean (Dore *et al.*, 1998; Popp *et al.*, 2002) except that in the Arabian Sea they are located at shallower depths. Going by its known isotopic signatures (Yoshida, 1988), nitrification has been proposed to be mainly responsible for the production of N_2O at shallow aphotic depths in the North Pacific (Dore *et al.*, 1998). The nitrification-related production may occur through two possible pathways: $NH_4^+ \rightarrow NH_2OH \rightarrow NOH \rightarrow NO \rightarrow N_2O$ and $NH_4^+ \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O$, and the relative importance of these pathways can be gauged from the oxygen isotopic composition of N_2O (Ostrom *et al.*, 2000; Popp *et al.*, 2002). The oxygen atom of NH_2OH is derived from O_2 (Dua *et al.*, 1979), whereas of the two atoms of oxygen in NO_2^- one each comes from O_2 and H_2O (Anderson and Hooper, 1983). Thus, if the oxygen isotopic compositions of O_2 and H_2O differ significantly, one can distinguish between the two formative pathways. The low $\Delta^{18}O$ (= $\delta^{18}O_{N_2O} - \delta^{18}O_{H_2O}$) values at Station ALOHA have been taken to support N_2O production from NO_2^-. Recently published enzymatic data from the Arabian Sea have been interpreted to invoke an important role of such nitrifier denitrification in N_2O production at low O_2 levels (Shailaja *et al.*, 2006). While concomitant data on the isotopic composition of O_2 and water are not available from this region, it would appear that significant production may also occur from the pathway involving NH_2OH. This is because the $\delta^{15}N$ and $\delta^{18}O$ often show contrasting behaviors, i.e. there is greater depletion of ^{15}N as compared to ^{18}O (Naqvi *et al.*, 1998a,b). In general, the $\delta^{18}O$ of N_2O is higher in the Arabian Sea (Naqvi *et al.*, 1998a,b; Yoshinari *et al.*, 1997) than in the Pacific Ocean (e.g., Kim and Craig, 1990; Popp *et al.*, 2002). Naqvi *et al.* (1998a) suggested that N_2O produced from NH_2OH-derived-NO should be slightly depleted with ^{15}N but enriched with ^{18}O. This pathway is also expected to lead to the concentration of ^{15}N at the central (α) position (Popp *et al.*, 2002; Stein and Yung, 2003), and so isotopomer analysis may help test this hypothesis.

4.2. Particulate and sedimentary organic nitrogen

Sediment trap studies in the deep sea (depth > ~1 km) carried out for several years at a number of sites in the North Indian Ocean showed that the PON undergoing sedimentation is more enriched in ^{15}N in the Arabian Sea than in the Bay of Bengal and, for that matter, most other parts of the ocean (Table 14.1; Gaye-Haake *et al.*, 2005; Schäfer and Ittekkot, 1993, 1995; Unger *et al.*, 2003, 2006). Denitrification in the former region and large influx of terrigenous material to the latter area are obviously responsible for this difference. The data also exhibit site-specific variations. For example, in the western Arabian Sea and southern Bay of Bengal, fractionation occurring during plankton growth under NO_3^--replete conditions affects the nitrogen isotopic composition of sinking particles in opposite directions, presumably due to different distances from the upwelling sites. At the western Arabian Sea trap site, which receives water upwelled off Oman, relatively low $\delta^{15}N$ values are characteristic of the peak SWM when the particle flux is at its maximum (Schäfer and Ittekkot, 1995). In contrast, at the southern Bay of Bengal trap site that is influenced by advection of water upwelled off the southwest coast of

India/west coast of Sri Lanka, a prominent $\delta^{15}N$ maximum is observed instead at this time (Unger *et al.*, 2006). N_2-fixation during the spring intermonsoon season can substantially lower the $\delta^{15}N$ of sinking PON as indicated by the time–series data from the southern Bay of Bengal (Unger *et al.*, 2006), as well as the shallower (100 and 500 m) drifting traps data collected from the Arabian Sea during the occurrence of the basin-scale *Trichodesmium* bloom mentioned in Section 3.3 (Montoya and Voss, 2006). In the latter instance, the material trapped at 500 m had an average $\delta^{15}N$ value of 1.9‰; in contrast, the $\delta^{15}N$ of the PON from the 100-m trap was inexplicably much higher (mean 7.1‰). As observed elsewhere (e.g. Altabet *et al.*, 1991; Thunnel *et al.*, 2004), the $\delta^{15}N$ values decrease slightly from the traps deployed in the mesopelagic to those in the bathypelagic realm (Gaye-Haake *et al.*, 2005). The sediment trap and surface sediment data follow similar trend of variability, but with the $\delta^{15}N$ for the near-bottom traps being consistently lower than the $\delta^{15}N$ for the underlying surface sediments by 2–3‰ except for the two shallowest, northernmost trap locations in the Arabian Sea and the Bay of Bengal (Fig. 14.17; Gaye-Haake *et al.*, 2005). This has been attributed to early diagenesis in sediments that removes isotopically lighter nitrogen; this interpretation is supported by a significant correlation between $\delta^{15}N$ and Degradation Index based on amino acid contents (Gaye-Haake *et al.*, 2005). Thus, sedimentary $\delta^{15}N$ is maximal (>11‰) in the central Arabian Sea and minimal (<4‰) in the northern Bay of Bengal with the values respectively decreasing and increasing southward to ~8‰ in the equatorial region. A local minimum (<6‰) is also observed over the western Indian continental margin, associated with a maximum in total nitrogen content (~0.5%). The sedimentary nitrogen content is generally higher closer to the coast
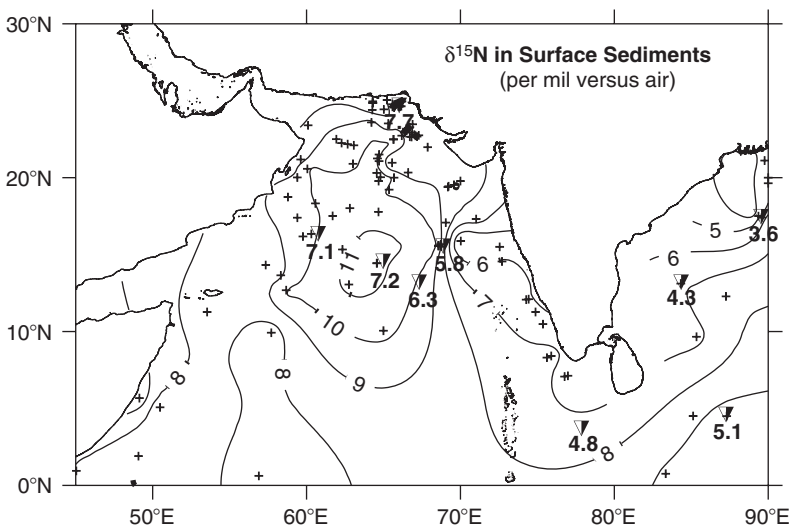
Figure 14.17 Distribution of $\delta^{15}N$ (‰ vs. air) in surface sediments of the North Indian Ocean (contours) and in sinking matter (bold numbers below the triangles that show locations of sediment trap moorings); the latter are long term weighted averages for the deepest traps (modified from Gaye-Haake *et al.*, 2005).

and in the Arabian Sea than in the Bay of Bengal, the latter in part due to the dilution caused by the river-derived sediments (Gaye-Haake *et al.*, 2005).

While the concentrations of suspended PON are somewhat higher in the Arabian Sea but still comparable and showing similar depthwise variability as in the Bay of Bengal, the nitrogen isotopic compositions of the PON are vastly different (Fig. 14.18) as observed for the settling and sedimentary PON, i.e. greater enrichment of ^{15}N in the Arabian Sea (Montoya and Voss, 2006) than in the Bay of Bengal (Kumar *et al.*, 2004). One intriguing aspect of the Arabian Sea data set is relative lack of variability in δ^{15}N below the surface layer with either depth or geographical location, with all values falling between 9 and 11‰ north of the equator. Surface-layer values are lower, but still substantially above (>6‰) the normal oceanic range (e.g., Altabet, 1989; Mino *et al.*, 2002; Montoya *et al.*, 2002). Exceptions from this general trend were noticed, though, in the region of abundant
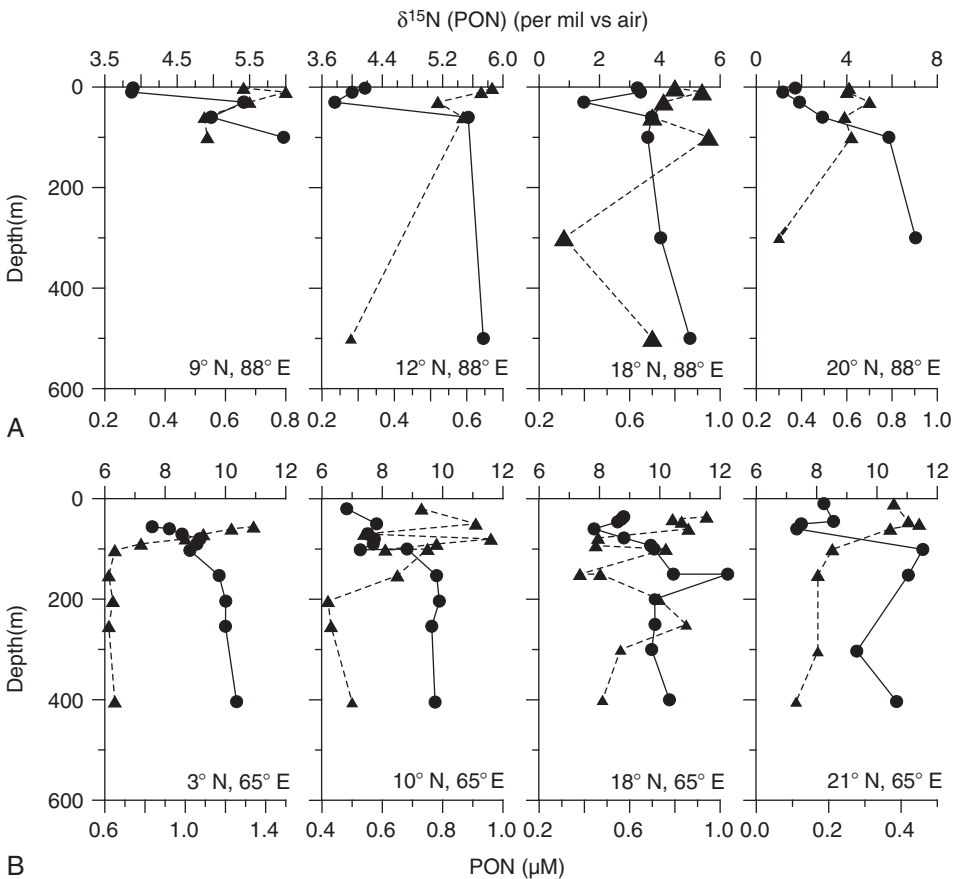
Figure 14.18 Vertical profiles of PON (▲) and its δ^{15}N (●) in (A) Bay of Bengal (upper panels; sampling period April–May 2003; Kumar *et al.*, 2004) and (B) Arabian Sea (lower panels; sampling period May, 1995; Montoya and Voss, 2006).

Trichodesmium blooms (Montoya and Voss, 2006). These results highlight the dominance of denitrification in determining the composition of particulate material over an area and a depth range much larger than those of the SNM. In the Bay of Bengal too the subsurface δ^{15}N data show little north-south variability with most values hovering around 6‰ (Fig. 14.18). Moreover, as in case of the Arabian Sea, the δ^{15}N of PON in Bay of Bengal also falls by >2‰ towards the surface, with the vertical profiles often showing a minimum at the base of the euphotic zone that has been ascribed to isotopic discrimination during NO_3^- uptake by phytoplankton (Kumar *et al.*, 2004). The general association of the lowest values (2–3‰) in coastal waters with salinities <32 has been interpreted to reflect riverine inputs in the Bay of Bengal. However, at higher salinities, the range is still quite large (2–7.6%), and while NO_3^- supplied through vertical mixing is believed to be the primary source of nitrogen (Kumar *et al.*, 2004), atmospheric deposition of fixed nitrogen, which has been reported to support a significant fraction of new production in the region (Schäfer *et al.*, 1993), could also significantly influence isotopic compositions of both suspended and sinking materials. Unfortunately, isotopic data on DIN are not yet available from the Bay of Bengal to assess the relative importance of these sources.

4.3. Glacial–interglacial changes in denitrification

The isotopic discrimination effect associated with denitrification (enrichment of ^{15}N in sediments) has been applied to reconstruct changes in mid-depth redox conditions on the geological time scale. This approach involves the assumption that NO_3^- brought up to the euphotic zone through upwelling and vertical mixing is locally consumed on an annual basis such that the signature of elevated δ^{15}N is transferred to sinking PON and ultimately preserved in sediments. This premise, however, needs to be qualified with some caveats: (1) As discussed above, several independent approaches point to substantial N_2-fixation in the Arabian Sea. However, the overall balance between denitrification and N_2-fixation, that determines the isotopic composition of sinking PON, is apparently strongly in favor of the former process such that organic matter synthesized in the surface layer and preserved in underlying sediments is on average more enriched in ^{15}N than is typical of areas that do not contain intense ODZs. Thus, the sedimentary δ^{15}N is more correctly a proxy of the balance between denitrification and N_2-fixation rather than of denitrification alone, and although it is not known if and how this balance changed with time, the implicit assumption is that during periods of intense denitrification in the past it was similar to what exists today. (2) As shown previously, early diagenesis causes an enrichment of ^{15}N in surface sediments relative to sinking PON by 2–3‰. This effect is, however, not seen at locations receiving high PON flux (Altabet *et al.*, 1999; Ganeshram *et al.*, 2000; Gaye-Haake *et al.*, 2005). Most of the data from the Arabian Sea come from such sites; but even for those locations where the near-bottom waters are exposed to relatively higher O_2 concentration and/or the sediments receive low PON flux, one may assume that the offset is time invariable so that changes observed within a record would still provide insights into denitrification variability. The qualitative similarity of the deep-sea records (e.g., from the core V34-101 raised from ∼3 km depth and

having a core top total nitrogen content of only 0.07%) to other records from the region justifies this assumption (Altabet *et al.*, 1999). (3) Sites of the most intense upwelling in the Arabian Sea are found along its western boundary (off Oman and Somalia), which are located outside the denitrification zone. The water that upwells here is derived from the south and its NO_3^- is not expected to be isotopically heavy. However, as this water advects $\sim$1000 km from the coast before its NO_3^- is fully consumed (Naqvi *et al.*, 2006a,c), substantial enrichment of ^{15}N must occur due to fractionation during phytoplankton uptake. Altabet *et al.* (1999) did, in fact, record surface δ^{15}N–NO_3^- values as high as $\sim$10‰ at an offshore station arising from this effect, whereas Schäfer and Ittekkot (1995) observed a related seasonal minimum in δ^{15}N of sinking PON at their western Arabian Sea trap site. Thus, even though on the basin-scale the upwelled NO_3^- is fully consumed annually, one may expect gradients in sedimentary δ^{15}N along the trajectory of flow of the upwelled water similar to those observed in the equatorial Pacific (Altabet and François, 1994). Since the bulk of the vertical flux occurs during the SWM season (Haake *et al.*, 1993), this potentially important effect deserves a detailed investigation. Altabet *et al.* (1999) argued that despite the large gradients in δ^{15}N of NO_3^- within the ODZ, the homogenous distribution of heavy δ^{15}N values just below the euphotic zone, facilitates uniform preservation of the denitrification signal all over the Arabian Sea. This in a way is analogous to the above-mentioned uniform distribution of δ^{15}N in the SPM, even though the processes that bring about such a homogenization are not understood.

Over the past ten years, a number of sedimentary time series of δ^{15}N have been published from several sites in the Arabian Sea representing a range of oceanographic settings and sedimentation rates (Agnihotri *et al.*, 2003; Altabet *et al.*, 1995, 1999, 2002; Ganeshram *et al.*, 2000; Suthhof *et al.*, 2001). These records show that large enrichment of δ^{15}N in sediments has been prevalent not only throughout the Holocene, but also all interglacial periods over the past 1 Ma. In contrast, lighter isotopic values ($\sim$5–6‰), comparable to those found in non-reducing environments today, characterize sediments that accumulated during the glacial stages indicating that denitrification weakened or was absent during such periods (Altabet *et al.*, 1995, 1999, 2002; Ganeshram *et al.*, 1995, 2000; Suthhof *et al.*, 2001). Sedimentary δ^{15}N exhibits a nearly normal distribution about a median of $\sim$6.5‰, with the present day values being close to the upper end of the range, and without a long term trend (Altabet *et al.*, 1999). Spectral analysis of the records indicates clear cyclicity associated with the three Milankowitch frequencies of 100, 41 and 23 kyr, significantly coherent with records of proxies of ice volume (foraminiferal δ^{18}O) for all three frequencies, and of the SWM strength (lithogenic grain size) for all but the eccentricity one, thus testifying the roles of both hydrography and productivity in controlling Arabian Sea denitrification (Altabet *et al.*, 1995, 1999). Higher resolution records indicate variations on an even shorter (millennial) time scale, closely linked with the climatic changes recorded by the Greenland ice cores (Altabet *et al.*, 2002; Suthhof *et al.*, 2001). For example, the δ^{15}N records in two sediment cores from the Oman Margin generated by Altabet *et al.* (2002) show oscillations that are remarkably similar in structure and timing to the Dansgaard/Oeschger events recorded in the Greenland (GISP2) ice cores (Fig. 14.19). When smoothened with a 3000 yr
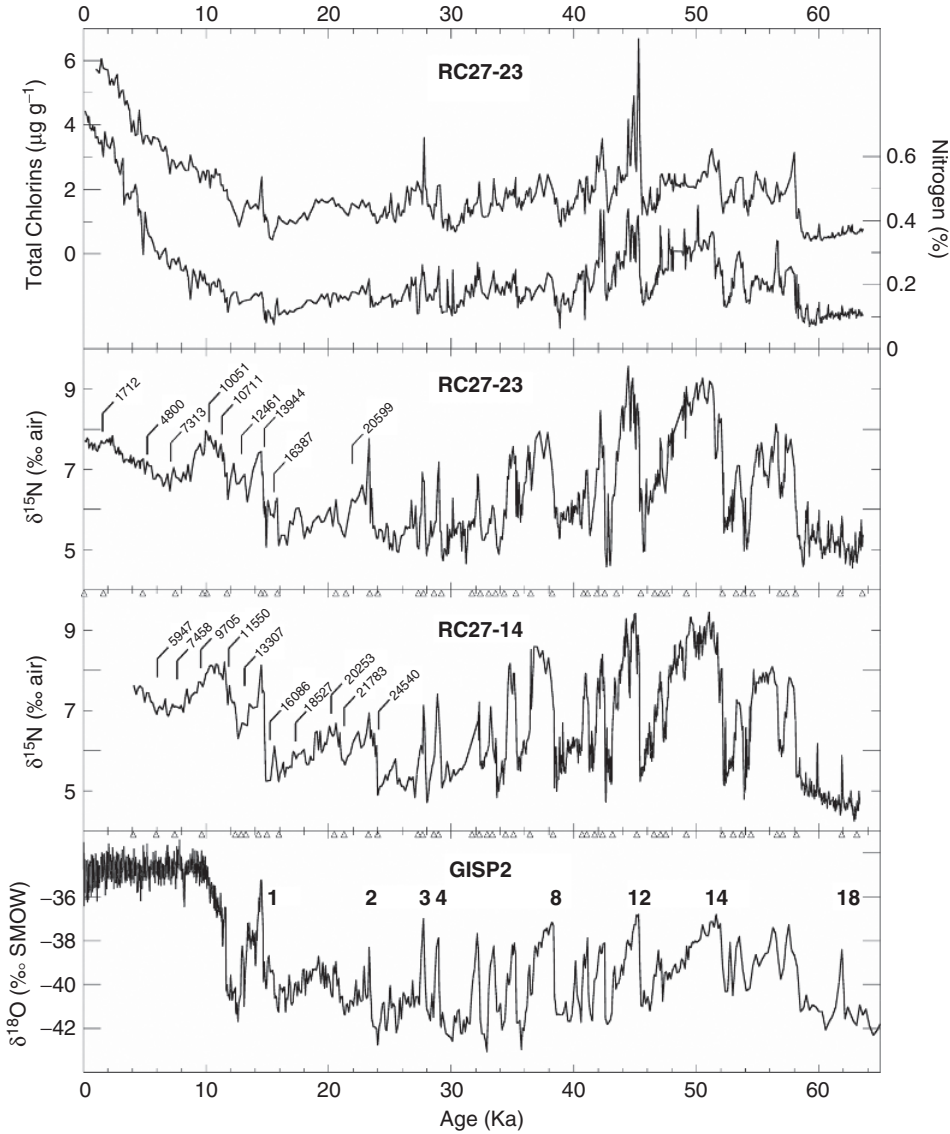
Figure 14.19 Records of $\delta^{15}N$, a denitrification proxy, from cores RC27-14 and RC27-23 (two middle panels), and of sedimentary nitrogen and total chlorin contents, proxies of productivity (upper panel), from RC27-23, compared with the GISP2 $\delta^{18}O$ record (lower panel) for the past 65,000 years. Radiocarbon dates converted to calender years for the two cores are marked on the $\delta^{15}N$ records. Numbers (1−18) on the bottom panel refer to Dansgaard−Oeschger events (from Altabet *et al.*, 2002).

running average − to account for the dampening effect of the residence time of nitrogen in the sea − the Arabian Sea denitrification records are remarkably similar to the Antarctic temperature and CO_2 records, leading Altabet *et al.* (2002) to conclude that changes in denitrification in the ODZ of the Arabian Sea, and probably in other

similar areas of the eastern tropical Pacific, may have a major impact on global climate. Furthermore, it has been suggested that such changes probably also contributed to the variability in atmospheric N_2O content as recorded in polar ice (Altabet et al., 1995, 2002; Flückiger et al., 2002; Suthhof et al, 2001).

The potential role of nitrogen cycle process in modulating glacial–interglacial changes through controls on inventory of fixed nitrogen, first proposed by McElroy (1983), has also been invoked by Falkowski (1997) and Broecker and Henderson (1998), who emphasized an enhanced N_2-fixation in the glacial ocean facilitated by greater aeolian supply of iron, as opposed to, or in addition to, reduced denitrification, favoured by Altabet et al. (1995, 1999, 2002) and Ganeshram et al. (1995, 2000). However, in order to account for the full amplitude of the glacial–interglacial atmospheric CO_2 change, the oceanic combined nitrogen inventory should have increased by at least 30%, provided that the ratio between inorganic carbon to organic carbon export decreased concurrently to keep $CaCO_3$ flux to deep sea at or below the present day level (Gruber, 2004; Sigman and Boyle, 2000). Moreover, for the increased nitrogen inventory to be effective for CO_2 sequestration there is an additional requirement of increased N:P ratio over the Redfield value – otherwise the production would become phosphorus limited. Gruber (2004) argued that the feedbacks within the nitrogen system do not allow very large departures from this ratio in today's ocean. He concluded that while changes in N_2-fixation and denitrification probably contributed to increased oceanic sequestration of CO_2 during the glacial periods, this was in all likelihood not the dominant mechanism.

More recently, Altabet (2007) has examined high resolution $\delta^{15}N$ records from several sites considered to be sensitive to oceanic average $\delta^{15}N$ and found no detectible change over the last 3 kyr or so (roughly the residence time of nitrogen in the ocean) implying a balanced marine nitrogen budget over the latest Holocene. This observation does not conform to the widely held view that nitrogen budget in today's ocean in severely out of balance with losses through sedimentary and water column denitrification far exceeding inputs (by >200 Tg N year^{-1}) from the atmosphere (mainly N_2-fixation) and land (Codispoti et al., 2001); a sustained imbalance of this magnitude is expected to produce detectable change in oceanic mean $\delta^{15}N$ over the investigated time period (Altabet, 2007). In contrast, most of the longer $\delta^{15}N$ records that extend to the last glacial maximum show a 2–3‰ increase over deglaciation followed by ~1‰ decrease in the early Holocene (see also Deutsch et al., 2004; Gruber, 2004). This seemingly global signal is also seen in the Arabian Sea data (e.g., Fig. 14.19), but the timing of the peak also corresponds to the intensified SWM in response to maximal summer insolation (Prell, 1984) that probably led to an intensified denitrification in the region. Altabet (2007) postulated that a transient deglacial imbalance in the global oceanic nitrogen ocean could have led to 10–30% decrease in the oceanic combined nitrogen inventory during this period before the feedback processes restored the balance. The mid-Holocene minimum in $\delta^{15}N$ seen in the Arabian Sea records (Fig. 14.19) is, however, not found in records from the other suboxic zones. The slow, secular increase in $\delta^{15}N$ since ~6 kyr B.P. implies a gradual intensification in the Arabian Sea denitrification that does not seem to have significantly affected oceanic combined nitrogen inventory.

5. Nitrogen Budgets

There have been several attempts to quantify key nitrogen fluxes in the Arabian Sea (Somasundar et al., 1990; Naqvi et al., 1992; Bange et al., 2000; Naqvi et al., in press). The budget given in Table 14.2 is reproduced from Naqvi et al. (in press), which in turn is not much different from that of Bange et al. (2000). Note that estimates for both water column denitrification and N_2-fixation are probably conservative, but the difference between the two terms will perhaps not change much, should the two estimates be revised upward in view of the following constraint. The estimated net loss of combined nitrogen (32.5 Tg N year^{-1}) must be balanced by NO_3^- inputs through circulation. The net NO_3^- flux by waters flowing in from the south (38 Tg N year^{-1}) computed from the hydrographic data matches very well with this deficit (Bange et al., 2000); notwithstanding the uncertainties involved in various calculations, this provides some confidence in the overall budget.

The budget for the Bay of Bengal (Table 14.3), also from Naqvi et al. (in press), is comparatively more uncertain. Due to the massive river runoff one would expect this region to receive copious amounts of nutrients from land, but the estimated riverine flux of DIN, based on measurements in the Hooghly estuary, is only ~0.5 Tg N year^{-1}, an order of magnitude lower than the estimate (4.2 Tg N year^{-1}) of Seitzinger et al. (2002) for inputs by the South Asian rivers. The riverine flux of PON (3.5 Tg N year^{-1}) is larger, but also more uncertain. Inputs of DON by rivers and DIN by groundwaters still remain to be measured, but they are unlikely to be quantitatively very important. In contrast, the atmospheric deposition of DIN has been reported to range from 0.3 to 2.5 (average 1.4) Tg N year^{-1} (Schäfer et al.,

Table 14.2 Combined nitrogen budget for the Arabian Sea

	Tg N year^{-1}	Tmol N year^{-1}
Inputs		
River runoff (DIN)	0.06	0.004
Atmospheric deposition (wet and dry)	1.5	0.107
N_2-fixation	3.3	0.236
Exchange with Red Sea	0.7	0.05
Total	5.6	0.4
Losses		
Pelagic denitrification		
Open-ocean	30	2.143
Indian shelf	4	0.286
Sedimentary denitrification	3.5	0.25
N_2O loss to atmosphere	0.4	0.029
Sedimentary burial	0.2	0.014
Total	38.1	2.721

Table 14.3 Combined nitrogen budget for the Bay of Bengal

	Tg N year^{-1}	Tmol N year^{-1}
Inputs		
River runoff		
DIN	0.5	0.036
PON	3.5	0.25
Atmospheric deposition (wet and dry)	1.4	0.1
N$_2$-fixation	0.5	0.036
Total	5.9	0.421
Losses		
Sedimentary denitrification	3	0.214
N$_2$O loss to atmosphere	0.07	0.005
Sedimentary burial	1	0.071
Total	4.1	0.291

1993). Rates of N$_2$-fixation in the Bay of Bengal have not been measured so far. However, as already stated, it is probably much lower than in the Arabian Sea and is tentatively taken as 0.5 Tg N year^{-1}.

The major modes of nitrogen losses from the Bay of Bengal are sedimentary denitrification and burial. Note that the estimate for sedimentary denitrification (3 Tg N year^{-1}) is based on only one measurement at the outer shelf off Chennai (Naik, 2003), while the burial in sediments was computed from the carbon burial using a C:N value of 10.

While accurate uncertainty estimates associated with the above flux estimates cannot be provided, the numbers are probably valid within a factor of two. Taking this into account, the fluvial and aeolian inputs of nitrogen into the Bay of Bengal may approximately match losses through burial and sedimentary denitrification. However, the input terms have been, in all likelihood, anthropogenically enhanced and will most certainly rise further in future due to the ongoing rapid increases in fertilizer and fuel consumption associated with growths of population and economy (see Seitzinger and Harrison, this volume; Paerl and Piehler, this volume).

6. CONCLUDING REMARKS

The work carried out during the past few decades has greatly improved our understanding of nitrogen cycling in the Indian Ocean, but several shortcoming still remain and new questions have emerged that should be answered by future research. These relate to (a) distributions and fluxes, (b) processes, and (c) anthropogenic impact. We now have a fairly good knowledge of the spatial and temporal variability of major inorganic nitrogen forms, but additional information is needed from some areas (e.g., the upwelling zone off Indonesian islands and the shelves of Pakistan and Myanmar) and during certain periods (e.g., Indian Ocean Dipole event). In contrast,

data on DON are severely lacking from most areas. Moreover, we know very little about the composition of DON, its reactivity/bioavailability and turnover rates. As for the fluxes, there exists large uncertainty on the supply side. As discussed above, the few directly determined rates of N_2-fixation focusing on *Trichodesmium* are much lower than suggested by the geochemical and isotopic data. The contribution of small diazotrophs is yet to be evaluated. Riverine inputs of both dissolved and particulate nitrogen to the ocean are poorly quantified, and no information is available on groundwater inputs so far. The situation concerning the loss terms is only slightly better. Despite several modeling and field studies including direct measurements, the water column denitrification rate for the Arabian Sea perhaps carries an uncertainty of up to $\pm 50\%$, and sedimentary denitrification rates are largely unknown for most areas.

With regard to the nitrogen cycle processes, the recently observed mismatch between nitrate deficits and N_2 excess within the Arabian Sea ODZ and the occurrence of significant deficits within the deeper oxycline in the North Indian Ocean provide a reflection of our inadequate quantitative understanding of the various potential pathways of N_2 production. The role of anammox vis-à-vis denitrification, in particular, remains to be evaluated for this region. Another hitherto neglected aspect of nitrogen cycling is the link between nitrogen and trace metal cycles (see Hutchins and Fu, this volume). Biologically-mediated nitrogen transformations involve a variety of enzymes that require metal cofactors. For example, reduction of NO_3^- by both denitrifying bacteria and phytoplankton, as well as N_2-fixation by diazotrophs, requires Fe. The low Fe levels in the Southern Ocean are well known to limit PP (Martin *et al.*, 1990). In the Arabian Sea, Fe supply via atmospheric dust deposition has been believed to maintain sufficiently high dissolved Fe levels for it not to be limiting (Measures and Vink, 1999; Smith, 2001). However, results of both modeling (Wiggert *et al.*, 2006) and field measurements (Naqvi *et al.*, manuscript in preparation) indicate possible limitation of PP by Fe in the nutrient-replete upwelled water in parts of the western Arabian Sea during the late SWM. The biogeochemical and ecological implications of such Fe deficiency in the Arabian Sea and in other parts of the Indian Ocean suggested by Wiggert *et al.* need to be investigated in detail. Within the ODZ, pronounced maxima in total dissolved Fe and Fe(II) concentrations have been found to coincide with the SNM. As Fe(II) is thermodynamically unstable even at submicromolar O_2 levels, active biological reduction of Fe(III) has been hypothesized as a possible explanation of the anomalous observation (Moffett *et al.*, 2007), which should be verified by future workers.

One other remarkable aspect of the biogeochemistry of the Indian Ocean is the large contrast in redox conditions prevalent within the OMZs of the Arabian Sea and Bay of Bengal despite very subtle difference in the ambient O_2 levels. In addition to understanding, through modeling, physical processes supplying O_2 to the OMZ that help the Bay of Bengal remain delicately poised just above the denitrification threshold, future research should target the fundamental question as to what environmental factors trigger the transition from oxic to suboxic (denitrifying) metabolisms. Inevitably this will involve a comprehensive study of ecology of microbes involved, which will also provide insights into the formative mechanisms of the bacteria-rich INL and the clear, relatively sterile zone just above this feature that are ubiquitous to all suboxic zones.

The global nitrogen cycle is being greatly impacted by human activities, and in the open ocean domain this impact is not expected to be more severe anywhere than in the North Indian Ocean. A more sluggish deep circulation and stronger stratification resulting from global warming are expected to lead to a decrease in O_2 content of deep water in conformity with the observed trend in recent years (Joos et al., 2003). The global warming is also projected to cause an intensification of the SWM, the effects of which are reported to be have already become discernible in terms of intensified summer winds, upwelling and phytoplankton blooms in the western Arabian Sea (Goes et al., 2005). Together with the increased nutrient loading from land, through both river runoff and atmospheric deposition, it is expected to result in an increase in O_2 demand at depth. Ultimately, however, how the global warming modifies the biological productivity, and the intensity and spatial extent of the OMZ in the North Indian Ocean will depend upon alterations of the cross equatorial meridional cells, which supply subsurface nutrient-rich waters from the south to the upwelling zones (McCreary, 2006). Due to the potential feedback to global climate through increased production of greenhouse gases, and the impact on regional marine living resources that may have serious socio-economic impacts, there is an urgent need to build coupled physical–biogeochemical models capable of predicting future evolution of the Indian Ocean biogeochemistry including redox nitrogen transformations under various scenarios of global change.

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CHAPTER 15

Nitrogen in Inland Seas

Edna Granéli *and* Wilhelm Granéli

Contents

1. Introduction

Inland or enclosed seas are characterized by limited water exchange with the open ocean. External nitrogen loading can be measured more accurately than for an open system, which makes inland seas ideal for budget calculations and modelling, similar to lakes. A few inland seas are well studied, and could serve as models for larger, open systems. Examples in this review will be taken mainly from the Baltic Sea. The Baltic Sea has a long history of research, is well studied from a systems point of view, has undergone dramatic changes during the last 100 years, and the focus on research and management has been on nitrogen during the last decades. For other inland seas information is much less complete.

We will treat external N loading (riverborne N, atmospheric deposition, N_2 fixation), N uptake by phytoplankton, denitrification and N losses to sediments and adjacent seas. It would be impossible to treat nitrogen in isolation. Thus, the question of nitrogen versus phosphorus as the limiting nutrient will be discussed. Comparisons between the Baltic Sea and the Mediterranean Sea, an inland sea with characteristics very different from the Baltic Sea, will be made at the end of the

chapter. Discussions will be limited to more principal differences: positive versus negative freshwater balance, estuarine versus inverse estuarine (Mediterranean type) circulation, nutrient trap versus nutrient escape (Wulff *et al.*, 1990), N/P ratios and limiting nutrients.

2. THE MODEL INLAND SEA—THE BALTIC SEA

Inland seas make up only a small percentage of the surface and volume of the oceans (2.9% and 0.8%, respectively). Ketchum (1983) includes in his review of enclosed seas the Mediterranean Sea, the Black Sea, the Red Sea, the Baltic Sea, the Bering Sea, the Okhotsk Sea, the Japan Sea, the East China Sea, the Gulf of St. Lawrence and the Gulf of California. The Baltic Sea is the second largest brackish–water system in the world (after the Black Sea), but it is among the smallest of the inland seas (Table 15.1).

There are several excellent review articles on the Baltic Sea (Elmgren, 1989, 2001; Jansson, 1997; Jansson and Dahlberg, 1999; Leppäkoski and Mihnea, 1996; Wulff *et al.*, 2001) some of which treat nutrients or nitrogen in particular (Elmgren and Larsson, 2001; Granéli, 2004; Granéli and Granéli, 1999; Larsson *et al.*, 1985; Wulff *et al.*, 1990). The Baltic Sea is comprised of a number of distinct basins (Fig. 15.1), the main three being the Bothnian Bay, the Bothnian Sea and the Baltic Proper. The Bothnian Bay has the lowest salinity (Fig. 15.2) and is oligotrophic, while the Bothnian Sea is a transition area to the more nutrient-rich Baltic Proper.

Eutrophication of the Baltic Sea is well described in several review papers (Elmgren, 1989, 2001; HELCOM, 2003; Jansson, 1997; Jansson and Dahlberg, 1999; Leppäkoski and Mihnea, 1996; Nausch *et al.*, 1999; Rosenberg *et al.*, 1990; Wulff *et al.*, 1990). Besides increased nutrient loading and concentrations, these changes include: decreased Secchi depth (from 10 m in summer 1914–1939 to 7 m in 1969–1991) (Sanden and Håkanson, 1996), decreased depth penetration of macro-alagae, e.g., *Fucus vesiculosus* (Kautsky *et al.*, 1986), increased primary production (Elmgren, 1989; Rahm and Danielsson, 2001; Stigebrandt, 1991) and deep-water oxygen consumption (Pers and Rahm, 2000), increased macrofauna biomass above

Table 15.1 Baltic Sea

Location	Lat 54–66°N	Mean depth	55 m
Area[a]	390,000 km^2	Max depth	459 m
Drainage area[b]	1.7×10^6 km^2	Volume	21,000 m^3
Theoretical water turnover	$\approx$25 year	Sill depth	18 m
Population in drainage area	85×10^6	Salinity in central part above halocline	6–7 PSU

[a] Excluding the Kattegat.
[b] Forests 48%, arable land 20%.

Figure 15.1 The Baltic Sea with drainage area and basin definition.
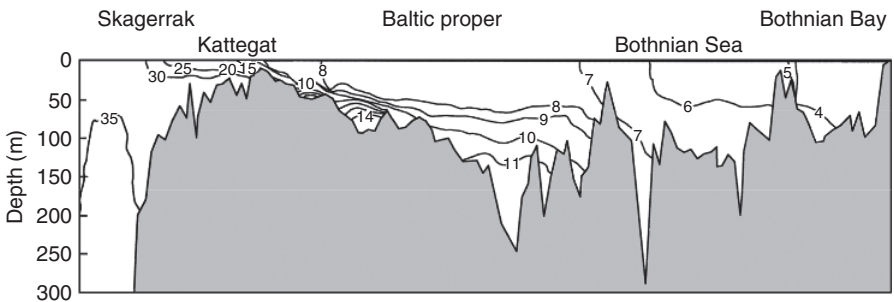
Figure 15.2 Salinity isoplets in the Baltic Sea, the Kattegat and the Skagerrack (redrawn from Andersson *et al.* (1992)).

the halocline and reduced or eliminated below (Rosenberg *et al.*, 1990), increased fish catches (Hansson and Rudstam, 1990; Jansson and Dahlberg, 1999) and virtual elimination of the most important top carnivores, seals (Elmgren, 1989).

   Due to its shallowness and strong permanent vertical density stratification the Baltic Sea has as some of its characteristics: (1) strong influence from benthic processes and (2) irregular (a few years to decades) cycling between deep-water anoxia and periods with below-halocline ventilation and good oxygen conditions. This makes the Baltic Sea different from e.g., the Black Sea and other permanently anoxic basins, as well as inland seas with a higher and more uniform salinity, and without widespread deep-water anoxia (e.g., the Mediterranean Sea). Even though

the Baltic Sea has the advantage of being probably the most intensively studied marine area in the world, nitrogen analyses of good quality only exist since the end of the 1960s. Much of the more recent nitrogen research in the Baltic Sea has been motivated by applied aspects, principally eutrophication, and includes international efforts to calculate external loading (Ehlin, 1999; HELCOM, 2004), as well as research on nitrogen management, its costs and its possible effects on the Baltic Sea eutrophication problem (Elmgren and Larsson, 2001; Granéli and Granéli, 1999; Gren et al., 1997; Hart and Brady, 2002; Löfgren et al., 1999; Ollikainen and Honkatukia, 2001; Savchuk and Wulff, 1999; Sundell, 1997).

2.1. Nitrogen in surface and deep water—Concentrations and pools

Concentrations of nitrogen and phosphorus have increased markedly in the Baltic Proper since measurements started 30–40 years ago (Fig. 15.3, see also Nausch et al., 1999; Perttilä et al., 1995; Rahm et al., 1996). Compared to conditions before 1950, concentrations of N and P have probably increased even more, but there are no data to verify this. In deep water, below the halocline (>60–80 m in the Baltic Proper), dramatic and rather rapid changes in nutrient concentrations may take place. This is caused by intermittent inflow of oxygenated, saltier deep-water, which will replace oxygen-deficient or anoxic (H_2S-containing), stagnant deep water. Complete renewal of deep-water only occurs with intervals of several years and stagnation periods may be more than decade-long. As a consequence there may be an initial build-up of nitrate in oxygenated deep-water, which is subsequently denitrified and instead ammonia will accumulate under anoxia.

Most of the water masses in the Baltic Sea are found above the halocline, e.g., in the Baltic Proper only 20% of the volume is below the halocline. Deep-water entrainment and especially internal nutrient turnover processes (phytoplankton uptake, N_2 fixation, sedimentation, denitrification) have a profound influence on concentrations in surface water. Decreases in nutrient concentrations in surface waters of the Baltic Sea have been interpreted as consequences of reduced external loading. However, the total amount of N and P in the water mass and active
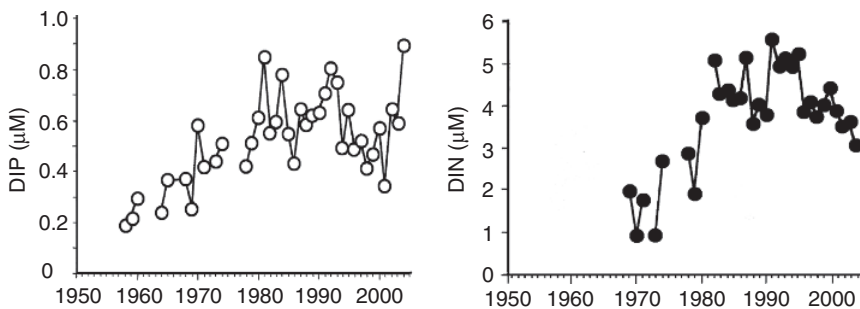
Figure 15.3 Inorganic nitrogen ($NO_2 + NO_3 + NH_4$) and phosphorus (PO_4) in surface water (0–10 m) of the Baltic Proper during winter (at the Landsort deep, redrawn from Larsson and Andersson (2004)).

sediments, as well as the amounts turned over annually, are several times larger than the annual external addition. This means that changes in the balance between internal nutrient sources and sinks will influence concentrations. Nutrients accumulated in deep water will at least partly be transported to surface water. This means that the length of "stagnation" periods, as well as the magnitude of irregular intrusions of deep water, are important for nutrient conditions also in surface waters (Larsson and Andersson, 2004; Pitkänen *et al.*, 2001).

The mean N pool size in 1971–1981 in the Baltic Sea as a whole was 5400 kt Tot-N, with 3900 kt in the Baltic Proper (change 1971–1981 + 121 kt N year^{-1} according to Wulff *et al.*, 1990. Larsson and Andersson (2004) gave a similar estimate for the Baltic Proper in 1995–2003, the N pool fluctuating between 3200 and 4300 kt, with a decreasing trend of roughly 100 kt N year^{-1}. Thus, over a period of less than 10 years, the pool size of N in the water masses of the Baltic Sea can change by more than 1000 kt. However, in our N budget we will assume that the net change is 0.

Net ecosystem production is approximately 50 g C m^{-2} year^{-1} in the Baltic Proper (Sandberg *et al.*, 2000; Stigebrandt, 1991), which, applying the Redfield ratio, would require 2200 kt N year^{-1} (Eilola and Stiegebrandt, 1999; Rahm *et al.*, 2000), while phytoplankton primary production (approximately 100–150 g C m^{-2} year^{-1} in the Baltic Proper and the Bothnian Sea) would require around 5000 kt N year^{-1}. Since a large fraction, on the order of 75% (Carlsson and Granéli, 1993; Carlsson *et al.*, 1993; Eilola and Stiegebrandt, 1999; Granéli *et al.*, 1999; Larsson and Andersson, 2004), of the total N in the water is in dissolved organic form, the N demand of phytoplankton exceeds the available DIN pool even during the spring bloom, when the store of DIN and DIP above the halocline is maximal (Eilola and Stiegebrandt, 1999).

2.2. Exchange with the north sea

Inflow of river water into the Baltic Sea exceeds evaporation by approximately 500 km^3 year^{-1}, while 1000 km^3 year^{-1} leaves the Baltic Sea as surface water to the Kattegat. This means that 500 km^3 year^{-1} of saltier water enters the Baltic Sea at deeper levels (Stigebrandt, 2001). The net N outflow is 100–140 kt N year^{-1} (Larsson *et al.*, 1985; Wulff *et al.*, 1990, 2001). We will use 150 kt N year^{-1} for our N budget. The N loss through the Danish Straits and the Öresund is on the order of 10% of the total N load to the Baltic Sea (see below). Thus 90% of the N load is retained in the system.

2.3. Nitrogen loading from river water including sewage—DON availability

Figures for N loading in rivers have constantly been modified, usually upwards (Grimvall and Stålnacke, 2001), not because there has been an upward trend in river-transported nutrients, but because more reliable measurements have become available. The mean riverine load of total N for 1980–1993 to the whole Baltic Sea (excluding the Kattegat but adding direct sewage emissions) can be estimated to 800 kt N year^{-1} using data from Stålnacke *et al.* (1999). The load was directly

proportional to water flow and showed a variation from roughly 600–1000 kt N year^{-1}. The corresponding Helsinki Comission (HELCOM, 2004) mean figure for 1994–2000 would be 640 kt N year^{-1}, with 77% from monitored rivers, 17% from unmonitored rivers and coastal areas and only 6% from municipalities, industries and fish farms along the coastline, discharging directly into the Baltic Sea. Although the HELCOM (2004); Stålnacke *et al.* (1999) figures do not cover an overlapping time period, there is no reason to believe that river transport of N has changed dramatically during the last 2–3 decades (Grimvall and Stålnacke, 2001). We will use 700 kt N year^{-1} as a reasonable figure for river plus sewage loading to the Baltic Sea.

Probably a substantial—but unknown—fraction of the riverine N load, as well as N in direct sewage emissions, is lost through denitrification and burial in the coastal zone, and will thus not reach the offshore water masses. In the Gulf of Gdansk, into which the River Vistula discharges (the second largest river draining into the Baltic Sea) it is estimated that 23% of the N load is retained, even though the water residence time is only 15 days (Witek *et al.*, 2003). Most of the retention is due to denitrification. Similarly, Neumann (2000), using a modelling approach, calculated that the Pomerian Bight, into which the Oder river discharges, retained through denitrification 65% of the riverine N load and that less than 15% of the load was exported to the offshore regions.

The discharge-normalized N load from rivers has been rather constant during the last two or three decades, irrespective of changes in fertilizer use, sewage treatment etc (Grimvall and Stålnacke, 2001; HELCOM, 2004; Löfgren *et al.*, 1999; Stålnacke *et al.*, 1999). However, compared to the beginning of the 20th century, the riverine N load has probably increased several folds. Larsson *et al.* (1985) estimated that the increase was 4× for N and 8× for P, when comparing the1980s with conditions before 1900, while Jansson and Dahlberg (1999) put the increase between 1940 and the 1990s to 3× and 5× for N and P, respectively. This last figure for N fits more or less with an estimated 30% natural background loss of N from the drainage area (HELCOM, 2004) compared to present losses. The increase in riverine N load most likely occurred before more systematic nitrogen monitoring was implemented, probably mainly during 1950–1970, a period when fertilizer use increased dramatically (Löfgren *et al.*, 1999; Nausch *et al.*, 1999). In the Daugava River (Latvia) nitrate increased from 0.2 mg N l^{-1} 1950–1965 to more than 1.0 mg N l^{-1} 1985–1995 (Grimvall and Stålnacke, 2001).

A large part of the total dissolved nitrogen in river water is in organic form. Riverine DON (roughly 1/2 to 1/3 of the total N load (HELCOM, 2004) may not be directly (hours–days) available to bacteria and phytoplankton after entering the Baltic Sea. However, since little N leaves the system in dissolved or particulate form through the outlets, and since there is certainly much less DON-export than the DON that enters and is produced internally in the Baltic Sea, there must be processes that mineralizes DON to DIN and/or transforms DON to particulate form, which will then partly be permanently buried in sediments. Since the residence time of N in the Baltic Sea is several years, a large fraction of DON could be mineralized through photochemical and microbiological processes (see Chapter 7 by Mulholland and Lomas, this volume).

Stepanauskas *et al.* (2002) made a survey of the availability to bacteria of DON from rivers around the whole Baltic Sea. They found that in summer the mean proportions of DIN, DON and PON in river water was 50%, 40%, and 10%. Although the share of DON may be somewhat lower in winter, DON is still a large fraction of N in river water and even higher in Baltic Sea surface water. Through regrowth bioassays with bacteria, Stepanauskas *et al.* (2002) showed that as a mean 30% of the DON in river water was potentially available to bacteria within 2 weeks. Also different phytoplankton species have been shown to utilize DON and PON from river water indirectly but also by direct incorporation of humic compounds (Carlsson and Granéli, 1993; Carlsson *et al.*, 1995; Granéli and Moreira, 1990; Granéli *et al.*, 1985, 1999).

2.4. Atmospheric deposition

For the Baltic Sea recent figures indicate that nitrogen deposition from the atmosphere may contribute up to 300 kt N year^{-1} (220–300 kt N year^{-1}), thus roughly half the amount transported to the Baltic Sea in river water (HELCOM; Hertel *et al.*, 2003; Jansson, 1997; Larsson *et al.*, 1985; Marmefelt *et al.*, 1999). Correcting for the Kattegat, a reasonable figure for present atmospheric deposition of N to the Baltic Sea is 250 kt N year^{-1}.

As atmospheric deposition of N occurs directly on the whole surface of the Baltic Sea, a proportionally smaller fraction than is the case for riverborne nitrogen will be intercepted in the coastal zone. This may increase the relative importance of atmospheric nitrogen compared to land-derived sources of nitrogen. Also, different from N in river water, the proportion of DON is probably low in atmospheric deposition, although most studies have neglected organic N totally. There is a marked south to north decreasing gradient in atmospheric N deposition, from 1000 kg km^{-2} year^{-1} in the SW Baltic Proper to less than 300 kg N km^{-2} year^{-1} in the Northern Bothnian Bay, with approximately equal total amounts of NH_4 and NO_3 and most (70–80%) of the nitrogen in wet deposition (Granat, 2001; Hertel *et al.*, 2003).

There are no long-term trends available for atmospheric deposition, but HELCOM reports a mean deposition of 300 kt N year^{-1} (including the Kattegat) for 1996–2001, with a variation between 230 and 330 kt N year^{-1}. Although emissions have decreased during this period, there is no indication of a decreased deposition of N over the Baltic Sea (Bartnicki *et al.*, 2003). The average nitrogen deposition on the Baltic Sea has most likely doubled to tripled during the last 50 years (Granat, 2001).

2.5. Cyanobacteria and nitrogen fixation

The Baltic Sea has as one of its most characteristic features large summer blooms of several species of heterocyst-bearing, colonial, N_2 fixing cyanobacteria. There are species of the genera *Aphanizomenon* and *Anabaena* (Janson and Granéli, 2002), well known from eutrophic lakes. Although *Aphanizomenon* normally has the largest share of the total biomass of N_2 fixing cyanobacteria, the most characteristic and

conspicuous species is *Nodularia spumigena*. To what extent there are also other N_2 fixers, e.g., smaller noncolonial cyanobacteria without heterocysts, is uncertain. Results from size-fractionated N_2 fixation studies indicate that this could be the case, but little direct evidence exists (Wasmund *et al.*, 2001 but see Montoya *et al.*, 2004 (see also Carpenter and Capone, Chapter 4, this volume).

Cyanobacteria blooms and N_2 fixation have been intimately linked to eutrophication of the Baltic Sea, a scenario well known from lakes receiving sewage with high concentrations of phosphorus. It has thus been thought that cyanobacteria blooms in the Baltic Sea are essentially a man-made problem, and that N_2 fixation has been provoked by anthropogenic discharges of P to the Baltic Sea, driving the N/P ratio to low values, a condition usually thought to favour cyanobacteria. Recent studies have shown that cyanobacteria are very good competitors for inorganic nitrogen as well in these waters (Stolte *et al.*, 2002). However, historic records, dating more than a century back, and paleoecological investigations, clearly show that cyanobacteria blooms have been a phenomenon intrinsic to the Baltic Sea for several thousand years (Bianchi *et al.*, 2000; Finni *et al.*, 2001; Poutanen and Nikkilä, 2001). Unfortunately, older observations (Finni *et al.*, 2001), and especially paleoecological indicators, are only qualitative or semiquantitative. Thus, even if there were cyanobacteria blooms in the Baltic Sea long before man could have influenced nutrient conditions to any great extent, it is generally (but not unanimously) believed that blooms have become more widespread, of prolonged duration and with higher biomasses after the middle of the last century (Poutanen and Nikkilä, 2001). This question is of great concern when discussing limiting nutrients for the Baltic Sea and possibilities to reduce cyanobacteria blooms through nutrient management (Bianchi *et al.*, 2000; Elmgren and Larsson, 2001).

N_2 fixation occurs mainly in the Baltic Proper, where the N/P ratio is low compared to the Redfield value during summer (Granéli *et al.*, 1990; Wulff *et al.*, 2001; Fig. 15.4). Because of the patchy nature of cyanobacteria blooms, strong
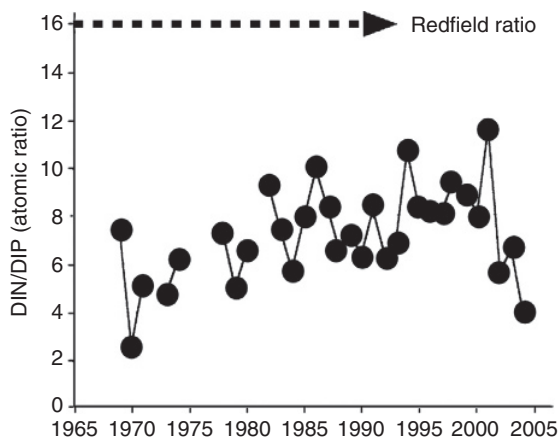
Figure 15.4 The DIN/DIP ratio of surface water (0–10 m) in the Baltic proper (at the Landsort deep) during winter (redrawn from Larsson and Andersson (2004)).

vertical gradients in biomass, rapid changes in distribution and great interannual variations due to weather conditions (Savchuk and Wulff, 2001), it has been difficult to get reliable figures of N_2 fixation through rate measurements *in situ*. Early figures, based on the acetylene-reduction method, were approximately 100 kt atmospheric N fixed annually for the Baltic Proper (Niemistö *et al.*, 1989), but newer figures based on the ^{15}N tracer technique indicate over 3 times this value (e.g., Wasmund *et al.*, 2001), nearly 400 kt N year^{-1}.

N_2 fixation can be quantified by measuring the increase in total N in the euphotic layer during periods when other sources of phytoplankton N (deep-water entrainment, additions from sediments, rivers and coastal point-sources) are insignificant or can be quantified. Using this approach Larsson *et al.* (2001) estimated that N_2 fixation in the Baltic Proper was 180–430 kt N year^{-1}, sufficient to sustain 30–90% of the pelagic net community production during summer.

Stable N isotope ratios may indicate the presence of N_2 fixation, since atmospheric N has a distinct (close to zero) $\delta^{15}N$ signal. Thus, low $\delta^{15}N$ values in seston and sediments have been interpreted as a consequence of incorporation of nitrogen from cyanobacteria N_2 fixation into the plankton, and subsequent sedimentation of plankton remains with a large fraction of N from N_2 fixation. Several studies using the stable N isotope technique indicate that N_2 fixed from the atmosphere (by cyanobacteria) is an important and even underestimated N source for the Baltic Proper. Rolff (2000) showed that during summer a depleted nitrogen isotopic signal, indicative of utilization of fixed nitrogen from cyanobacteria, was propagated through all size-classes of plankton. Based on the strength of the "N_2 fixation signal", the author drew the conclusion that N_2 fixing cyanobacteria are more important as a nitrogen source than previously assumed. Struck *et al.* (1998) calculated that in the top 5 cm of central Baltic Proper sediments 20% or 60% of the N content could be of cyanobacterial origin, depending on assumptions. However, some caution is necessary when using N isotopic signals in sediments and seston for discussions about the importance of N_2 fixation by cyanobacteria, as a low $\partial^{15}N$ signal may have other explanations than N_2-fixation (compare similar discussion for the Mediterranean Sea in Krom *et al.* (2004).

In summary, it can be concluded that there is no reliable figure for N_2 fixation in the Baltic Sea. It may in addition vary substantially between years. A lower limit is 100 kt N year^{-1}, while the upper limit is more uncertain, it could be as high as 500 kt N year^{-1}. We will use 300 kt N year^{-1} as a compromise.

2.6. Nitrogen sinks—Denitrification and burial

As shown above only small amounts of N leave the Baltic Sea in dissolved and particulate forms through the "outlet". Some 650,000 tons of fish are removed annually from the Baltic Sea through commercial fisheries, which is equivalent to only 15 kt N year^{-1} (Hjerne and Hansson, 2001). The N pool in the water masses of the Baltic Sea, some 5000 kt N, has shown decadal changes of on the order of 1000 kt N, both an increase and a decrease. However, mean changes are at present <100 kt N year^{-1} (Larsson and Andersson, 2004; Larsson *et al.*, 1985; Savchuk and Wulff, 2001; Wulff *et al.*, 1990). Neither exchange with the Kattegat/North Sea,

nor changes in the N pool in the water mass can account for any large fraction of the total annual N load on the Baltic Sea, which amounts to well over 1000 kt N year^{-1} (river + atmospheric + N$_2$ fixation, conservatively estimated to 700 (range 600–800) + 250 (range 200–300) + 300 (range 100–500) kt N year^{-1} = 1250 kt N year^{-1}). This means that internal processes, denitrification and permanent sediment burial, are of overriding importance for the fate of nitrogen introduced into the Baltic Sea and must remove some 1100 kt N year^{-1} (with outflow to the Kattegat removing 150 kt N year^{-1} and a mean net accumulation in the water mass of 0 kt N year^{-1}).

There are heavy accumulations of *Aphanizomenon* and *Nodularia* in the Baltic Sea, and these cyanobacteria need microzones with low oxygen tension to be able to fix nitrogen. However, there are no indications that such blooms are associated with denitrification (Hietanen *et al.*, 2002), even though the genetic potential for denitrification in cyanobacterial aggregates seems to be present (Tuomainen *et al.*, 2003). Denitrification is instead most likely mainly a process that occurs in above-halocline sediments, and is based on a sequence of sedimentation, ammonification, nitrification and denitrification, which is promoted by macrofaunal reworking of surface sediments.

There are surprisingly few direct measurements of denitrification in water or sediment of the Baltic Sea (Brettar and Rheinheimer, 1992; Stockenberg and Johnstone, 1997). The first attempts to quantify denitrification in the Baltic Sea were made by Rönner (1985) and by Schaffer and Rönner (1984), and were based on models/indirect calculations. These authors mainly considered denitrification below the permanent halocline in the Baltic Proper, where anoxic conditions are common. Their estimate was 410 kt N year^{-1}, with most of the denitrification taking place in the sediments. This is most probably an overestimation of denitrification below the halocline.

Most calculations of denitrification are indirect, and are based on N budgets, denitrification being the difference between input and output, when accumulation of N in the water and permanent sediment incorporation of N have been taken into account. There are also modelling approaches to denitrification (Savchuk and Wulff, 2001; Schaffer and Rönner, 1984), but assumptions made in these models have often been shown to be unrealistic Jansson (2001). In one of the earliest attempts to make a N budget for the Baltic Sea, Larsson *et al.* (1985), ended up with an unexplained net sink of 423 kt N year^{-1}, while their denitrification estimate was 470 kt N year^{-1}. The outcome of a mechanistic N model for the Baltic Proper was a denitrification sink of more than 1200 kt N year^{-1}, almost exclusively (94%) taking place in the sediments (Savchuk and Wulff, 2001). Although this figure for denitrification may be closer to the truth than the first attempt by Schaffer and Rönner (1984), the model most likely seriously underestimated permanent burial of N.

In contrast to denitrification, permanent burial of N is probably mostly taking place in sediments below the halocline. Accumulation bottoms only account for approximately 40% of the Baltic Proper, and even less in the other major basins, while transportation and erosion bottoms make up some 40% and 20% of the Baltic Sea, respectively (Carman and Cederwall, 2001). Sediment nitrogen burial in Baltic Proper offshore sediments has been estimated to 91–245 kt N year^{-1} (Jansson,

2001). Since the Bothnian Bay and Sea are less productive than the Baltic Proper, are shallower, and have a less developed stratification, burial may be low. Assuming burial to be in proportion to accumulation bottoms and half the magnitude per area compared to the Baltic Proper, the Bothnian Bay and Sea would accumulate some 16–42 kt N year^{-1}. Thus, permanent burial in the Baltic Sea would be maximum some 300 kt N year^{-1}, leaving at least 800 kt N year^{-1} for denitrification (external loading including N$_2$ fixation less losses to the Kattegat and sediment burial). Burial in inshore areas, e.g., estuaries, is unknown, but could perhaps be significant (Neumann, 2000; Witek et al., 2003). Stockenberg and Johnstone (1997) estimated benthic denitrification in the Bothnian Sea and Bothnian Bay to be 50 kt N year^{-1}. Thus most denitrification must take place in the Baltic Proper. Accepting a total denitrification of 800 kt N year^{-1} for the Baltic Sea would mean that something like 65% of the external load of N (1250 kt N year^{-1}) would be denitrified. This is high compared to estuaries, but is not incompatible with figures for lakes (Seitzinger, 1988). Denitrification in the Baltic Proper could be proportionally higher than in estuaries and most lakes due to the longer residence time of nitrogen in the Baltic Sea compared to the more rapidly flushed lakes and estuaries. Another way to check if estimated total denitrification in the Baltic Sea is reasonable, is to calculate what rate is necessary per area to eliminate 800 kt N year^{-1}. Discounting denitrification in the Bothnian Bay and Sea, 750 kt N year^{-1} would remain. The Baltic Proper (excluding the Kattegat) covers 270,000 km^2. Denitrification would thus have to be at least 3 t N km^{-2} year^{-1} (3 g N m^{-2} year^{-1}, 0.2 mol N m^{-2} year^{-1}), which is compatible with values measured in other systems (Seitzinger, 1988; Seitzinger et al., 1984).

An important but unresolved question is if eutrophication, with increased below-halocline anoxia, will promote or reduce denitrification in the Baltic Sea (Rönner, 1985). In the latter case there would be more N available for phytoplankton and eutrophication would be a self-accelerating process. If the water below the halocline is totally anoxic, denitrification will stop and ammonia accumulate. With ample oxygen and an increased macrobenthic biomass (which has been one of the consequences of eutrophication of the Baltic Sea) above the halocline, denitrification should be promoted. Denitrification increases with increasing ecosystem production, and most likely faster than the increase in production. Thus denitrification would be very low in an ultraoligotrophic system (Krom et al., 2004), but would eliminate an increasing proportion of the external N load when the system undergoes eutrophication. N$_2$ fixation will never keep pace with denitrification, and the system will appear more and more N-limited. This gradient is clearly seen in the Bothnian Bay—Bothnian Sea—Baltic Proper (see also CONCLUSIONS).

2.7. A nitrogen budget for the baltic sea

From the above discussion of the different fluxes of N to and from the Baltic Sea (excluding the Kattegat), as well as N pools in the Baltic Sea, it can be concluded that (Fig. 15.5):

(1) Total loading (including N$_2$ fixation) has in this review been estimated to 1250 kt N year^{-1}, which is not very different from earlier estimates: Larsson
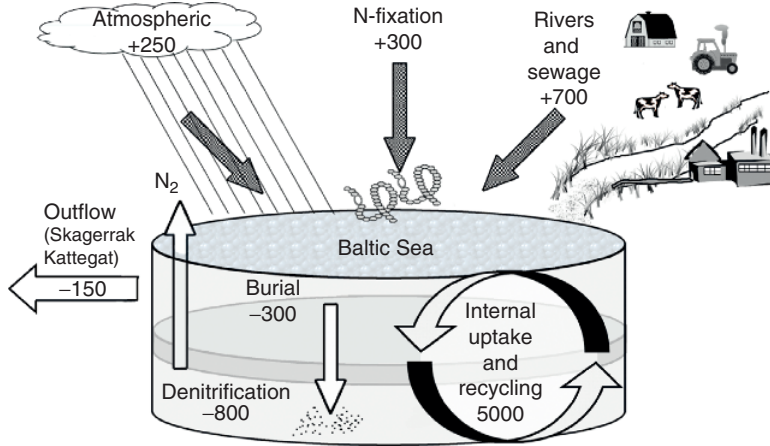
Figure 15.5 A nitrogen budget for the Baltic Sea (excluding the Kattegat) in kt N ton year^{-1}.

    et al. (1985) 1200 kt N year^{-1}, Enell and Fejes (1995) 1300 kt N year^{-1},
    Grimvall and Stålnacke (2001) 1250 kt N year^{-1} (corrections made for the
    Kattegat).
(2) While the external loading of nutrients shows a huge excess of N in relation to
    the Redfield ratio, by internal adjustments (large sinks for N but relatively
    smaller for P) the N/P ratio in the water masses (of the Baltic Proper) is well
    below the Redfield ratio, especially for DIN/DIP.
(3) The residence time of N in the Baltic Sea system (including water and
    sediments) is short (5 years) compared to the hydraulic/salt residence time
    (25 years) and is also much shorter than for phosphorus (>10 years). The
    Baltic Sea thus has a much longer "memory" for P than for N, and a change in
    N loading will affect N in the Baltic Sea much faster than a corresponding
    change in P loading. However, this would not be valid if changes in external
    N loading are compensated by changes in the balance between N$_2$ fixation
    and denitrification (at present not known).

2.8. Nitrogen versus phosphorus—Limiting nutrient controversy

Limnologists have for at least 50 years maintained the view that phosphorus is the
ultimate limiting nutrient for lake ecosystem production, while the nitrogen hypoth-
esis has dominated for marine systems since Ryther and Dunstan (1971). The P
regulation theory states that shortage of nitrogen will ultimately be compensated
through N$_2$ fixation, and N$_2$ fixers will be limited by phosphorus. This could create
the situation that phytoplankton growth/biomass accumulation is N limited (time
scales of days–weeks), while ecosystem net production is P limited (time scale of
months—years). What is the situation in the Baltic Sea, which is brackish, and thus
neither a lake, nor a marine system? The Baltic Sea is composed of several distinct
basins, with rather different nutrient conditions. The northernmost Bothnian Bay is
most lake-like, with very low salinities and high N/P ratios, receiving mostly

unpolluted river water, due to limited human population and agricultural activity in the drainage area. Sediments in the Bothnian Bay are more likely to retain P than in the more southerly basins, due to good oxygen conditions, low SO_4 and favourable Fe/P ratios. The Baltic Proper, which receives most of the anthropogenically derived external loading of nutrients, is characterized by very low inorganic N/P ratios, both above and below the halocline (Fig. 15.4). Here algal growth and maybe also ecosystem net production seems to be N limited. Is this a ''natural'' or a secondary phenomenon, and is really N the ultimate limiting nutrient? N limitation could, as is the case in hypertrophic lakes, be a secondary phenomenon, caused by low N/P ratios in sewage, internal loading of P from the sediments and loss of N through denitrification. On the other hand, sediment records show clear signs of deep-water anoxia and cyanobacterial blooms thousands of years ago (Bianchi et al., 2000), indicating that low N/P ratios may be a natural phenomenon in the Baltic proper. The question of N versus P limitation has haunted Baltic ecologists and environmental managers for at least 2 decades and it still remains unresolved.

Bioassay and mesocosm experiments, as well as physiological indicators (phosphatase activity) usually point strongly to N limitation of algal growth rate and biomass accumulation in the Baltic Proper (Granéli et al., 1990), except that cyanobacteria may be P limited (Moisander et al., 2003; Rydin et al., 2002). In the oligotrophic, low-saline Bothnian Bay, enrichment experiments point to P limitation (Hagström et al., 2001), in accordance with the high N/P ratio in the water (Wulff et al., 2001). These experimental studies also show that N_2 fixation can be greatly enhanced by P addition (Moisander et al., 2003; Rydin et al., 2002). However, N_2 fixing cyanobacteria are not present during the spring bloom, when the winter store of DIN is totally consumed, leaving some excess DIP. The spring bloom is thought to contribute proportionately more to net sedimentation (new production) and O_2 consumption than summer primary production based on regenerated N (Elmgren and Larsson, 2001).

P loading has most likely increased more than N loading, may be 8 versus 4 times the loading that existed a century ago (Larsson et al., 1985). This could have accentuated N limitation. On the other hand the N/P ratio in the Baltic Sea is not primarily a function of the N/P ratio in external loading, which is still high in comparison to the Redfield ratio (HELCOM, 2004; Wulff et al., 2001). The change in N/P ratio is thus caused by internal processes, that force P to remain in the water, while N is lost through denitrification and permanent burial (Wulff et al., 2001). Caraco et al. (1989) have shown that the ability of lake sediments to retain P is related to sulphate concentration and Blomqvist et al. (2004) have launched the theory that N limitation in brackish waters, in contrast to P limitation in lakes, can be explained by an unfavourable Fe/P ratio in brackish/marine shallow systems, due to formation of iron sulphides in anoxic sediments. There may be other explanations too, e.g., that the Baltic Sea has an unusually efficient denitrification, due to its shallowness (Larsson, 1988). A similar case has been made for the Adriatic Sea in contrast to the Eastern Mediterranen, where denitrification seems to be very low (Krom et al., 2004).

It is very difficult to make experimental tests of the hypothesis that net marine ecosystem production is N limited, in the same way as the lake ecosystem P limitation hypothesis has been tested through whole lake fertilization experiments

(Schindler, 1977). In this respect the Himmerfjärden experiments are unique. External N and P loadings, as well as ratios between these, have been manipulated in a controlled way for a semi-enclosed bay (Himmerfjärden) of the Baltic Proper, receiving substantial amounts of tertiary (both N and P removal) treated sewage (Elmgren and Larsson, 1997, 2001; Granéli *et al.*, 1990). These full–scale experiments showed that chlorophyll and algal biomass in the bay were reduced when N in sewage was reduced.

2.9. Management of nitrogen

Because P may regulate the growth of cyanobacteria, it has been argued that P reduction is important, even if N regulates overall ecosystem production (Elmgren and Larsson, 2001). However, during the last decades focus has changed somewhat from P to N management with respect to Baltic Sea eutrophication. Many sewage treatment plants have been equipped with N stripping facilities, even though most anthropogenic N entering the Baltic Sea is diffuse, emanating from agriculture (NH_4 and NO_3) and transportation/industry (NO_x). Politically set goals to half the anthropogenic load of N to the Baltic Sea in 10 years have been decided in different Baltic countries, as well as by multinational protocols. These N reduction goals were ambitious/optimistic and have not nearly been reached.

   Savchuk and Wulff (2001) modelled effects from a drastic reduction in P or N (50% each 5 years) alone or in combination. The model showed that P reduction alone in 20 years caused a reduction in primary production and N_2 fixation of approximately 30%. If both N and P were reduced, primary production was similar to the P alone case, whereas N_2 fixation increased initially. N reduction alone had no effect on primary production and caused a strong (40–50%) increase in N_2 fixation. Eilola and Stiegebrandt (1999), using a biogeochemical reactor model, came to the conclusion that DON would compensate for N deficiencies and that N thus is not limiting at the systems level. Hence they concluded that P reduction would be the only effective remedy for the Baltic Sea. Unfortunately such models are based on oversimplified biological assumptions and there is no way at present to judge the realism in the produced forecasts. Elmgren and Larsson (2001) advocated both N and P reductions, based on the assumption that P regulates N_2 fixation/cyanobacteria, while N regulates the spring bloom and thus oxygen consumption in deep water. Their arguments are based on empirical observations, e.g., the occurrence of cyano–bacteria blooms (based on paleoecological evidence) and N limitation for several thousand years in the Baltic Proper, as well as results from systems scale nutrient manipulations in the Himmerfjärden Bay (Elmgren and Larsson, 1997, 2001).

3. Comparisons with Other Enclosed Seas

   It is particularly instructive to compare the Baltic Sea with the Mediterranean Sea. The two inland seas of course differ with respect to size (area and depth) and climate, but of more principal interest are the differences in exchange with the open

ocean, freshwater balance, sill depth, river versus atmospheric N loading, nutrient status and nutrient limitation.

While the Baltic Sea is characterized by a net outflow of freshwater, and thus has a typical estuarine circulation, in the Mediterranean Sea evaporation exceeds rain plus river water input. This means that there is a net inflow of Atlantic water through the Strait of Gibraltar and that salinity in the Mediterranean Sea as a mean is higher than in the Atlantic Ocean. In the Baltic Sea there is an inflow of saltier water from the Kattegat/Skagerrak at depth, while freshened water flows out in a surface layer. In the Mediterranean Sea, on the contrary, saltier water leaves as a deeper current through the Strait of Gibraltar. This can be called inverse estuarine (Crispi *et al.*, 2001) or Mediterranean type circulation. There is also another difference: The Baltic Sea has shallow connections (the Sound and the Danish Belt Sea) to the Kattegat/Skagerrak (18 m), while the Gibraltar Sound is much deeper (300 m). This means that nutrients will get trapped in the Baltic Sea, while nutrients will be flushed out of the Mediterranean Sea (Wulff *et al.*, 1990). As a consequence the Baltic Sea has to some degree always been more eutrophic than the open ocean (cyanobacteria blooms thousands of years ago), and has become even more eutrophic due to nutrients of anthropogenic origin being trapped. The Mediterranean Sea (with the exception of smaller basins like the Adratic Sea) on the other hand is less nutrient rich than the Atlantic Ocean to which it is connected, and is in fact one of the more pronounced ocean deserts on the globe. There is a west–east gradient in the Mediterranean Sea, the Eastern Mediterranean being ultra oligotrophic with extreme Secchi depths (Berman *et al.*, 1985).

There are several mechanisms contributing to the nutrient poor status of large parts of the Mediterranean Sea and especially the Eastern Basin. There is e.g., an unbalanced fresh water inflow, which has become even more accentuated after damming of the Nile. Most river water enters in the west, leaving the eastern part without riverborne nutrients and far from the more nutrient rich Atlantic water entering through the Strait of Gibraltar. It has also been suggested that there is a ''biological pump'' transporting N to deep water through sedimentation and thus causing a successive impoverishment of the Mediterranean surface water moving eastwards (Crispi *et al.*, 2001).

In comparison to calculations of river input of nutrients to the Baltic Sea (which still after decades of efforts are not definite), similar figures for the Mediterranean Sea are very uncertain. The drainage basin of the Mediterranean Sea is to a large extent arid, and river discharge has diminished due to water use for irrigation etc. This is especially evident with respect to the Nile. However, this does not necessarily mean that total riverine and coastal point source loadings have decreased, since increases in N concentrations in river water have most likely more than compensated a decrease in runoff. UNEP (1997) has estimated the riverine N input to the Mediterranean Sea to 1000 kt N year^{-1}, and the atmospheric deposition is almost identical. Total N loading (river + atmosphere) is thus for the Mediterranean Sea $\approx$2000 kt N year^{-1}, while the figure for the Baltic Sea, excluding N$_2$ fixation, is $\approx$1000 kt N year^{-1}, thus half the Mediterranean value. However, the Baltic Sea is much smaller (0.37 $\times$ 10^6 km^2) than the Mediterranean Sea (2.52 $\times$ 10^6 km^2), which means that the external N loading per km^2 sea surface is several times higher for the Baltic Sea. Although

new figures for the Eastern Mediterranean (Krom *et al.*, 2004) indicate that the N loading on the Mediterranean may be substantially larger than the (UNEP, 1997) estimate, it is still low compared to the Baltic Sea. However, the difference in eutrophication between the Baltic Sea and the Mediterranean Sea is probably not only related to nutrient loading per unit area, but has also to do with differences in limiting nutrients and in nutrient exchange with the outside.

Since the Mediterranean Sea is surrounded by rather dry areas, atmospheric input of nutrients in dust is significant. In the SE Mediterranean (Herut *et al.*, 2002) calculated that dry fallout supplied 2.5 times more DIN than wet deposition and 2 times more than riverine N input (see also Krom *et al.*, 2004). Also for P, dry atmospheric input was a dominating source, and since atmospheric deposition has a high N/P ratio (Markaki *et al.*, 2003) this means that in the eastern Mediterranean water the N/P ratio is very high compared to the Redfield value (Krom *et al.*, 2004). In the ultraoligotrophic eastern Mediterranean atmospheric N deposition was several times higher than export production (as annual PON flux). Thus N does not seem to limit phytoplankton production, a conclusion supported by bioassay experiments (Diaz *et al.*, 2001). Atmospheric nutrient inputs to the Baltic Sea, as is the case for the Mediterranean Sea, have high N/P ratios and for the Baltic Sea also riverine N/P ratios are well above the Redfield ratio. Nevertheless N/P ratios in the water are very different in the two inland seas: markedly above 16:1 in the Mediterranean Sea and well below in the Baltic (Proper). This difference is due to internal sinks and sources. In the Baltic Sea the sink has a very high N/P ratio due to denitrification but not very efficient P binding to sediments. For the Mediterranean Sea the sink must have a low N/P ratio, indicating inefficient denitrification (Krom *et al.*, 2004) and/or efficient P sequestering to particulate material. The reason behind this may have to do with oxygen conditions and organic carbon availability. Since the Mediterranean Sea is so nutrient-depleted, denitrification may be carbon limited, while low production, great mean depth (1470 m) and absence of the strong density stratification characteristic for the shallow Baltic Sea, prevent reducing conditions that would liberate P from sediments.

In the Baltic Sea N_2 fixation is of great significance, both because the N_2 fixers (colonial cyanobacteria) are dominating components of the plankton community, and because N_2 fixation is an important (10% to >30%) source of N for phytoplankton. That N_2 fixation is a significant fraction of the N input to the Baltic Proper has been explained by low N/P ratios in the water and high P availability. This would suggest that N_2 fixation should be low in the Mediterranean Sea, because N/P ratios are high, which would select against N_2 fixing cyanobacteria, and because P is very low, which would also decrease the potential for N_2 fixation. However, $\partial^{15}N$ values of PON and NO_3 in the Mediterranean Sea on the contrary indicate that biological N_2 fixation is an important source of new N, especially in the eastern basin (Pantoja *et al.*, 2002). This contradicts Markaki *et al.* (2003), who suggested that atmospheric deposition would be more than adequate to cover the demand of new N for export production. On the other hand losses of N through the Strait of Gibraltar are higher than atmospheric plus riverine N inputs, suggesting the need for N_2 fixation to balance the deficit (Pantoja *et al.*, 2002). According to these authors up to 20% of N in the western Mediterranean Sea, and as much as 90% in the eastern basin, may be

derived from N_2 fixation. However, Krom *et al.* (2004) have argued that N_2 fixation is insignificant in the Eastern Mediterranean, and that low $\delta^{15}N$ values have other explanations than N_2 fixation, a conclusion consistent with the Mediterranean as a P limited inland sea.

4. Conclusions and Future Research Prospects and Needs

Nitrogen sources and sinks in the Baltic Sea are fairly well identified and quantified compared to other inland seas. However, there are serious uncertainities in the estimations of N_2 fixation, denitrification and N burial. Further, the influence of P on N and vice versa are not well understood, preventing us from making realistic forecasts of future conditions in the Baltic Sea under various N loading scenarios. There are still questions as to what has been most important for eutrophication, the increase in P or N or both, and when changes took place. However, most serious is our limited understanding of how the Baltic Sea system will react to reductions in N loading and how long it will take to reach a new equilibrium. In deeper, large lakes (e.g., Lake Vättern, Lake Washington, the Great Lakes) rather drastic reductions in external P loading have resulted in remarkably increased transparency and changes in nutrient concentrations, while in shallow lakes the sediment P memory may delay recovery for decades through internal P loading.

A comparison between contrasting marine ecosystems, e.g., the Mediterranean Sea and the Baltic Sea, with respect to primary production/Secchi depth, N_2 fixation, N/P ratios in the water (N vs P limitation of phytoplankton growth) and denitrification, can be instructive (Fig. 15.6). The N/P ratio in external loading is probably always much greater than the Redfield ratio (for most aquatic ecosystems), whether loading is mainly through atmospheric deposition or through riverborne N. However, the N/P ratio in the water is usually below the loading ratio. In an extremely oligotrophic system this difference is smaller than in a very productive/eutrophic system. In the Mediterranean Sea the N/P ratio is thus ≈ 30 in the water, compared to 16 in the open ocean and <10 in the Baltic Proper. In the Mediterranean Sea both N_2 fixation and denitrification are probably low, while in the Baltic Sea both processes contribute to a large fraction of N sources and sinks.

With increasing primary production denitrification increases out of proportion to nitrogen loading (atmosphere + riverborne). N_2 fixation will not increase proportionally to denitrification, driving the N/P ratio in the water to sub-Redfield values and creating a nitrogen "gap" and N limitation of phytoplankton growth. This process is reinforced through decreasing P immobilization by sediments with increasingly more eutrophic conditions. The same phenomenon can be observed in a gradient from ultraoligotrophic to hypertrophic lakes. The residence time of N in the system is obviously of importance. Here inland seas form a transition from estuaries to major oceans, making them very valuable model systems. There should
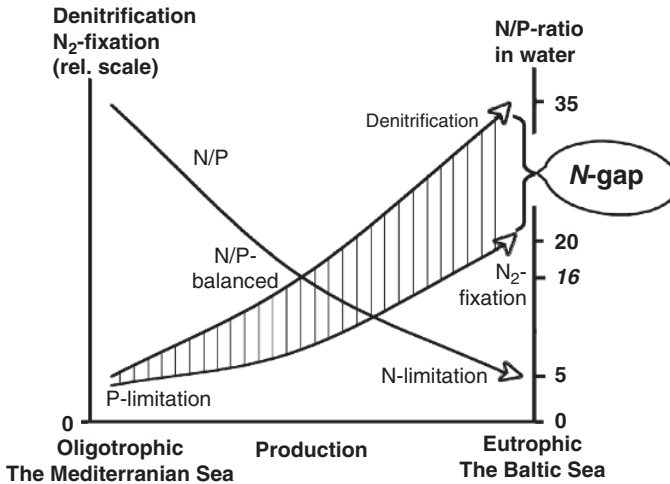
Figure 15.6 Schematic picture of a gradient of Inland seas or Lakes from ultraoligotrophic to hypertrophic. As production increases, denitrification increases, and N_2 fixation cannot close the N–gap, causing the N/P ratio to decrease and phytoplankton to appear N– instead of P-limited. External N/P-loading ratio (atmospheric deposition, riverborne load) is always much greater than Redfield ratio.

be much more to be learnt from studies of N in inland seas, especially if more systems were as in depth investigated as the Baltic Sea.

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> CHAPTER 16

THE NITROGEN CYCLE IN THE NORTH PACIFIC TRADES BIOME: AN EVOLVING PARADIGM

David M. Karl, Robert R. Bidigare, Matthew J. Church, John E. Dore, Ricardo M. Letelier, Claire Mahaffey, *and* Jonathan P. Zehr

Contents

> # 1. PROLOGUE

The systematic transformation of nitrogen (N) from one form to another is referred to as N-cycle. This cycle is a key component of the much larger and interconnected hydrosphere-lithosphere-atmosphere-biosphere N-cycle of planet Earth (Boyer and Howarth, 2002; Galloway *et al.*, 2004; Karl and Michaels, 2001). Because N is a macronutrient required for the growth of all living organisms, the marine N-cycle is inextricably coupled to the production and decomposition of organic matter on both regional and global scales. Consequently, most N-cycle processes are coupled both to the flow of energy and to other bioelement cycles, most notably carbon (C), hydrogen (H), oxygen (O), phosphorus (P) and sulfur (S), as well as to the global cycles of many trace elements (e.g., iron, zinc, cobalt, copper,

cadmium, to name a few) (see Hutchins and Fu, Chapter 38, this volume). While this chapter and the volume that it is part of focus specifically on N, it is prudent to broaden the perspective and to acknowledge these critical nutrient element inter-connections and well established metabolic interdependencies.

In open ocean marine ecosystems, N-cycle processes are driven almost exclusively by the metabolic activities of microorganisms, especially *Bacteria* and *Archaea*. Some abiotic N transformations can occur (e.g., photolytic alteration), but these are quantitatively negligible in open ocean ecosystems. Microorganisms require N as a nutrient source, but the redox potential of some N-containing substrates also provides an energy mediated pathway whereby microbes intersect the N–cycle (Fig. 16.1). The microbial transformations between these redox end-members and the intermediate oxidation states of the other stable N species are either energy-requiring (N reductions) or energy-yielding (N oxidations), and thus they have important metabolic and ecological consequences. In this regard, nitrite (NO_2^-) is a key redox intermediate (Fig. 16.2) even though NO_2^- concentrations and turnover rates are rarely measured in the field.
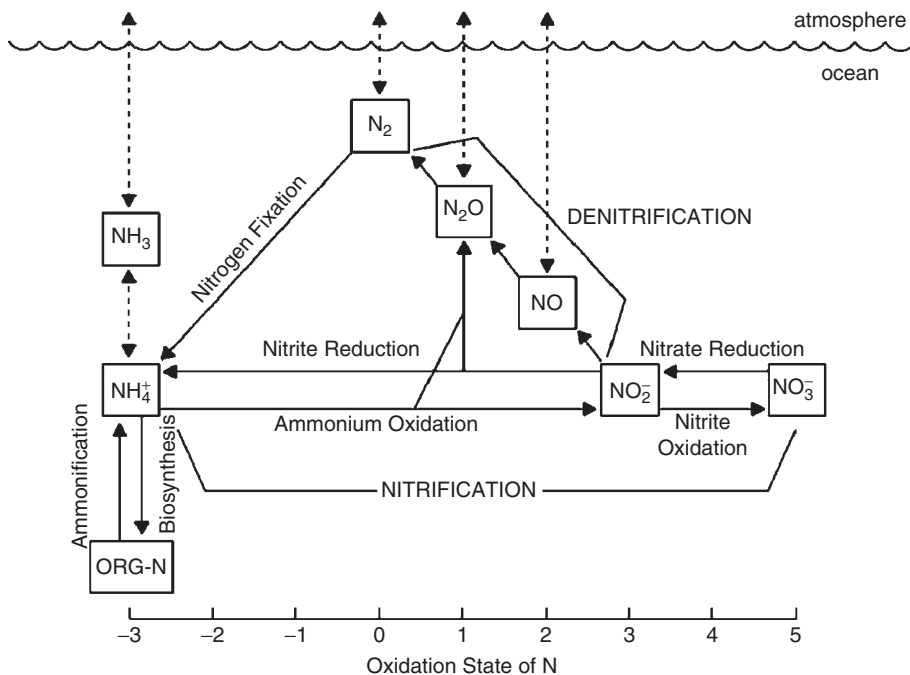
Figure 16.1 Schematic view of the marine N-cycle showing major pools and transformations. Shown on the bottom is the oxidation state of N in each of the major pools. Changes in the valence state of N require (reductions) or release (oxidations) energy and are often coupled to metabolism. Adapted from Capone (1991) and Karl and Michaels (2001). ORG-N = organic N; NH_2OH, not shown, is the precursor for NH_4^+ oxidation to N_2O.
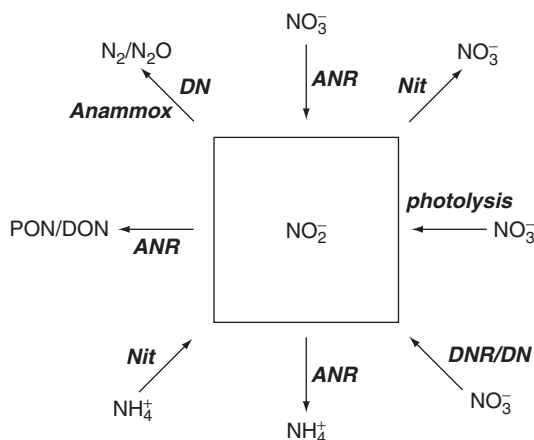
Figure 16.2 The redox intermediate, nitrite (NO_2^-), occupies a central position in the marine N-cycle. Shown are the various processes that are either sources or sinks for NO_2^-. Abbreviations include (clockwise from top): ANR = assimilatory nitrate reduction, Nit = nitrification, photolysis = UV-driven photocatalysis, DNR/DN = dissimilatory nitrate reduction/denitrification, ANR and Nit = as above, Anammox = Anaerobic ammonium oxidation.

Some dissolved N species are transported great distances via ocean circulation, while others are locally generated and short-lived due to rapid metabolic turnover. Some N-containing molecules are gaseous thereby linking them to the atmosphere via thermodynamically driven air-sea exchange reactions. In fact, the most abundant form of N in the sea is dissolved gaseous dinitrogen (N_2), which accounts for more than 95% of the total N inventory in seawater (Table 16.1). The relative stability of the triple bond of N_2 ($N{\equiv}N$) renders this form of N nearly inert to most organisms. However, selected prokaryotes can reduce N_2 to NH_4^+ (a process called N_2 fixation), thereby enriching the ecosystem with a form of fixed N that is broadly available to most, if not all, microbes. In addition, fixed N can be converted back into N_2 via denitrification and anaerobic ammonium oxidation (anammox), leading to a loss of bioavailable N from those habitats unless local rates of N_2 fixation exceed denitrification plus anammox rates. This mobilization of N_2 gas does not directly depend upon ocean mixing. Therefore, both N_2 fixation and denitrification can decouple N from other bioelement cycles which depend on ocean mixing; this decoupling has potentially important biogeochemical implications (Gruber, 2004, Chapter 1, this volume).

Organic N is an important component of the marine N-cycle (Figs.16.1 and 16.3A and B; Table 16.1) (see Aliwahri and Meador, Chapter 3, this volume). In the marine environment, organic nitrogen exists in a more or less continuous spectrum of molecular and particle sizes from simple low molecular weight (LMW) compounds (e.g., urea and amino acids) through more complex high molecular weight (HMW) ''dissolved'' species (e.g., protein, colloids and gels) to true particles ranging

Table 16.1 Representative N Inventories for Open Ocean, Trades Biomes and Selected Production–Consumption Pathways

Substrate pool	Representative concentrations	Possible production pathways
Gaseous N		
N_2	0.4–0.5 mmol l^{-1}	Denitrification (including anammox), air–sea exchange
N_2O	5–10 nmol l^{-1}	Nitrification, denitrification, air–sea exchange
"Fixed" DIN		
NO_3^-	0.1–10 nmol l^{-1}	Nitrification, lightning, precipitation
NO_2^-	<1 nmol l^{-1}	Nitrification, assimilatory and dissimilatory nitrate reduction, photolysis
NH_4^+	1.5–30 nmol l^{-1}	Ammonification, biological N_2 fixation, precipitation
"Fixed" DON		
Total pool	5–6 μmol l^{-1}	Cell death/autolysis, excretion-exudation, grazing, viral lysis, biological N_2 fixation, hydrolysis of PON
– Combined amino acids	0.10–0.50 μmol l^{-1}	
– Free amino acids	1–10 nmol l^{-1}	
– ATP	50–150 pmol l^{-1}	
– DNA	0.2–4 μg l^{-1}	
– Urea	0.05–0.20 μmol l^{-1}	
Particulate N		
Total PON	0.3–0.5 μmol l^{-1}	Auto- and heterotrophic cell production and death, grazing, viral lysis, molting, condensation of DON
– DNA	2–5 μg l^{-1}	
– ATP	40–60 pmol l^{-1}	
– Biomass	0.1–0.3 μmol l^{-1}	
– Chlorophyll	0.05–0.15 μg l^{-1}	

Concentrations are all for near-surface waters (0–50 m).

from submicron (e.g., viruses, bacteria and some non-living particles) to meters or more (e.g., pelagic fishes, squids and whales). Typically, the operational boundary between LMW and HMW dissolved organic N (DON) is 1000 Daltons (~1 nm) and the operational distinction between DON and particulate organic N (PON) is operationally defined, for example if microfine glass fiber filters are used then the boundary is approximately 0.5–1.0 μm. For this reason, "dissolved" organics as reported in the scientific literature may also contain some small particles (Table 16.1). DON is just one subcomponent of the much larger dissolved organic matter (DOM) pool in seawater. DOM contains a broad spectrum of organic molecules, only some of which contain N. Isolation of LMW- and HMW-DOM combined with isotopic (^{15}N, ^{13}C, ^{14}C), structural and biochemical characterization can yield insights into the age, lability and compartmentalization of N within this large organic N pool (Benner *et al.*, 1997; Loh *et al.*, 2004; Meador *et al.*, 2007).

1.1. The North Pacific trades biome

The marine environment is large by any worldly measure. For more than a century, oceanographers have recognized that there are predictable physical and chemical properties, and biotic communities that vary systematically from near-shore environments to the oceanic realm and from the equator to the poles. This has led to a recognition and definition of specific biogeographical provinces, or biomes, within which processes can be scaled in time and space after adequate representative sampling (Longhurst, 1998). With the advent of Earth-orbiting satellites designed to measure key ocean parameters such as sea surface temperature, topography, winds, light and color—the latter as a surrogate of photoautotrophic community biomass—a wealth of information on global ocean environmental conditions as they relate to the ecological geography of the sea now exists. Based on these criteria, there
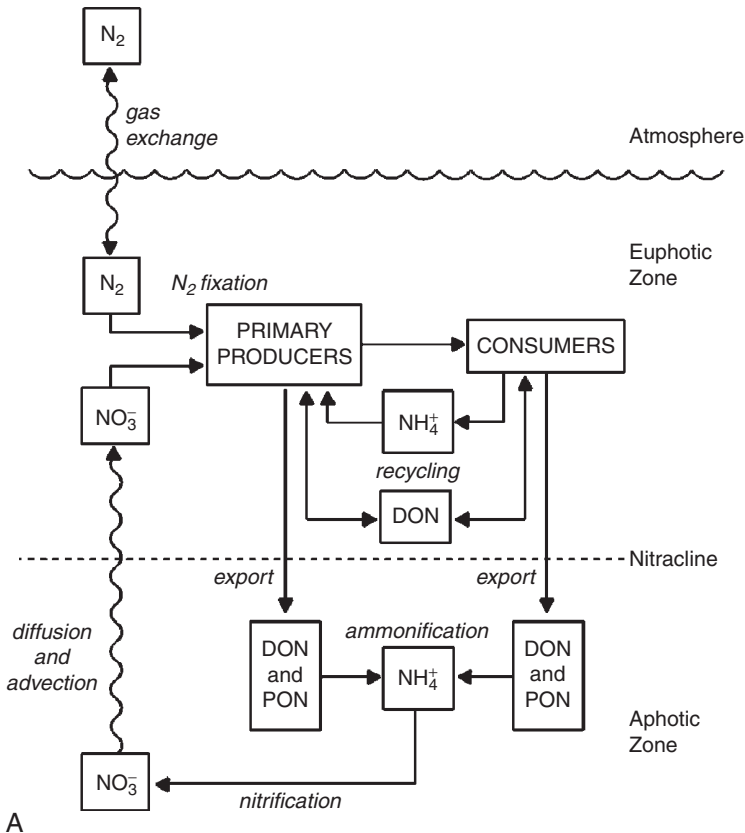
Figure 16.3 Microbial food web processes sustain the marine N-cycle in the North Pacific trades biome. Shown are: (A) a schematic view of the various sources, transformations and sinks for key N pools and

(Continued)

N Resource Partitioning in the NPSG
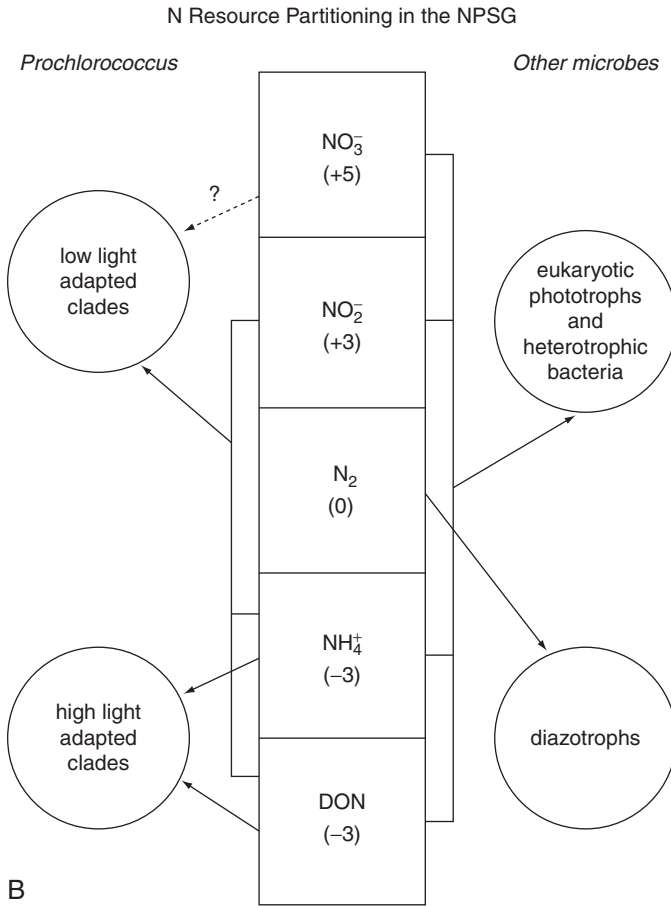
Figure 16.3 cont'd (B) a hypothetical microbial N-resource partitioning in the euphotic zone among the various N pools and components of the microbial assemblage.

appear to be four primary biomes in the ocean: a coastal boundary biome and three oceanic zones (polar, westerlies and trades; Longhurst, 1998). Biological processes in both the polar and westerlies regions are characterized by strong seasonal cycles that are established by changes in upper water column stratification and light. As the mixed-layer shoals above a critical depth, a vernal phytoplankton bloom results (Siegel *et al.*, 2002; Sverdrup, 1953), leading to a temporal pulse of carbon and energy through the food web and export of particulate matter to the deep sea. The N-cycle in these seasonally forced biomes is characterized by two distinct phases. At the start of the spring bloom, NO_3^- is present in high concentrations and reduced forms, including NH_4^+ and DON, are low. As the mixed-layer becomes NO_3^- limited for phytoplankton growth because of net uptake in early summer, the system evolves into a lower biomass, nutrient-limited system fueled primarily by locally regenerated NH_4^+ and DON.

In contrast, the trade wind regimes support an N-cycle with fundamentally different dynamics than those observed in the polar and westerlies biomes. A key feature of the physical habitat in the trades biome, which extends from approximately 30°N to 30°S in each ocean basin and collectively represents about 45% of the total area of the ocean, is the presence of a seasonally stable pycnocline/thermocline caused by the radiation balance of positive downward heat flux across the sea surface. The dynamic topography of the North Pacific Ocean reveals systematic basin-scale variations that drive the large scale anticyclonic (clockwise) circulation of water around the core of the trades biome at approximately 20°N (Fig. 16.4A). The anticyclonic circulation effectively isolates the upper portion of the water column from large volume water exchange with the bordering current systems. This stratification leads to a permanent vertical separation of light (above) and nutrients (below), and results in a condition of extreme oligotrophy in near-surface waters including low nutrient flux, low standing stocks of particulate matter, low net rates of organic matter production and low rates of export (e.g., Fig. 16.4B). These are characteristic features of the trades biomes worldwide.

The dynamics of the N-cycle in the trades biomes is distinct from those in other oceanic regions because gross primary production is supported largely by locally recycled NH_4^+ and DON, with a subsidy via local N_2 fixation and stochastic nutrient entrainment events. Due to the rapid and efficient recycling of most N and associated bioelement pools, these oligotrophic regions sustain high N turnover rates despite low ambient pool concentrations and PON standing stocks. The stable vertical structure present in trades biomes may facilitate depth-dependent niche specialization and support the co-existence of a diverse microbiota that contribute to unique metabolic and ecophysiological processes that in many ways epitomize the marine N-cycle (Fig. 16.3B).

1.2. The "new" versus "regenerated" nitrogen paradigm

In 1967, Dugdale and Goering formalized their now unifying concept of new and regenerated primary production of organic matter in the sea (Fig. 16.5). In their model, new production was defined as that portion of total primary production that was supported by allochthonous N sources such as upwelled NO_3^- or locally fixed N_2. Regenerated production, which typically ranges from 70 to 90% of total production in most open ocean systems, was defined as that portion of total primary production that was supported by NH_4^+ or DON. They assumed that total N assimilation could, as a first approximation, be treated as the sum of NH_4^+ plus NO_3^- uptake, and that these two rates could be quantitatively measured in separate but simultaneous incubation experiments (a few hours to 1 day in duration) following the addition of the appropriate ^{15}N-labeled substrate. While the uptake of $^{15}NH_4^+$ could be equated to locally regenerated N, the uptake of $^{15}NO_3^-$ represented N that was imported to the euphotic zone by upward advection and diffusion. In their presentation, they were very explicit on several important issues including a warning that if local nitrification or local N_2 fixation were later found to be important processes in the regional N-cycle of interest, then there would be a need to reconsider the model assumptions. N_2 fixation and nitrification fundamentally alter the
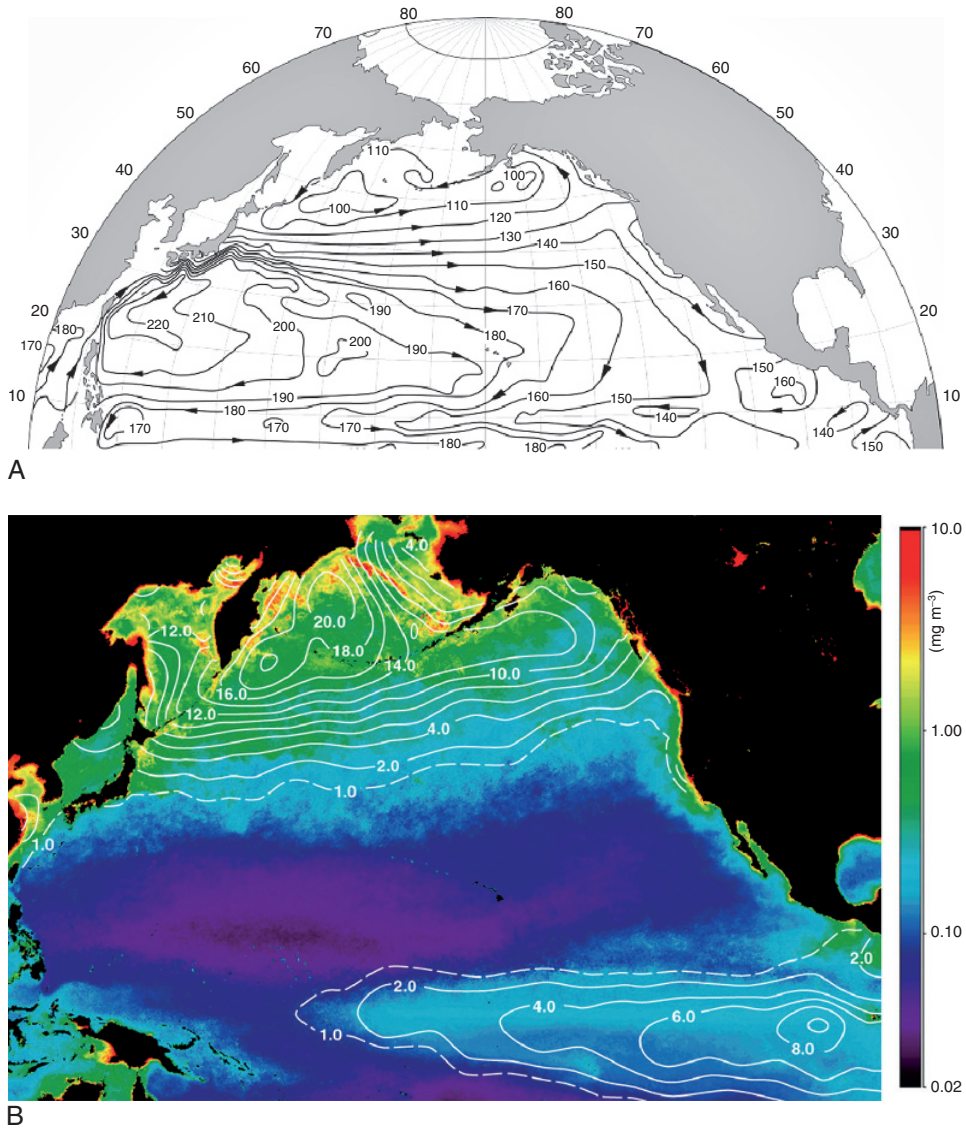
Figure 16.4 Map of the North Pacific Ocean basin showing several important features of the trades biome. (A) Dynamic topography of the sea surface in dyn-cm relative to 1000 dbar based on historical hydrographic observations. Arrows show the direction of geostrophic flow. From Wyrtki (1975). (B) Sea surface distributions of chlorophyll (mg m^{-3}) for the Pacific Ocean in 2003 from 15°S to 65°N latitude as derived from the AQUA MODIS satellite-based sensor system (4 km resolution). Superimposed on ocean color, in white contour lines, is the mean annual surface nitrate concentration (mmol NO$_3^-$ m^{-3}) based on the World Ocean Atlas (2001) Ocean Climate Laboratory/NODC. Areas of high NO$_3^-$ (and presumably NO$_3^-$ flux) correspond to areas that are enriched in chlorophyll as a result of net plant growth. The North Pacific trades biome is the central region of low standing stocks of plants (<0.1 mg m^{-3}; blue–purple areas) and low ambient NO$_3^-$ concentrations (<1 mmol m^{-3}).
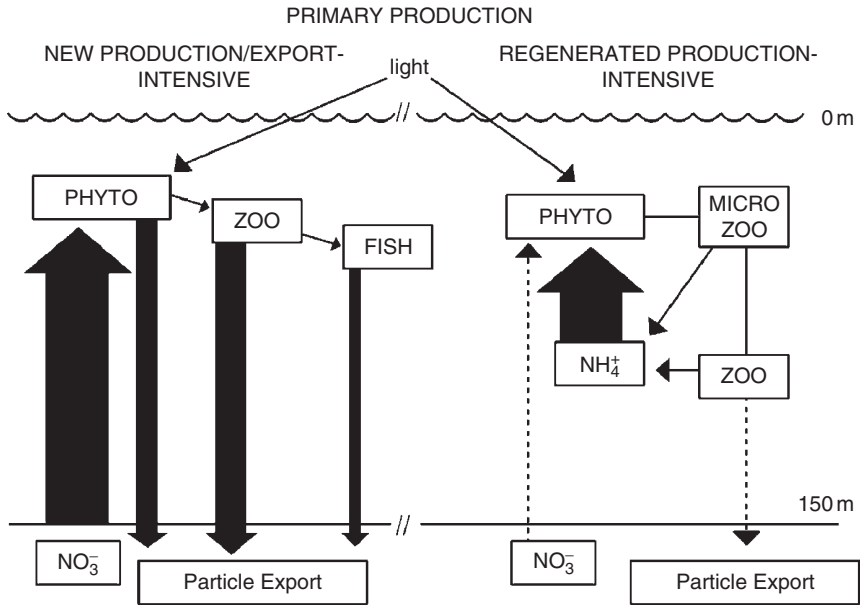
Figure 16.5 A conceptualized view of the new versus regenerated N model based on the classic work of Dugdale and Goering (1967). Shown are two contrasting marine ecosystems: (Left) an upwelling habitat where allochthonous NO_3^--supported new production dominates total primary productivity, and (Right) an open ocean habitat where locally produced NH_4^+-supported regenerated production dominates total primary productivity. New production-intensive biomes also support much greater export per unit area, usually in the form of sinking particulate matter, than remineralization-intensive systems like the North Pacific trades biome.

conceptual views of new (i.e., NO_3^-) vs. regenerated (i.e., NH_4^+) sources of N. For example, new NH_4^+ excreted during the growth of N_2 fixing microorganisms freely mixes with the pool of regenerated NH_4^+, and regenerated NO_3^- formed locally via nitrification and becomes indistinguishable from truly new NO_3^- imported into the region from allochthonous sources. In addition to the advective-diffusive fluxes of NO_3^- from beneath the euphotic zone and N_2 fixation, new N for the North Pacific trades biome is also derived from continental sources transported great distances as both NO_3^- aerosols and in association with Asian dust.

The new vs. regenerated N conceptual framework led to a convenient field protocol wherein paired incubations using ^{15}N–NH_4^+ and ^{15}N–NO_3^- were used to estimate the fraction of total N assimilation that is supported by new N, the so-called "f-ratio" (Eppley and Peterson, 1979). On less frequent occasion, investigators also included an estimate of the uptake of DON using a "model" compound such as urea. However, it is impossible to relate the uptake of this single compound to the potential assimilation of the entire DON pool which has not yet been fully characterized (Hansell and Carlson, 2002). Ironically, this major "advance" in our conceptualization of the marine N-cycle led to a situation where N_2 fixation was relegated to a "negligible term" in the N assimilation budget and was all but ignored

in most field studies of the marine N-cycle. Only recently has there been a renewed interest in quantitative measurements of N_2 fixation in the North Pacific trades biome. It is now recognized as a significant pathway of new production rivaling the flux of N from NO_3^- in selected oligotrophic regions. Furthermore, euphotic zone nitrification can locally produce "recycled" NO_3^- from NH_4^+. Both processes will be discussed later in this chapter. The time has come for a reconsideration of the new vs. regenerated N paradigm (Karl, 2000; Yool *et al.*, 2007).

1.3. Hawaii Ocean Time-series (HOT) program

In recent years, several long-term oceanic monitoring programs have been established to make systematic repeated observations of key physical and biogeochemical parameters including those related to oceanic N-cycle processes (Karl *et al.*, 2003). One of these programs, the Hawaii Ocean Time-series (HOT), began sampling in October 1988 at a deep ocean station dubbed Station ALOHA (A Long-term Oligotrophic Habitat Assessment) which is located in the trades biome of the North Pacific Ocean at 22°45′N, 158°W (Karl and Lukas, 1996; Karl and Winn, 1991). HOT was built on the foundation of previous field research in the North Pacific gyre dating back to the Challenger expedition in the late 19th century, and including several predecessor time-series programs (Table 16.2). As part of this program, approximately monthly measurements are made to develop a climatology of physical and biogeochemical properties including water mass characteristics, nutrient inventories, microbial community structure, primary/export production and the net metabolic balance of the sea. In this comprehensive on-going study, numerous N-cycle state variables have routinely been measured and others, including several key rate measurements, have been made on a less frequent basis (Table 16.3). This chapter will summarize these HOT program N-cycle accomplishments in the much broader context of microbial ecology and ocean biogeochemistry of the North Pacific subtropical gyre, including the presentation of a research prospectus for the future. We will primarily focus on results obtained during the past two decades, and on earlier "benchmark" achievements that guided us in the experimental designs and interpretations of our observations.

2. Distributions of Major Nitrogen Pools and Selected Nitrogen Fluxes

2.1. Dissolved and particulate nitrogen inventories

Required growth elements, like N, have uneven distributions in the open sea, both in time and in space. Consequently, inventory measurements should ideally integrate over these expected spatial and temporal variations, but this is not usually feasible in most expeditionary style investigations. Remote sensing of selected N species (e.g., NO_3^-) would be highly desirable and is now possible using novel mooring-based instrumentation (Johnson and Coletti, 2002; Johnson *et al.*, 2007).

Table 16.2 List of Selected Key Publications, Programs, Expeditions and Intellectual Breakthroughs that have Contributed to our Current Understanding of the Marine N-cycle in the North Pacific Trades Biome

Date(s)	Program/Person(s)/Event	Discovery, data sets, and ecological significance
1961	R. C. Dugdale, D. W. Menzel and J. H. Ryther	Discovery of N_2 fixation in the sea (*Deep-Sea Res.* 7: 297–300)
1967	R. C. Dugdale and J. J. Goering	Formulation of the new (NO_3^--based) vs. regenerated (NH_4^+-based) paradigm for marine primary productivity (*Limnol. Oceanogr.* 12: 196–206)
1968–1985	J. McGowan, T. Hayward, E. Venrick and others, CLIMAX time-series	Pioneering research on rates and regulation of primary production, including nutrient limitation; studies of environmental heterogeneity and phytoplankton community structure; dynamics of deep chlorophyll maximum layer; studies of N_2 fixation; DON distributions and dynamics; studies of primary NO_2^- maximum layer; analytical methods development and improvement centered at, or near, the CLIMAX site (28°N, 155°W)
1969–1970	K. Gundersen, D. Gordon, R. Fournier and others	Biogeochemical time-series at oceanic site dubbed Sta. Gollum (22°N, 158°W)
1978–1981	P. Bienfang, J. Szyper and others	Biogeochemistry time-series at two Hawaii Ocean Thermal Energy Conversion (OTEC) program sites (20°N, 156°W and 21°N, 158°W)
1979	R. W. Eppley and B. J. Peterson	Refinement of the equivalance of new and export production under steady-state conditions (*Nature* 282: 677–680)
1982–1988	G. A. Knauer, J. H. Martin and others, VERtical Transport and EXchange (VERTEX) program	Pioneering research on the relationships between particulate matter production, export and remineralization; ^{15}N tracer studies; particle-associated nitrification; trace element (Fe) controls on primary production; establishment of an 18-month ocean time-series at 33°N, 139°W

(Continued)

Table 16.2 List of Selected Key Publications, Programs, Expeditions and Intellectual Breakthroughs that have Contributed to our Current Understanding of the Marine N-cycle in the North Pacific Trades Biome (*continued*)

Date(s)	Program/Person(s)/Event	Discovery, data sets, and ecological significance
1983	E. Carpenter and D. Capone	Publication of "Nitrogen in the Marine Environment"
1985	R. W. Eppley and others, Plankton Rate Processes in Oligotrophic Oceans (PRPOOS) program	Determination of phytoplankton growth rates and regulation of primary production; plankton community size distributions; ^{15}N tracer studies
1986–1987	P. Betzer, E. Laws and others, Asian Dust Inputs to Oligotrophic Seas (ADIOS) program	Role of dust (Fe) deposition and atmospheric forcing on plankton processes
1988–present	D. Karl, R. Lukas and others, Hawaii Ocean Time-series (HOT) program	Establishment of deep ocean, physical-biogeochemical time-series at Station ALOHA (22°45'N, 158°W) as one component of the Joint Global Ocean Flux Study (JGOFS) program
1991	E. Wada and A. Hattori	Publication of "Nitrogen in the Sea: Forms, Abundances, and Rate Processes"
1992	E. Carpenter, D. Capone and J. Rueter	Publication of "Marine Pelagic Cyanobacteria: *Trichodesmium* and other Diazotrophs"
2002	D. Hansell and C. Carlson	Publication of "Biogeochemistry of Marine Dissolved Organic Matter"
2006	D. Karl and others	Establishment of the Center for Microbial Oceanography: Research and Education (C-MORE) for comprehensive studies of marine microbial biogeochemistry

Table 16.3 Selected HOT Program N-cycle Measurements (hahana.soest.hawaii. edu/HOT/methods)

Property/Process	Method
State variables	
NO_3^-/NO_2^-	Surface waters (0–125 m): chemiluminescence
	Deeper waters: segmented-flow autoanalyzer
DON	UV photolysis followed by autoanalyzer
N_2O	Gas chromatography/electron capture
PON	High temperature combustion/gas chromatography
^{15}N-PON	Isotope ratio mass spectrometry
$^{15}N/^{18}O$-N_2O	Isotope ratio mass spectrometry
Dissolved/particulate C:N:P	As above, for DON/PON
Rates	
Autotrophic PON production	^{14}C-HCO_3^- and O_2 based *in situ* incubation, extrapolation to N
Heterotrophic PON production	^{3}H-leucine based *in situ* incubation, extrapolation to N
Total microbial production	^{32}P-phosphate based *in situ* incubation, extrapolation to N
PON export and subeuphotic zone remineralization	Free drifting and moored sediment traps
Nitrification	Substrate changes during timed incubations, ^{14}C-HCO_3^- uptake/inhibitors
N_2 fixation	^{15}N-N_2 based *in situ* incubation

Despite the fact that N is an essential nutrient and potentially the growth rate limiting nutrient in the sea, only NO_3^- exhibits the predicted "nutrient-like" profile (i.e., depleted near the surface with increasing concentrations at depth; Figs. 16.6 and 16.7A). Typically, there are NO_3^- deficits in the euphotic zone (0–175 m) where new organic matter is produced and exported, and NO_3^- excesses in regions below the approximately 0.1% light level where net organic matter decomposition and nitrification occur. Vertical profiles of NO_3^- in the North Pacific trades biome have barely detectable concentrations (<10 nM) in the upper 0–100 m and essentially no vertical gradient; hence, there is no upward diffusion over this depth range. Eppley *et al.* (1990) observed near-surface (0–30 m) enrichments of NO_3^- (28–40 nM) compared to mid-euphotic zone depth (50 m) minima of approximately 10 nM, a feature that they interpreted to be the result of atmospheric deposition of NO_3^-. At Station ALOHA, the 0–100 m inventory of NO_3^- displays both seasonal and interannual variability (Fig. 16.7B) which may be a result of stochastic mixing events.

Below 100 m, NO_3^- increases to a maximum of approximately 42 µM at 800 m, the core of the oxygen minimum zone; at greater depths the NO_3^- concentrations decrease to about 36 µM near the seafloor (Fig. 16.7C). It is important to note that
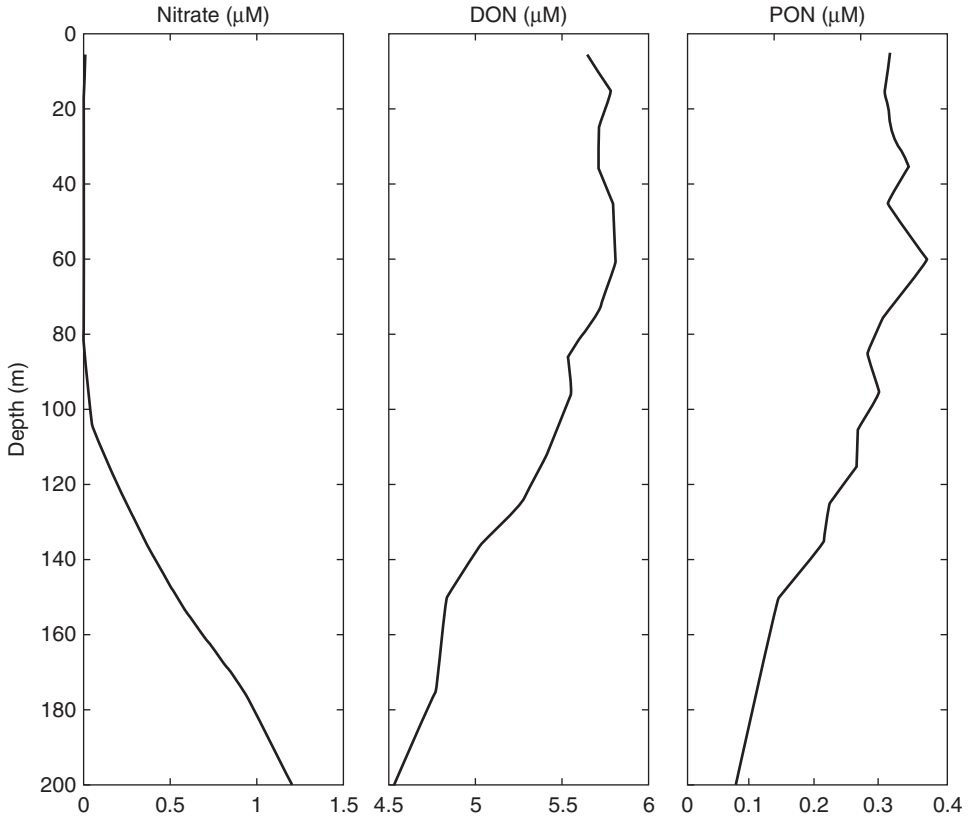
Figure 16.6 Representative upper ocean profile of NO_3^-, DON and PON at Station ALOHA based on 17-year time-series observations. Note accumulation of reduced N, especially DON in near-surface and decreases with depth. In the near-surface waters at Station ALOHA, DON accounts for nearly 95% of the total fixed N inventory.

deep water (>2000 m) NO_3^- concentrations in the North Pacific Ocean are nearly twice as high as they are in the North Atlantic Ocean. This is a consequence of the different ages of the water masses and, consequently, the greater amount of time to accumulate NO_3^- from the combined effects of the biological pump and coupled ammonification/nitrification (Fig. 16.7C). In the trades biome of both major ocean basins, the temporal variability in the horizontal and vertical distributions of NO_3^- is relatively low compared to the dynamics observed in the coastal, polar or westerlies biomes. The vertical and horizontal stabilities of NO_3^- in the North Pacific trades biome are manifestations of a finely tuned balance between NO_3^- input (primarily via upward diffusion and local nitrification) and NO_3^- removal (primarily via uptake into living biomass and subsequent export by gravitational settling of particulate matter).
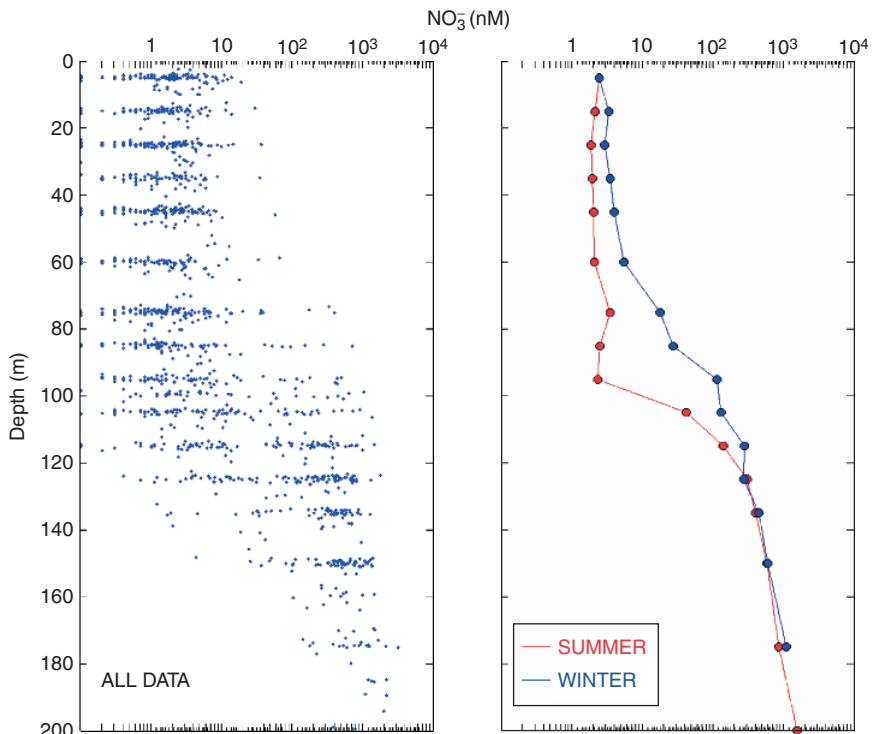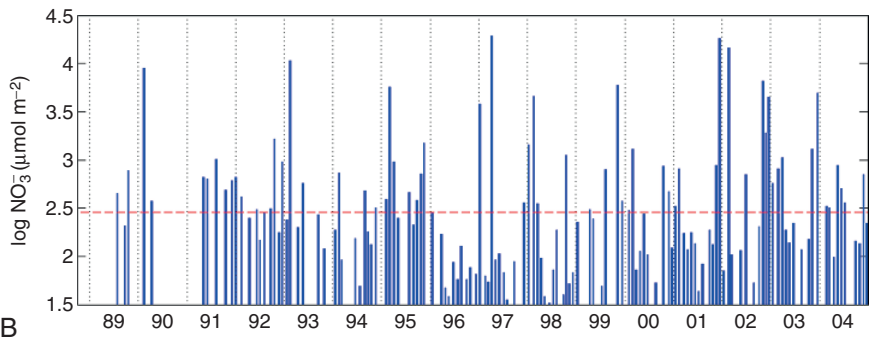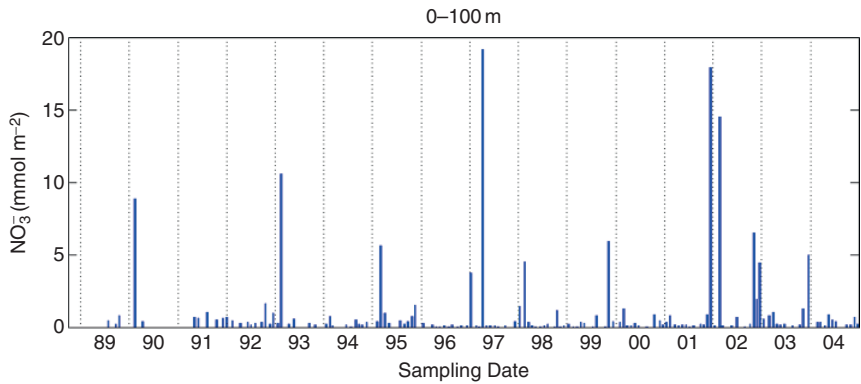
Figure 16.7 Seasonal and interannual variations in NO_3^- concentrations at Station ALOHA. Shown are: (A) the 16–year data set on NO_3^- (nM) in the upper 200 m as well as the summer vs. winter climatologies. Note the log scale in both graphs. (B) integrated (0–100 m) inventories of NO_3^- showing aperiodic injections of NO_3^- into the upper euphotic zone. Note the lower graph presents the data on a log scale to emphasize the extreme temporal variability in NO_3^- inventory which exceeds a factor of 300 over the 16–year observation period,

(Continued)
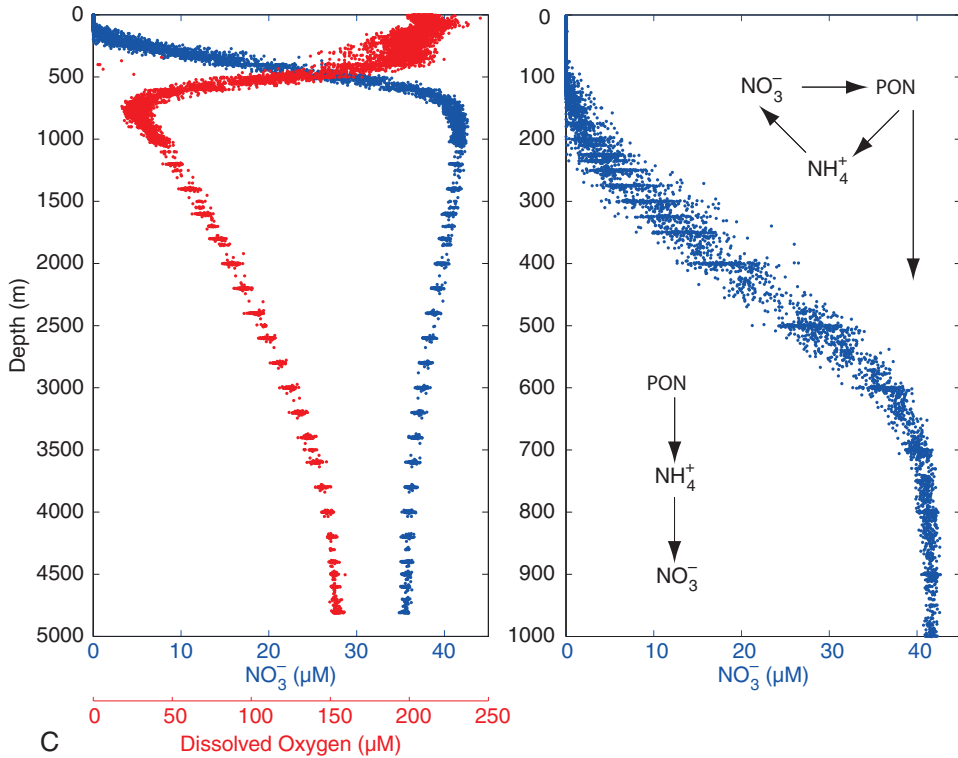
Figure 16.7 cont'd (C) full water column NO_3^- and dissolved oxygen profiles showing the relationships between the two pools. On the right hand plot is an enlargement of the upper 1000 m where most of the N transformations take place.

In a series of papers, Villareal and colleagues have documented a novel mechanism to supplement the net upward flux of NO_3^- in the trades biome, namely the vertical migration of diatom (*Rhizosolenia*) mats (Pilskaln *et al.*, 2005; Singler and Villareal, 2005; Villareal *et al.*, 1993, 1999). Estimates of the quantitative significance of this process, by their field observations, appear to be increasing over time and currently average 40 μmol N m^{-2} d^{-1} (Pilskaln *et al.*, 2005), a value that approaches 20% of the N export measured using sediment traps (Karl *et al.*, 1996). Because *Rhizosolenia* mats are rare and probably an aperiodic occurrence, it is difficult to estimate the annual flux of N by this process with certainty. Regardless of its quantitative role for the ecosystem as a whole, the evolution of this strategy for NO_3^- acquisition is testament to the acute selective pressures that act on microbial communities under nutrient stressed conditions. This phytoplankton-based vertical migration process, if selective for NO_3^- relative to other inorganic nutrients, would lead to a decoupling of N from the otherwise linked cycles of C and P and would thus have significant ecological implications. However, to our knowledge there are no published data on phosphate co-transport by this or similar mechanism in mat-forming diatoms. Other possible mechanisms for aperiodic enhancements in the rates of N delivery and removal will be examined in greater detail in a subsequent section of this review.

NH_4^+, NO_2^- and DON are also characterized as "nutrients," despite the fact that none of them has a "nutrient-like" profile in the open sea (e.g., DON; Fig. 16.6). The most important reason for this is that NH_4^+, NO_2^- and selected compounds in the DON pool have much shorter residence times in the water column, in part, due to the fact that they are either partially or fully reduced and contain additional potential metabolic energy. Essentially all of the "fixed" or reactive N that is exported from the surface ocean to greater depths, whether in dissolved or particulate form, is in the -3 valence state (R-NH_3 or NH_4^+), whereas essentially all of the fixed N in deep water is in the $+5$ valence state (NO_3^-). This indicates an important role for deep water nitrification (org-N/$NH_4^+ \rightarrow NO_3^-$) as a key metabolic process even though we have not yet identified the site(s) where active nitrification occurs or characterized the diversity of deep water nitrifiers.

DON and PON concentrations are both maximal in near-surface waters where inorganic nutrients are assimilated into organic matter, a process that is ultimately sustained by photosynthesis (Fig. 16.6). The decreasing concentrations of PON and DON with increasing water depth are manifestations of energy limitation of sub-euphotic zone waters and net remineralization processes. In the North Pacific trades biome, DON is usually the larger of these two organic pools, and is the largest reservoir of fixed N in near-surface open ocean habitats (Bronk, 2002; Karl et al., 2001a). As such, it represents both a potential source of N for microbial growth and, because it is in the most reduced (-3) valence state, a potential source of energy for those microbes capable of utilizing it. Consequently, the near-surface enrichments of DON are enigmatic and must be a result of the longer term accumulation of semi-labile or refractory organic compounds.

The total DON pool is poorly characterized at the present time, and is likely to consist of numerous individual compounds or compound classes of varying concentration and bioavailability (Aluwihare et al., 2005; McCarthy et al., 1997, 1998). The most dramatic result was the discovery that most of the HMW-DON is present in the form of amides, rather than the previously-held view of DON as a complex spectrum of heterocyclic compounds formed from condensation reactions. Several candidate amide-containing compounds thought to be important are proteins, chitin and peptidoglycan, the latter being a major constituent of bacterial cell walls. Recently, Aluwihare et al. (2005) have reported that the HMW-DON pool (which accounts for approximately 30% of the total DON at their study site in the North Pacific trades biome) consists of two chemically distinct pools of amide based on selective hydrolysis, ^{15}N-NMR spectroscopy and chemical analysis of degradation products. Their results indicated that nearly half of the HMW-DON pool in surface waters consists of N-acetyl amino polysaccharides (N-AAP). Since peptidoglycan is rich in N-acetyl glucosamine and N-acetyl muramic acid, they were expected to be present in—if not to dominate—the N-AAP pool, but were found in only negligible concintrations (Aluwihare et al., 2005). It is conceivable that the N-AAPs in seawater are resistant to degradation, both chemical and microbiological, due to condensation. Using ^{15}N and molecular techniques, Meador et al. (2007) reported that the $\delta^{15}N$ of surface HMW-DON was relatively invariant throughout the tropical Atlantic (4.1 $\pm$ 0.6‰) and tropical Pacific (5.4 $\pm$ 0.8 ‰) Oceans, and showed little correlation with sources or concentrations of N supporting

primary production. However, the δ^{15}N of the dissolved protein fraction was consistently δ^{15}N-depleted relative to bulk HMW-DON in regions where N$_2$ fixation was the dominant source of new N. This suggests that there is a small, rapidly recycled component of the HMW-DON pool that is more labile than the bulk pool (Meador *et al.*, 2007). This pioneering research continues.

Particulate nitrogen in the open sea is most likely organic in origin (i.e., PON) and is expected to be as complex in molecular structure as DON. Although few data exist on bulk PON characterization (Bronk, 2002), several specific N-containing components of the total PON pool have been measured, some routinely (Table 16.1). Because living microbial cells (biomass) can comprise a major, but variable percentage of total PON in seawater (from ~10 to 80%, depending upon depth and geographical location), a portion of the PON pool must consist of the major cellular N reservoirs, namely protein, nucleic acids and, for bacteria, cell walls. While DON and PON can be interconverted, the two pools are likely to have different sources and sinks. Based on ^{13}C-NMR and δ^{13}C isotopic analyses, the HMW-DOM and POM at Station ALOHA appear to be different in both molecular composition and source (Sannigrahi *et al.*, 2005).

The concentrations and dynamics of the near-surface DON and PON pools have been studied at Station ALOHA since 1988. Church *et al.* (2002) reported that the 0–175 m dissolved organic C (DOC) and DON (but not dissolved organic P) increased at rates of 303 and 33 mmol m^{-2} year^{-1}, respectively, for the period 1993–1999. The accumulated DOM had a mean C:N molar ratio of 27.5. By comparison, the C:N ratio of isolated HMW-DOM in the North Pacific trades biome is 14–15, suggesting that the highly aged (based on ^{14}C content) LMW-DOM is more carbon rich (Loh *et al.*, 2004). However, the true C:N ratio of the DON pool (as opposed to bulk DOM) is neither known nor easily determined because the DOC and DOC-N, DOC-N-P and DOC-P sub-pools cannot currently be separated. For example, the N content of HMW-DOM isolated from Station ALOHA varied between 0.95 and 1.69 wt% with no clear depth trends between 20 and 4000 m; molecular analyses identified carbohydrate and amino acids as major compound classes (Sannigrahi *et al.*, 2005).

With regard to PON, Hebel and Karl (2001) have reported regular seasonal changes in the 0–75 m inventories of PON at Station ALOHA, with significantly greater concentrations in summer and fall and minimum concentrations in winter. The average molar C:N:P stoichiometries for 0–75 m particulate matter were 122:17.1:1 (winter), 119:18.4:1 (spring), 140:20.2:1 (summer), and 143:21.8:1 (fall), indicating a tendency for high C:N and high N:P ratios relative to the canonical Redfield ratio of 106C:16N:1P (Hebel and Karl, 2001). They also reported that the contemporary HOT program data set indicates a 70–100% increase in the POC and PON inventories throughout the euphotic zone in comparison to those reported more than 20 years ago during the GOLLUM program (1969–1970) using similar methods. These long-term trends reported by Hebel and Karl (2001), as well as the sub-decadal variations in DOC and DON reported by Church *et al.* (2002), are consistent with enhanced N$_2$-supported new production and increased retention of N-containing compounds. Additional data on these key processes in the marine N-cycle, and the ecological implications of decade-scale enhancements in N$_2$ fixation are discussed later in this chapter.

2.2. Nitrogen assimilation and particulate nitrogen production

Several comprehensive studies of N assimilation in the North Pacific trades biome have been conducted over the past several decades. Gundersen and his colleagues (1974, 1976) were the first to establish N_2 fixation as a source of new N to the open ocean ecosystem, and concluded that it was a more important source of fixed N than wet deposition from the atmosphere (see Case Studies section). They also made measurements of the rates of nitrification, denitrification and assimilatory nitrate-reduction. These latter experiments involved the addition of fairly high concentrations of exogenous N substrates (NH_4^+, NO_2^-, NO_3^-) and extended incubations (days to months), so the rates reported must be viewed as "potential" rates at best.

Eppley *et al.* (1977) measured *in situ* rates of assimilation of NH_4^+, urea and NO_3^- in the upper photic zone on several expeditions to the central North Pacific gyre near the Climax site using ^{15}N tracer techniques. Their experiments were based on 24-h incubations and did not account for isotope dilution (substrate recycling during the incubation period). Assimilation of NO_3^- in surface waters was negligible due to low ambient NO_3^- concentrations, but potential uptake rates under conditions of NO_3^- saturation were in the range of 1–8 nM d^{-1}.

A decade later, similar ^{15}N tracer measurements were conducted at the Climax site during the PRPOOS expedition (Sahlsten, 1987). The experimental design called for large volume (4 liter), short-term (3–4 h) incubations under simulated (light/temperature) *in situ* conditions; diel variability in N assimilation rates was also determined. Several interesting results were obtained: (1) the average ($n = 3$) percentages of NO_3^-, urea and NH_4^+ to the total N assimilated were 14%, 32%, and 54%, indicating that regenerated N supports approximately 85% of the N demand, (2) the total daily rate of N assimilation was 12.5 mmol N m^{-2} day^{-1} integrated over the euphotic zone and (3) < 3 μm size fraction accounted for approximately 75% of the NH_4^+ uptake.

As one component of the decade-long VERTEX program, an oceanic time-series station (33°N, 139°W) was occupied for an 18-month period from October 1986 to May 1988. During this observation period, the site was visited on 7 occasions (~90-day interval) for approximately 1 week per expedition to retrieve and redeploy a free-drifting sediment trap array, to collect water samples and to conduct experiments relevant to C- and N-cycle processes (Harrison *et al.*, 1992; Knauer *et al.*, 1990). The uptake and assimilation of $^{15}NO_3^-$ and $^{15}NH_4^+$ substrates were measured during incubation experiments that were designed to assess, and correct for, isotope dilution of the added tracers. Photoautotrophic N assimilation was measured using the ^{14}C into protein method, described later in this section. Measurements were also made of the concentrations of NO_3^-, NH_4^+, DON, PON, total microbial biomass, autotrophic biomass, heterotrophic biomass, primary productivity and the export of particulate matter (Harrison *et al.*, 1992). In many ways this was, at that time, the most comprehensive study of the marine N-cycle ever conducted in the North Pacific trades biome.

The total microbial community assimilation of NO_3^- and NH_4^+, as well as total photoautotrophic N uptake all showed significant depth and time dependence, with maximum rates of N assimilation in the near-surface waters during summer (Figs.16.8A and 16.8B, Table 16.4). The f-ratio was consistently low (≤ 0.1 in the
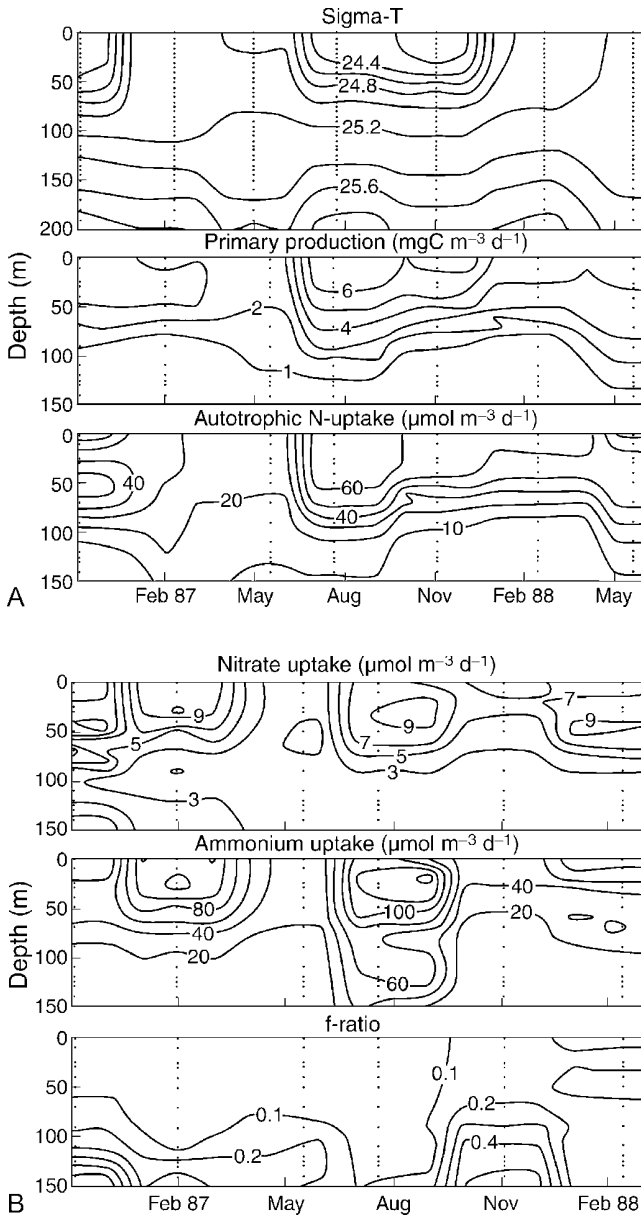
Figure 16.8 N assimilation data from the VERTEX program time-series station at 33°N, 139°W (adapted from Harrison *et al.*, 1992). Shown are: (A) Upper water column density, expressed as "sigma-T" showing changes in stratification of the water column, ^{14}C-HCO_3^--based primary production, autotrophic N-uptake based on the ^{14}C-HCO_3^- into protein method (see text for details) and (B) NO_3^- and NH_4^+ uptake rates based on ^{15}N tracer experiments and calculated f-ratios.

Table 16.4 Selected N Flux Estimates in the North Pacific Trades Biome

Location/Date	*Process*: Method	N-assimilation rates (mg N m^{-2} day^{-1})	Reference
27°58′N, 154°54′W Aug 1973	*Selected substrate assimilation*: Uptake of ^{15}N-labeled substrates at various depths between 0–75 m	NH$_4^+$: 3–22 NO$_3^-$: 0 Urea: 0.8–19 N$_2$: 0.03–0.7	Mague *et al.* (1977)
18°44′N, 157°W Feb 1980	*"Net" Total microbial production:* Conversion of ^{3}H-adenine incorporation into DNA to N using N:DNA ratio of 7.6, integrated to 1% surface irradiance	71.5	Winn and Karl (1984)
26°N, 155°W Mar–Apr 1986 and Oct 1986 (ADIOS)	*Total photo-autotrophic N assimilation:* Uptake of ^{14}C-HCO$_3^-$ into protein, integrated to 1% surface irradiance	58.7–84.9	Laws *et al.* (1989)
33°N, 139°W 7 cruises Oct 1986–May 1988 (VERTEX)	*Selected substrate assimilation*: Uptake of ^{15}N-labeled substrates	NO$_3^-$: 8.26 ± 2.94 (annual = 3080) NH$_4^+$: 83.7 ± 57.3 (annual = 31,220)	Harrison *et al.* (1992)
22°45′N, 158°W various cruises to Sta. ALOHA from Oct 1988 to Dec 2005 (HOT)	*"Net" Total microbial N assimilation:* Conversion of ^{32}P-based production to N using N:P of 16:1	8 cruises 0–75 m: 116 ± 32 75–175 m: 51.8 ± 28	Björkman and Karl (2003)
	"Net" photoautotrophic N assimilation: Conversion of ^{14}C-based production to N using C:N of 6.6:1	Net = 88.2 (annual = 32,193)	Karl *et al.* (2002)
	"Gross" photoautotrophic N assimilation: Conversion of O$_2$-based production to N using a PQ = 1.1 and a C:N of 6.6:1 both integrated to 1% surface irradiance	Gross = 175 (annual = 63,875)	Williams *et al.* (2004)
	"Net" heterotrophic production: Conversion of ^{3}H-leucine incorporation rates to N using 265 g N per mol leucine incorporated	Net = 12.3–19.5	Church *et al.* (2004)
	"Net" N$_2$ fixation: ^{15}N$_2$ assimilation into PON, integrated 0–100 m	0.56–1.62	Dore *et al.* (2002)
	NO$_3^-$ and N$_2$-based new production: Model estimation using δ^{15}N mass balance of exported PON for period 1990–2000	N$_2$-based: mean = 1.89 NO$_3^-$-based: mean = 2.07	Dore *et al.* (2002)
	PON export from euphotic zone: free-floating sediment traps at 150 m	3.1–5.6	Karl *et al.* (1996)

upper 0–50 m) throughout the year, indicating a strong metabolic preference ($\geq$90% of the total) for NH_4^+. The uptake of NO_3^- was less variable than that of NH_4^+, and could account for the annual PN export measured using sediment traps. The seven–cruise mean 0–150 m integrated rates of NO_3^-, NH_4^+ and autotrophic-N assimilation were 8.26, 83.7 and 59.2 mg N m^{-2} day^{-1}, indicating a significant non–photosynthetic uptake (~40%) of [NO_3^- + NH_4^+], presumably chemoorganoheterotrophic assimilation of NH_4^+. Unfortunately, neither DON uptake nor N_2 fixation were measured in this study.

Allen *et al.* (1996) compared NO_3^- uptake rates based on net changes in [NO_3^-] during timed incubations to rates estimated from $^{15}NO_3^-$ tracer experiments, in which approximately 55 nM NO_3^- was added to the samples; ambient [NO_3^-] was $\leq$10 nM. The addition of the ''tracer'' stimulated NO_3^- uptake to rates that approached 25 nM N day^{-1} compared to the measured net NO_3^- uptake rates that were not significantly different from zero for 24-h incubations of unspiked seawater samples. They also concluded that most of the total euphotic zone NO_3^- assimilation probably occurs within a few tens of meters near the top of the nitracline between the 0.1 and 1% light levels, hence, a sampling protocol for NO_3^- assimilation measurements needs to be designed accordingly (Allen *et al.*, 1996).

The rate of ^{14}C-bicarbonate incorporation into protein has also been used to estimate rates of phytoplankton N-assimilation (DiTullio and Laws, 1983). This method, applicable to all N-limited marine ecosystems, builds on the observation that the C:N ratio in protein is remarkably constant and that, under N limitation, a fairly constant percentage (85%) of phytoplankton N is incorporated into protein, with little or no N storage (DiTullio and Laws, 1983). Application of the method involves an incubation with ^{14}C-HCO_3^- followed by extraction and isolation of the ^{14}C-labeled protein fraction. From measurement of the ^{12}C/^{14}C ratio of the total dissolved inorganic carbon pool, the protein C assimilation rates can be estimated. Total phytoplankton N assimilation is then calculated from the theoretical ratios of C:N in protein and protein in phytoplankton under N limitation. In theory, this method has the ability to measure total N assimilation by photoautotrophic plankton regardless of substrate class utilized to support growth (fixed inorganic N plus organic N plus N_2), and the ability to measure phytoplankton N assimilation without interference from co-occurring chemoorganoheterotrophic bacteria. However, it should be emphasized that heterotrophic microorganisms will become partially labeled over time, especially if the microbial food web is active in the incubation bottles. A disadvantage of the method is that it can only be used to measure N assimilation in N-limited ecosystems. Furthermore, at the present time there is limited information whether photoautotrophic prokaryotes, including the two most abundant groups of marine picophytoplankton, *Prochlorococcus* and *Synechococcus*, have protein C:N ratios and protein cell quotas that are similar to the eukaryotic phytoplankton that form the basis for this method.

At a station located in the North Pacific trades biome (18°N, 156°W), DiTullio and Laws (1983) reported autotrophic N uptake rates of 7–11 μmol N m^{-3} h^{-1} for four samples collected in the euphotic zone. The integrated (0–120 m; to the 1% light level) rate of autotrophic N assimilation was 0.93 mmol N m^{-2} h^{-1} and the mean molar C:N assimilation ratio (C assimilation was measured using ^{14}C) was 7.84 ($\pm$0.23), compared

to the Redfield ratio of 6.6. Laws *et al.* (1984) later applied the "^{14}C into protein" method to estimate phytoplankton N assimilation during the PRPOOS program at several stations around the island of Oahu, Hawaii. Rates of assimilation ranged from 0.05 to 0.14 µmol N l^{-1} d^{-1} for near-surface waters, excluding one eutrophic sample (1.8 µg chl a l^{-1}) collected in Kaneohe Bay (Laws *et al.*, 1984). These rates were generally lower than corresponding rates of N assimilation based on the direct incorporation of ^{15}N-labeled NH$_4^+$ with corrections for isotope dilution over time (based on the method of Glibert *et al.*, 1982). The average molar ratio of ^{14}C-bicarbonate assimilation to N assimilation by ^{14}C into protein for these experiments was 7.3 ($\pm$2.1; $n = 6$), not significantly different from the Redfield C:N ratio of 6.6.

In a subsequent report, Laws *et al.* (1985) compared N assimilation based on ^{15}NH$_4^+$ and ^{14}CO$_2$ into protein at three sites near Oahu, Hawaii. Once again the uptake rates of ^{15}NH$_4^+$ exceeded total photoautotrophic N assimilation. They concluded that heterotrophic processes accounted for 50–75% of the NH$_4^+$ uptake, suggesting an intense competition between phototrophs and organotrophs for NH$_4^+$ in these habitats.

In most oceanic environments, including the North Pacific trades biome, primary production of organic matter was traditionally thought to be limited by the supply of fixed N (Caperon and Meyer, 1972; Eppley *et al.*, 1977; Ryther, 1959). This conceptual view of a N-controlled ecosystem assumes that the large reservoir of N$_2$ in the sea is inaccessible to primary producers or that the activities of N$_2$ fixing microorganisms are limited by some other major or trace nutrient (e.g., P or Fe). In most marine environments, nutrient elements are tightly coupled in such a way that alleviation of proximate N, P or Fe limitation will lead to immediate limitation by the next, as predicted by Liebig's Law of the minimum (Karl, 2002; Liebig, 1840). Consequently, single "nutrient addition" experiments may not reveal the fundamental processes controlling the ecosystem carrying capacity and structure; new experimental approaches may be necessary to address this important ecological problem. Suffice it to say that for the multiple required elements likely to limit rates of organic matter production in the sea (N, P and Fe), only N can be converted from a nearly inexhaustible, but relatively inert, pool (N$_2$) to a bioavailable form (NH$_4^+$), provided the required energy is available (either directly as sunlight or as reduced organic matter, which itself is ultimately derived from sunlight) and that N$_2$ fixing microorganisms are present and actively growing. Therefore, from this simple analysis it appears that the marine environment might only be N-limited in areas where energy (light) is also limiting, and in all other regions—including much of the surface ocean in the North Pacific trades biome—organic matter production should ultimately be limited by P or Fe, or both (Karl, 2002).

2.3. Particle export and sub-euphotic zone remineralization of nitrate

Once formed in the near-surface waters, PON has three possible fates. It can be: (1) locally remineralized back to inorganic N (primarily NH$_4^+$) by the combined activities of protozoan and metazoan grazers, bacteria and viruses, (2) converted to

DON and either accumulate or enter into the microbial food web remineralization pathway as in (1), or (3) exported from the local system via advection or gravitational settling.

With the development and extensive use of particle interceptor traps (PITs), also called sediment traps, a new phase of marine biogeochemistry was initiated (Honjo, 1978; Knauer *et al.*, 1979; Soutar *et al.*, 1977). In addition to obtaining "static" measurements of the inventories of DON and PON, oceanographers were now also able to record the downward vertical flux (and upward flux as well if sediment traps are deployed in an inverted configuration), of particulate matter. Returned samples could be interrogated using microscopes, elemental analyzers, mass spectrometers and DNA sequencers, to name a few tools, to characterize the materials collected at a given reference depth or to determine chemical and biological changes that occur as particles sink between selected reference depths. Early models developed to predict particle flux based on surface primary production and water depth (Berger *et al.*, 1987; Pace *et al.*, 1987; Suess, 1980), or particle flux at depth based on particle flux measured at the base of the euphotic zone (~150–200 m; Martin *et al.*, 1987) provided conceptual frameworks for more detailed, mechanistic studies during the VERTEX program and throughout the JGOFS era. Because much of the total global marine export occurs in open ocean habitats (Karl *et al.*, 1996; Martin *et al.*, 1987) it is important to understand the coupling between particle production and downward particle flux, and the factors that cause them to vary over time.

Eppley and Peterson (1979) elaborated further on the Dugdale and Goering (1967) conceptual framework for relating new production to export. If a system is in biological steady-state, or if the measurements are integrated over a sufficiently long period of time (months to years), then new production—*sensu* Dugdale and Goering (1967)—should be equivalent to the amount of primary production that is available for export (Eppley and Peterson, 1979). This export would be quantitatively balanced by the resupply of the production-rate-limiting nutrient(s). Unfortunately, these relationships appear to be much more complex than previously assumed (Karl *et al.*, 1996; Karl *et al.*, 2001b). Data collected during the ongoing HOT program indicate both seasonal and interannual variations in the flux of PON from the euphotic zone, with aperiodic decoupling from primary production that may be partly related to the hypothesized role of N_2 fixation, leading to a systematic alternation between N and P limitation and changes in microbial community structure and ecosystem processes (Karl, 1999; Karl *et al.*, 1996; Karl *et al.*, 2001a, b).

The vertical fluxes of particulate C, N and P at Station ALOHA reveal the following general trends (Christian *et al.*, 1997; Karl *et al.*, 1996): (1) export is maximum near the base of the euphotic zone (mean $\pm$ 1 SD = 2.30 $\pm$ 0.86 mmol C m^{-2} day^{-1}, 0.28 $\pm$ 0.11 mmol N m^{-2} day^{-1} and 0.013 $\pm$ 0.005 mmol P m^{-2} day^{-1} at a reference depth of 150 m), (2) POC flux ranges from 2 to 17% (mean = 6.7%) of the contemporary primary production, (3) C:N:P molar stoichiometry of the exported materials at the 150 m reference depth averages 177:21.5:1, indicating a deficit of N and P, relative to C, compared to the Redfield ratio of 106:16:1, (4) the molar ratios of C:N and C:P of the sinking particulate matter increase by 50% and 35%, respectively, with increasing depth over the range 150–500 m, resulting in a consistently longer solubilization length scale and a concomitant deeper penetration

of particulate C, relative to N or P, and (5) C, N and P fluxes all display both seasonal and interannual variability and may be punctuated by large, aperiodic pulses that are not well sampled or understood, as described in the previous section.

The base of the euphotic zone, approximately 150–200 m in the North Pacific trades biome, represents a key ecosystem boundary below which there is a net loss of sinking N and a net accumulation of NO_3^- with increasing water column depth. While several other processes can contribute to C-N-P bioelement export (Emerson *et al.*, 1997), the gravitational settling of particulate matter ultimately derived from photosynthesis is generally the most important. Sinking particles, containing N in the most reduced amine form (-3), are ultimately oxidized to NO_3^- ($+5$) by the combined activities of macro- and microorganisms, especially the NH_4^+- and NO_2^--oxidizing prokaryotes. However, the exact pathways of conversion, sites of oxidation and organisms involved are not well understood (see Figs.16.1, 16.7C and 16.9A). In one model, microorganisms associated with the sinking particles convert $PON \rightarrow NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$. In a second model, sinking particles are consumed by macrofauna who produce a new suite of sinking particles and produce an overlapping ladder or semi-continuous "rain of detritus." In a third conceptual model, sinking particles disaggregate, disintegrate or otherwise alter their sinking rates (and possibly achieve neutral buoyancy) so that free-living microorganisms at depth can catalyze PON remineralization (Fig. 16.9A). Each model has independent rate controls and sensitivities to changes in the nature of the initial sinking particle spectrum. Regardless of the mechanism(s) involved, the sequence of metabolic processes conforms to the von Brand *et al.* (1937) "diatom rotting" experiments (Fig. 16.9B). The coupled processes of ammonification (the production/release of NH_4^+ from PON) and nitrification (the stepwise oxidation of NH_4^+ to NO_2^-/NO_3^-) are the key processes in the conversion of PON to NO_3^-, both in this classic experiment and in the ocean as a whole. Ammonification is a very general and widespread process, whereas nitrification is probably more restricted.

The mesopelagic zone of the world ocean (defined as the habitat between the lower epipelagic zone and the upper bathypelagic zone, approximately 150–1500 m; Hedgpeth, 1957) is sometimes referred to as the "twilight zone" partly because the light flux in this region is—at most—equivalent to twilight and partly because "mystery abounds" as we know so little about it. One of the largest and yet unsolved mysteries involves the process of nitrification. Until recently, it was thought that chemolithoautotrophic nitrifying bacteria (e.g., *Nitrosomonas/Nitrobacter*, and related genera) controlled the oxidation of NH_4^+ to NO_3^- in subeuphotic zone habitats. However, after the pioneering discovery of non-thermophilic *Archaea* in marine ecosystems (Fuhrman *et al.*, 1992; DeLong, 1992), pelagic *Crenarchaeota* and *Euryarchaeota* were recognized as important components of the total microbial assemblage in the North Pacific trades biome, especially within the twilight zone (Karner *et al.*, 2001). And despite the ubiquity of pelagic *Archaea*, their metabolic and biogeochemical roles remained unresolved or, at best, only partially answered. Recently, Pearson *et al.* (2001) and Wuchter *et al.* (2003) provided independent evidence suggesting that mesopelagic zone *Archaea* may have an autotrophic metabolism, and even more recently a chemo-lithoautotrophic NH_4^+-oxidizing marine archaeon has been isolated documenting
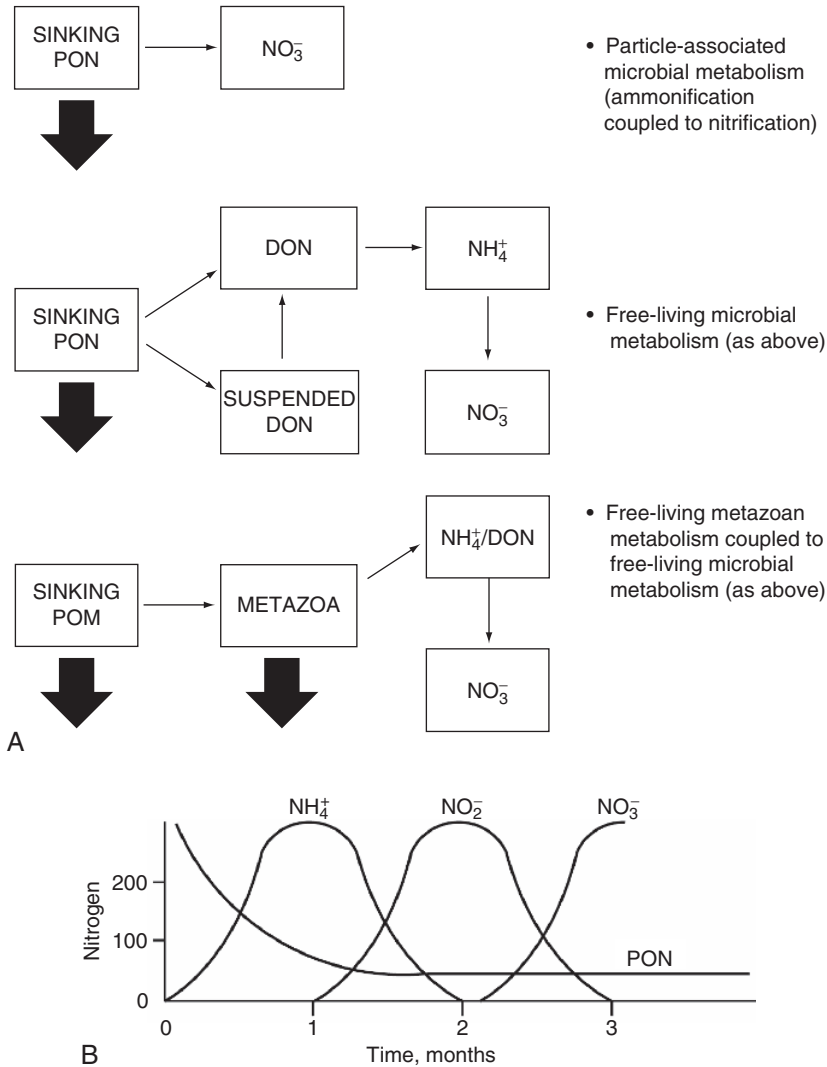
Figure 16.9 Coupled euphotic zone PON export and mesopelagic zone remineralization processes. (A) Shown are three conceptual models to account for the net decomposition of sinking POM and coupled nitrification showing the various and, potentially independent, roles of micro- and macroorganisms in the ocean's mesopelagic zone N-cycle (see text for details). (B) The observed stepwise remineralization of diatom-associated biomass (PON) to NH_4^+, NO_2^- and NO_3^- (all shown as arbitrary units of N) in the dark as particles aged over a period of three months. Redrawn from data in von Brand *et al.* (1937).

the first example of nitrification in the domain *Archaea* (Könneke *et al.*, 2005). Laboratory growth of the isolated Crenarachaeote strain SM1, which has been assigned the candidate status, *Nitrosopumilus maritimus*, demonstrated a quantitative conversion of NH_4^+ to NO_2^- as the sole energy source and incorporation of HCO_3^- as the sole carbon source. Furthermore, metagenomic surveys have

revealed that some *Archaea* carry a unique ammonia monooxygenase (*amoA*) gene (Schleper *et al.*, 2005; Venter *et al.*, 2004), and polymerase chain reaction (PCR) primers designed to specifically target archaeal *amoA* have detected its presence in a variety of coastal and open ocean habitats (Francis *et al.*, 2005). Mincer *et al.* (2007) have obtained the quantitative distribution of putative nitrifying genes and phylotypes in a picoplanktonic genome library from Station ALOHA. They report a deeply branching crenarchaeal group related to a hot spring clade indicating that the *amo*A containing archaea in the mesopelagic zone may be more diverse than previously reported. They also found a positive correlation between pelagic *Nitrospina* and crenarchaea suggesting a probable syntrophic relationship between the two. Even with this new discovery of archaeal ammonium oxidation, there still appears to be a division of metabolic labor during the process of nitrification (Costa *et al.*, 2006), despite one report to the contrary (Ram *et al.*, 2001). Consequently, it is tempting to hypothesize that *Archaea* may play a role in, or even control, subeuphotic zone nitrification, at least the oxidation of NH_4^+ (e.g., Ingalls *et al.*, 2006; Nicol and Schleper, 2006; Wuchter *et al.*, 2006; Beman *et al.*, 2008). Well designed *in situ* rate experiments that are able to distinguish between bacterial and archaeal NH_4^+ and NO_2^- oxidation, perhaps using specific inhibitors, will ultimately be necessary to test this novel hypothesis.

3. Selected Trades Biome Ecosystem Processes

3.1. Nitrous oxide production and sea-to-air gas flux

Nitrous oxide (N_2O) is a potent greenhouse gas (approximately 200 times more effective than CO_2 on a molar basis) that has also been implicated in stratospheric ozone depletion (Kim and Craig, 1990; Yoshida *et al.*, 1989) (see Bange, Chapter 2, this volume). Currently, N_2O accounts for about 5.5% of the enhanced radiative forcing attributed to all gases in the atmosphere (IPCC, 2007). Furthermore, while the atmospheric inventory of N_2O is increasing, its sources are not well understood causing a renewed interest in the role of marine ecosystems as a potential source for N_2O.

In the North Pacific trades biome, N_2O is a trace dissolved gas with typical concentrations ranging from 5 to 50 nM (Fig. 16.10A). N_2O concentrations in near-surface waters are generally in slight excess of air saturation, implying both a local source and a sustained ocean-to-atmosphere flux. At Station ALOHA, N_2O concentrations in the surface mixed-layer zone range from 5.1 to 9.3 μmol m^{-3}, equivalent to 83–137% of air saturation; deeper within the euphotic zone, the N_2O concentrations range from 6.8 to 14.4 μmol m^{-3}, equivalent to 95–187% of air saturation (Fig. 16.10B; Dore and Karl, 1996a; Popp *et al.*, 2002; unpublished HOT data). In selected oceanic regions, N_2O can exceed 300% saturation, relative to atmospheric equilibrium. An understanding of the processes maintaining the euphotic zone N_2O supersaturation has received the most interest (Capone, 1991), mainly because of the potential for a significant sea-to-air N_2O flux. Beneath the euphotic zone, at a depth approximately 500–1000 m, there is a peak in N_2O concentration that is usually co-located with the dissolved oxygen minimum (Ostrom *et al.*, 2000).
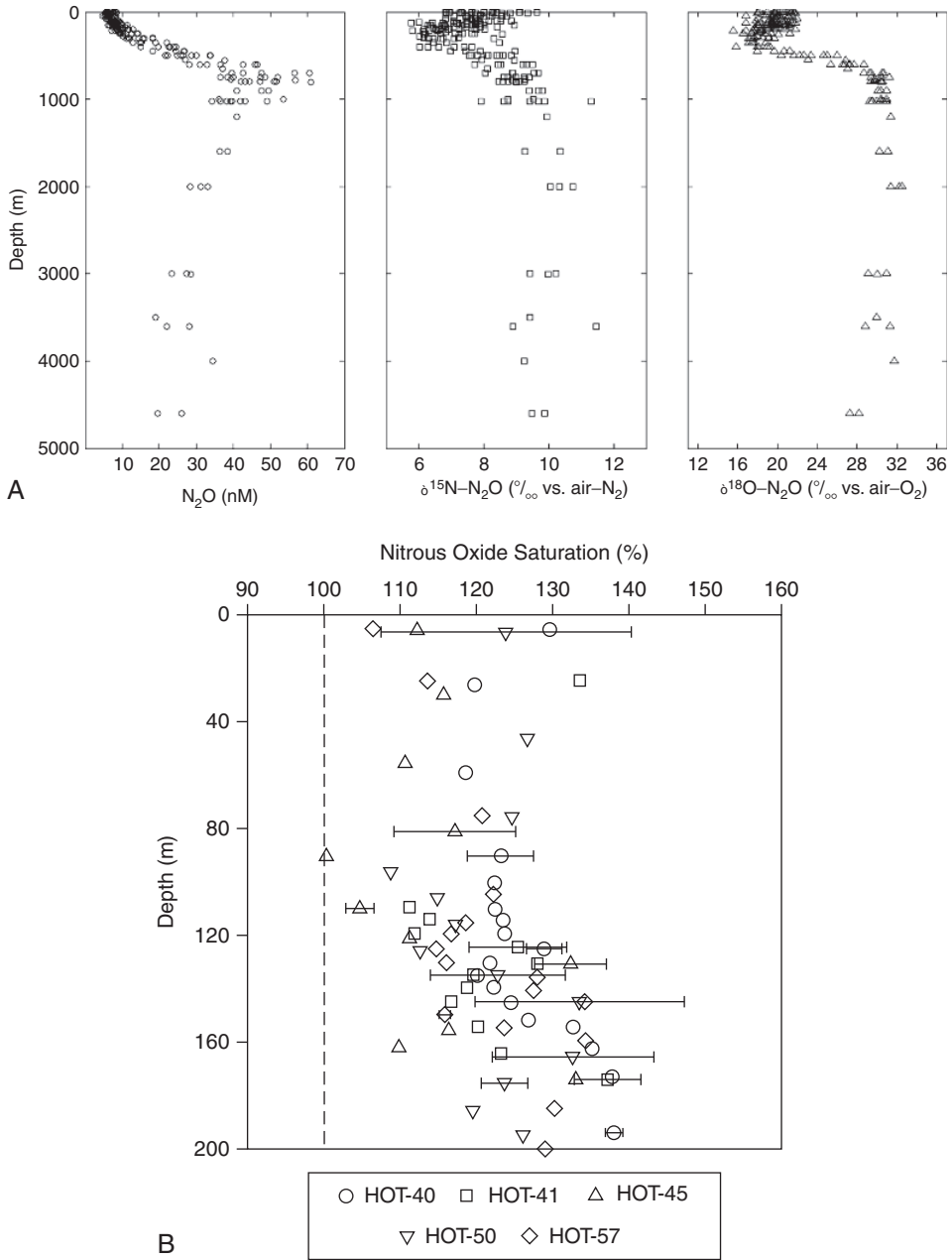
Figure 16.10 (A) Nitrous oxide (N_2O) concentrations and isotopic composition for water samples collected at Station ALOHA. [Left] Depth profile of N_2O showing a distinct mid-depth maximum of ~60 nM coincident with the dissolved oxygen minimum. [Center] ^{15}N isotope composition of N_2O. [Right] ^{18}O isotope composition of N_2O. Data from Dore *et al.* (1998) and B. Popp and J. Dore (unpublished). (B) N_2O saturation state, expressed as a percentage of air saturation, for the upper portion of the water column at Station ALOHA during the period September 1992–September 1994. The vertical dashed line indicates equilibrium (100% saturation) with atmospheric N_2O. With the exception of one measured value on cruise HOT-45, all determinations indicate significant N_2O saturation relative to the atmosphere which implies both a local source and a net ocean–to–air gas flux. From Dore and Karl (1996a).

The formation of N_2O in seawater has at least two fundamentally distinct pathways: nitrification by *Bacteria* and possibly *Archaea*, and bacterial denitrification (Ritchie and Nicholas, 1972; Vincent *et al.*, 1981; Yoshida *et al.*, 1989). In any given habitat, the relative contributions of these two competing oxidative vs. reductive metabolic pathways can vary spatially and temporally. Although relatively inert, N_2O—once formed—can be reduced to N_2 by selected bacteria. Dore and Karl (1996a) used N_2O concentration data and an empirically determined gas transfer model (Wanninkhof, 1992) to estimate the instantaneous sea-to-air flux of N_2O. They estimated a sea-to-air N_2O flux of 1.83–8.11 µmol m^{-2} day^{-1} for five separate HOT cruises between September 1992 and September 1994. Based on a simple 1-D box model and an eddy-diffusivity coefficient of 3.7×10^{-5} m^2 s^{-1} (Lewis *et al.*, 1986), they calculated that the upward vertical flux of N_2O into the euphotic zone was two to three orders of magnitude lower than would be required to sustain the estimated sea-to-air flux, and concluded that there must be a local, near-surface ocean source of N_2O in the North Pacific trades biome. They hypothesized that NH_4^+ oxidation (the first step in nitrification) was the most likely source and used published laboratory results of the maximum molar yield of N_2O during bacterial nitrification (N_2O production = 0.5% of total NH_4^+ oxidation rate; Goreau *et al.*, 1980; Lipschultz *et al.*, 1981) to conservatively constrain rates of *in situ* nitrification. Their model results yielded NH_4^+ oxidation rates of 0.34–1.59 mmol m^{-2} day^{-1} for the euphotic zone (0–175 m) at Station ALOHA, a range of values that was consistent with measured rates of nitrification based on changes in NO_3^-/NO_2^- during dark incubations (Dore and Karl, 1996a) and with those derived from ^{15}N tracer measurements (Olson, 1981a), all in the lower reaches of the euphotic zone (100–175 m).

In a subsequent study, Dore *et al.* (1998) confirmed the large N_2O source at Station ALOHA using direct measurements of the δ^{15}N and δ^{18}O of water column dissolved N_2O. Combining a 1-D eddy diffusive model with both N_2O concentration and dual isotopic gradient measurements they derived a revised net N_2O sea-to-air flux of 0.4 ± 0.2 µmol m^{-2} day^{-1}, approximately an order of magnitude lower than estimated in the earlier study. The ^{15}N and ^{18}O-depleted isotopic signatures of N_2O at the base of the euphotic zone suggested nitrification as the most probable source of N_2O in the surface ocean, a finding that gained additional support from later measurements of the δ^{18}O of H_2O and dissolved O_2 (Ostrom *et al.*, 2000), the δ^{15}N of NO_3^- (Sutka *et al.*, 2004), and the N-isotopomeric composition of dissolved N_2O (Popp *et al.*, 2002). Popp *et al.* (2002) also used the isotopomer mass balance to constrain the sea-to-air N_2O flux at 0.4–1.0 µmol m^{-2} day^{-1}, a value which agreed well with their gas transfer estimate based on wind speed and surface N_2O saturation state (1.1 ± 0.7 µmol m^{-2} day^{-1}). The differences between the various estimates of sea-air N_2O flux at Station ALOHA may be at least partially explained by differences in the eddy-diffusivity coefficients employed and the considerable temporal variability in the surface ocean N_2O saturation (Fig. 16.10B).

Near-surface ocean nitrification at rates that are likely to exceed the upward eddy-diffusion of NO_3^- from beneath the euphotic zone (e.g., Martin and Pondaven, 2006) has important implications for both the conceptual model of new vs. regenerated production (Dugdale and Goering, 1967) and for the

quantitative relationships between f-ratio (the fraction of total N assimilation that is supported by new N, usually measured as NO_3^-) and system export (see Fig. 16.5). Furthermore, because N_2O production scales on gross rates of NH_4^+ oxidation (rather than on net rates) any environmental variable that leads to an enhancement in the cycling rates of NH_4^+ would be expected to have a corresponding impact on N_2O production and hence on N_2O flux to the atmosphere. For example, N_2 fixation (which produces NH_4^+), Fe/P deposition, or even subtle shifts in microbial community structure or grazing rates could all impact NH_4^+/N_2O inventories.

3.2. Structure and dynamics of the primary and secondary nitrite maximum layers

Nitrite-N (NO_2^-) has an intermediate redox position between that of NH_4^+ and NO_3^-, thus NO_2^- often accumulates in selected depth strata when active N transformations are occurring (Rakestraw, 1936; Vaccaro and Ryther, 1960). In the global ocean, two such zones have been identified and studied (Fig. 16.11): (1) the primary NO_2^- maximum (PNM) zone that is usually located near the base of the euphotic zone worldwide and (2) the secondary NO_2^- maximum (SNM) zone that is most prominent in oxygen depleted waters (Codispoti and Richards, 1976; Fiadeiro and Strickland, 1968).

The formation and maintenance of the PNM appears to be complex, perhaps involving at least three independent and, in part, competing processes that include: (1) chemolithoautotrophic oxidation of NH_4^+ (Brandhorst, 1959; Olson, 1981b), (2) partial (incomplete) assimilatory NO_3^- reduction by phytoplankton (Kiefer *et al.*, 1976; Vaccaro and Ryther, 1960), and (3) partial dissimilatory NO_3^- reduction by chemoorganoheterotrophs (Wada and Hattori, 1972) growing in oxygen-depleted microenvironments or in the guts of mesozooplankton or fishes. This important aspect of the marine N-cycle has recently been reviewed by Lomas and Lipschultz (2006).

The production of significant amounts of extracellular NO_2^- by marine phytoplankton during NO_3^- assimilation was first demonstrated in unialgal cultures by Vaccaro and Ryther (1960). They also found that the highest NO_2^- concentrations appeared in cultures recovering from N deficiency and grown under reduced light. They suggested that in the open ocean near the base of the photic zone, where NO_3^- becomes plentiful but phytoplankton are light-limited, partial assimilatory reduction of NO_3^- to NO_2^- leads to the formation and maintenance of the PNM. Subsequent laboratory studies by Carlucci *et al.* (1970) supported their results, and this hypothesis was re-examined in a field study conducted by Kiefer *et al.* (1976). The latter study employed a simple box model using measured profiles of NO_2^-, NO_3^- and phytoplankton carbon, along with a literature-derived eddy diffusivity constant, to show that rates of NO_2^- production are consistent with the Vaccaro and Ryther phytoplankton assimilatory reduction hypothesis. Kiefer *et al.* (1976) concluded that the PNM "exists at a given depth because the cells above the maximum are depleted of nitrate, while the cells below the maximum receive insufficient radiant energy to maintain intracellular rates of NO_3^- reduction." When light levels are low, the rate of NO_3^- reduction to NO_2^- exceeds the rate of NO_2^- reduction to NH_4^+, the final precursor for N incorporation into cellular
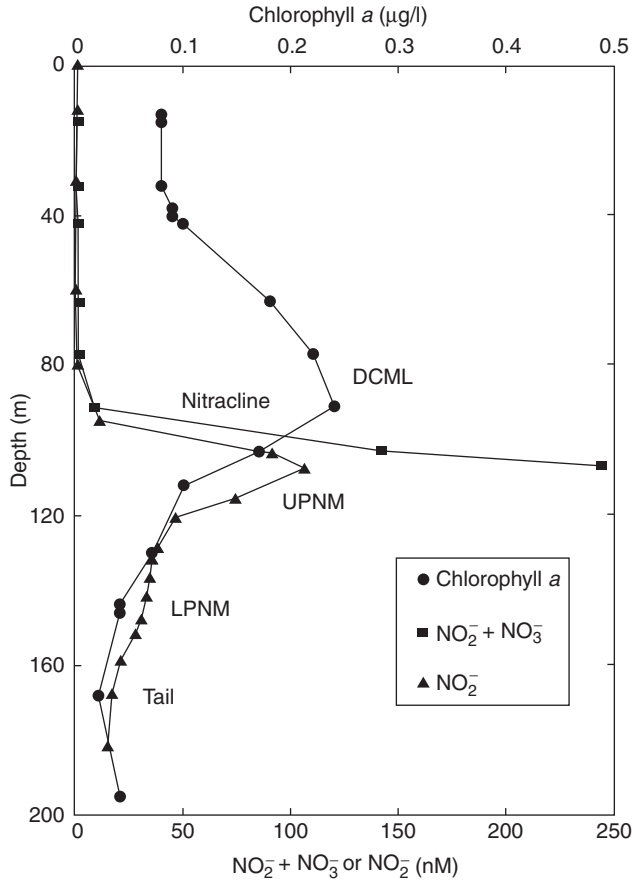
Figure 16.11 Vertical distributions of Chlorophyll *a*, $[NO_2^- + NO_3^-]$, and $[NO_2^-]$ for the water column at Station ALOHA in October 1992. The conspicuous NO_2^- maximum beginning at approximately 100 m is positioned below the deep Chlorophyll *a* maximum layer (DCML) and is coincident with the top of the nitracline. This primary nitrite maximum (PNM) is further divided into upper and lower regions (UPNM and LPNM, respectively) with a tailing of the LPNM. These major features are the result of competing microbiological NO_2^- production and utilization processes as shown in Fig. 16.2. From Dore and Karl (1996b).

materials. An intracellular pooling of NO_2^- occurs, and as a result of the slightly acidic intracellular milieu of phytoplankton, NO_2^- is protonated to HNO_2, a weak acid, which freely diffuses out of the cells as an uncharged molecule, nitrous acid. In seawater (pH ≈ 8.0), the HNO_2 dissociates to produce free NO_2^- and this process produces and sustains the PNM at a selected isolume (Kiefer *et al.*, 1976).

Indirect evidence further supporting the phytoplankton partial assimilatory reduction model was presented by Herbland and Voituriez (1979). They performed a statistical analysis of 123 measurements of the depths of the PNM and the top of the nitracline, and found a strong correlation. They also found a significant offset of

about 11 m between these two features, the PNM falling below the top of the nitracline. Moreover, in an earlier study conducted in tropical waters, they showed that NO_2^- is never detectable when NO_3^- is not present (Herbland and Voituriez, 1977). Thus, they concluded that the PNM "stands where the chlorophyll concentrations decrease in a light-limited regime and where NO_3^- is abundant" (Herbland and Voituriez, 1979).

The condition of low light level for phytoplankton NO_2^- production suggested by Vaccaro and Ryther (1960) is apparently not absolutely necessary. Field work by Wada and Hattori (1971) and laboratory-based culture studies by Olson *et al.* (1980) showed that NO_2^- production by phytoplankton is positively correlated with both NO_3^- concentration and light intensity. Furthermore, in boreal areas rich in surface NO_3^-, an annual accumulation of NO_2^- closely matches the annual depletion of NO_3^- as light levels increase during the spring (e.g., see Dore and Karl, 1992; Olson, 1981a; Verjbinskaya, 1932). It is therefore probable that the phytoplankton contribution to the PNM is not due to low light, but to *sufficient* light and *ample* NO_3^-, enabling phytoplankton cells to maintain a high rate of NO_3^- reduction (Dore, 1995). This view is consistent with the correlation of the depth of the PNM and the nitracline seen by Herbland and Voituriez (1979); indeed, the depth of maximal NO_3^- uptake measured by Eppley and Koeve (1990) for trades biome stations with NO_3^-–depleted surface layers and steep nitraclines was always a few meters below the top of the nitracline.

As mentioned previously, *Prochlorococcus* is the dominant phytoplankton group in the North Pacific trades biome. Recently, the full genome sequences of several representative *Prochlorococcus* ecotypes have been published (Dufresne *et al.*, 2003; Rocap *et al.*, 2003). It is important to point out that none of the three genomes sequenced contain nitrate reductase, the enzyme responsible for the reduction of NO_3^- to NO_2^-, the hypothesized mechanism for the existence of the PNM layer. This is not to say that *Prochlorococcus* does not contribute to the PNM, rather that we have no evidence to date that they can utilize NO_3^-. However, recent results suggest that a yet–to–be–isolated *Prochlorococcus* ecotype may contain nitrate reductase (Casey *et al.*, 2007). Furthermore, the deep living/dark-adapted ecotype of *Prochlorococcus*, as well as other microbes, can utilize NO_2^- as a source of N for biosynthesis so the net effect of phytoplankton/microbe metabolism would be to erode, not to produce or sustain, the PNM.

As an alternative to partial assimilatory NO_3^- reduction by phytoplankton, oxidation of NH_4^+ by *Bacteria* and *Archaea* (the first step in the 2-step process of nitrification) can produce NO_2^- as an intermediate product. Nitrifying bacteria were first isolated from the marine environment by Watson (1965) and are now known to be ubiquitous in the global ocean. Wada and Hattori (1971) used a sensitive chemical assay to measure changes in NO_2^- in incubated samples, to conclude that NH_4^+ was the major source of NO_2^- in the PNM in the central North Pacific Ocean. Miyazaki *et al.* (1973, 1975), using a ^{15}N tracer method, found that, in Sagami Bay and in the western North Pacific, NH_4^+ and NO_3^- were both important sources of NO_2^-.

In a benchmark study, Olson (1981a) used improved ^{15}N tracer methods to measure the production of NO_2^-, independently from NO_3^- and NH_4^+, as well as the simultaneous uptake of NO_3^- and NH_4^+ in a variety of marine habitats. He found

that in the North Pacific trades biome, NH_4^+ was the major source of NO_2^- for the PNM. In addition, he observed that a large fraction of the NH_4^+-oxidizing activity passed through a 0.6 μm filter but was retained on a 0.2 μm filter, suggesting that bacteria were responsible. The rate of NO_2^- production from NH_4^+ for experiments conducted in the North Pacific trades biome was reported to be 2.24–7.30 μmol m^{-3} d^{-1}, yielding a NO_2^- turnover time of 25 ± 10 days in the PNM zone.

In a companion paper, Olson (1981b) proposed a mechanism by which nitrifying bacteria might form and maintain the PNM, involving the differential photoinhibition of the two steps of nitrification, namely NH_4^+ oxidation and NO_2^- oxidation. Based on the observation that NH_4^+ oxidation occurred both within and below the PNM, but that NO_2^- oxidation occurred only below, he concluded that NO_2^- was accumulating because, at the light levels associated with the PNM, NO_2^- oxidation was inhibited while NH_4^+ oxidation was not (Olson, 1981b). In studies of coastal seawater samples, Olson found that NO_2^- oxidation was indeed inhibited by light of an intensity less than that required to inhibit NH_4^+ oxidation, and that the critical intensity was about 1% of surface irradiance, about the light level found at the PNM. The physiological mechanism for this differential inhibition by light on the two independent steps of nitrification is not clear, but may be related to differences in the sensitivity of ammonium oxidizers and nitrite oxidizers to photochemically produced carbon monoxide or to oxidation of intracellular components such as cytochrome C$_{554}$ (Vanzella *et al.*, 1990). Alternatively, differential recovery from photoinhibition, rather than differential photoinhibition, has been proposed as a mechanism for accumulating NO_2^- in the PNM (Guerrero and Jones, 1996).

Dore and Karl (1996b) presented a comprehensive data set on NO_2^- vertical distributions and temporal dynamics at Station ALOHA for the observation period September 1989 to November 1993. Their results revealed a novel double-peaked structure to the PNM, and they separated the previously reported single PNM into an upper and a lower zone (UPNM and LPNM, respectively). Comparisons between monthly cruises showed substantial variability in the vertical structures and NO_2^- concentrations in the UPNM and LPNM features (Figs. 16.12A and 16.12B). The authors suggested that the UPNM was a result of partial assimilatory NO_3^- reduction by phytoplankton (i.e., the Vaccaro and Richards model) while the LPNM was a result of differential nitrification (i.e., the Olson model). Dore (1995) developed a refined steady-state model that combined the processes of phytoplankton reduction of NO_3^- to NO_2^-, as well as bacterial oxidations of NH_4^+ to NO_2^- and NO_2^- to NO_3^-. This "hybrid" model made several simplifying assumptions. For example, it neglected mixing and diffusion, fixed the microbial community composition, assumed that NH_4^+ and NO_2^- oxidizing bacteria were uniform in the upper 200 m of the water column, and used an assumed NH_4^+ profile. Nevertheless the Dore (1995) model was able to simulate the general features of the PNM observed at Station ALOHA, including the large upper PNM (a result of phytoplankton reduction of NO_3^-), the vertical separation of oxidative and reductive processes, and the vertical asymmetry of NO_2^- within the broad PNM in this region (Dore, 1995). The lower portion of the PNM was not always as well reproduced, and sensitivity analysis showed that $[NH_4^+]$ had a large effect on the LPNM, but not on the UPNM, due to the dependence of NH_4^+ oxidation on substrate concentration.
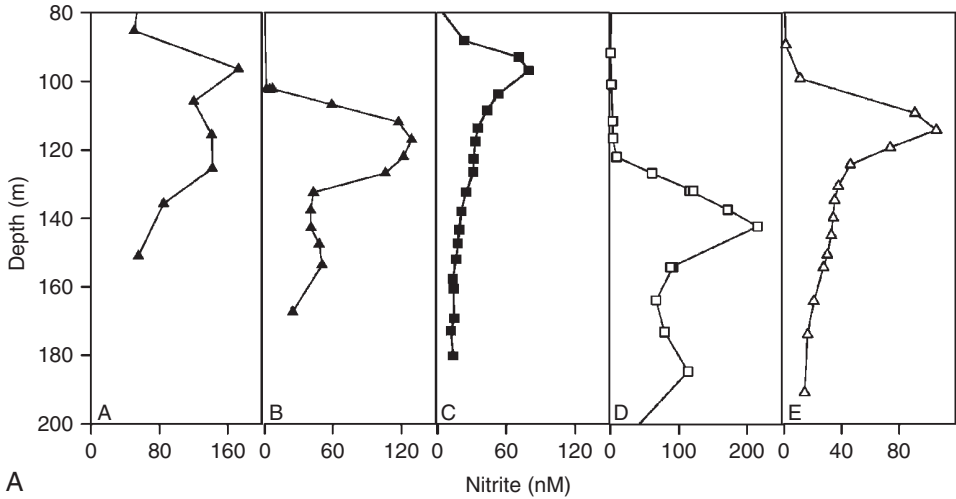
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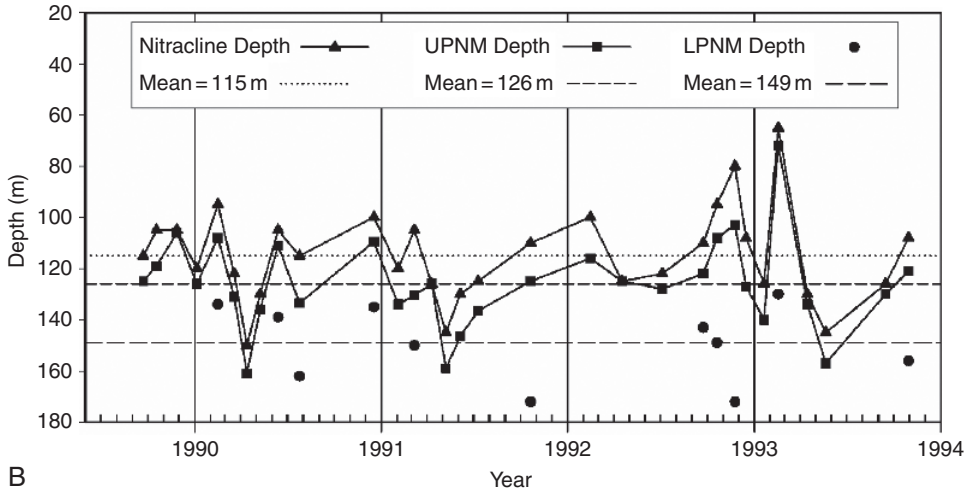
B

Figure 16.12 Temporal and spatial (depth) variations in the primary nitrite maximum layer at Station ALOHA. (A) Shown are five representative profiles of NO_2^- between 80 and 200 m from February 1990 to September 1992 displaying the two-peaked structure (U = UPNM and L = LPNM) of the general feature. Note concentration variations and changes in reference depths of the key features. A = February 1990, B = July 1990, C = December 1990, D = October 1991, E = September 1992. From Dore and Karl (1996b). (B) Temporal variations in the positions of the UPNM and LPNM relative to the nitracline. Shown also are the mean depths for these features over the observation period. From Dore and Karl (1996b).

Zafiriou *et al.* (1992) reported trace amounts of NO_2^- (0.4–1 nM) in the ''tail'' of the PNM layer in the NW Atlantic Ocean to a depth of at least 1000 m. The NO_2^- inventory in this region was roughly equivalent to that in the PNM feature itself. This mid-water NO_2^- pool was dynamic with an estimated turnover time of a few days. At Station ALOHA, Dore and Karl (1996b) likewise detected an
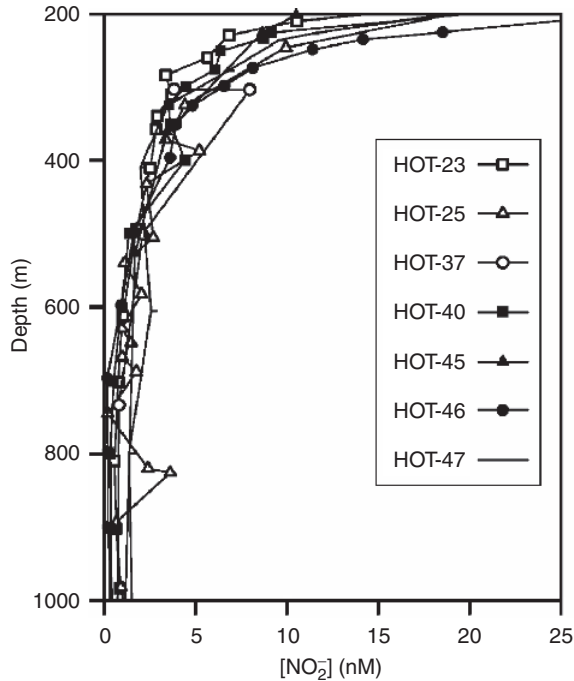
Figure 16.13 Mesopelagic zone profiles of nitrate from Station ALOHA during 7 cruises from February 1991 to May 1993. From Dore and Karl (1996b).

exponential decay of NO_2^- over the depth range of 200–1000 m with concentrations decreasing from >5 nM at 200 m to <1 nM at 1000 m (Fig. 16.13). The Station ALOHA data set, which included 7 separate profiles over a 2-year period, also displayed temporal variability suggesting that the mesopelagic zone NO_2^- pools are dynamic even at great depths. While the mechanism(s) is not known, fluctuations in $[NO_2^-]$ are undoubtedly biological in origin, most likely a result of coupled particle export–remineralization and nitrification processes.

3.3. New production by nitrogen fixation

Biological N_2 fixation was discovered in the early 20th century in a soil bacterium (Beijerinck, 1908). In his now classic monograph on "Oceanography: Its scope, problems and economic importance," Bigelow (1931) stated that "the possibility that so-called N_2 fixers may also fertilize seawater must be taken into account"; however, systematic investigation did not begin for at least another three decades. In the past decade, there has been an enormous effort to obtain accurate estimates of the rates of N_2 fixation and the controlling mechanisms of this vital ecosystem process (Capone, 2001; Gruber, 2005; Karl *et al.*, 2002; Mahaffey *et al.*, 2005;

Michaels *et al.*, 2001) (see Carpenter & Capone, Chapter 4, this volume). In part because of its sensitivity to climate variability and its potential role in the marine sequestration of carbon.

According to Marumo and Asaoka (1974), the North Pacific trades biome supports the growth of at least five different N_2 fixing cyanobacteria: *Trichodesmium thiebautii*, *Trichodesmium erythraeum*, *Oscillatoria* sp., *Katagnymene spiralis* and *Richelia intracellularis*. Based on observations made during an August–October 1969 expedition from 50°N to 15°S along the 155°W meridian, they reported that *T. thiebautii* and *Rhizosolenia styliformis* had the largest geographical range from 15°S to 40°N; none of the species were found north of 40°N. *T. thiebautii* was the only N_2-fixing cyanobacterium found south of 10°N.

Several pioneering field studies including Mague *et al.* (1974 and 1977) and Gundersen *et al.* (1976) established N_2 fixation as an important metabolic pathway in the marine N–cycle of the North Pacific trades biome. Selected results included: (1) N_2 fixation was widespread in the oligotrophic portions of the North Pacific, both in free-living non-heterocystous *Trichodesmium* assemblages and in diatom-*Richelia intracellularis* symbiotic associations, (2) there were intermittent spatial and temporal distributions of N_2 fixing organisms, with greater average abundance and activity in summer, (3) strong vertical zonation of N_2 fixation with greatest rates in the upper 40 m of the water column, but detectable activity to at least the 1% light level (~100 m), (4) significant O_2 inhibition of N_2 fixation with a pO_2 of 0.4 atm causing an approximately 75% inhibition of activity, (5) when present, *Trichodesmium* could meet 100% of its N requirement via N_2 fixation, and (6) during stratified summer conditions, approximately 3% of the total N assimilated (as determined by the combined uptake of ^{15}N-labeled NH_4^+, NO_3^-, urea and N_2) was supplied via N_2 fixation.

The establishment of HOT, in October 1988, marked the next and current phase of N_2 fixation research in the North Pacific trades biome. The extant HOT program N_2 fixation database now includes biological, biogeochemical and genomic information in addition to direct measurements of rates of N_2 fixation and environmental controls thereof. The HOT program interest in the process of N_2 fixation began during the HOT-9 cruise in August 1989 when a large (approximately 10^3 km^2) *Trichodesmium* "bloom" was encountered near Station ALOHA (Karl *et al.*, 1992). The HOT data set on the role of N_2 fixation was reported at an international symposium on the biology and ecology of diazotrophic microorganisms in the sea, the first special focus event of its kind (Carpenter *et al.*, 1992). Analysis of the near-surface water particulate matter showed concentration enrichments of 3375- to 7787-fold for C, N, ATP and chlorophyll *a* compared to non-bloom HOT climatologies; P was enriched only 583-fold, leading to unusually high C:P and N:P ratios (particulate matter C:N:P = 891:125:1 for bloom compared to 142:20:1 for non-bloom conditions; Karl *et al.*, 1992). In addition, dissolved N pools (NH_4^+, [$NO_2^- + NO_3^-$], DON) were all enriched within the bloom, in the case of NH_4^+, by 27-fold, whereas dissolved phosphate and silicic acid were not enriched. Although no rate measurements of N_2 fixation were made during this serendipitous encounter, these dissolved and particulate matter indices were all consistent with high rates of new N input. Based on measured ^{14}C assimilation and measured particulate C:N molar ratio of 7.1 in the bloom, and assuming a 2-m thick

near-surface layer of *Trichodesmium* that was active at this site for only 1 day per year, the authors concluded that N_2 fixation could supply 80–100 mmol N m^{-2} year^{-1}, compared to the primary production total N assimilation of approximately 2326 mmol N m^{-2} year^{-1} (Karl *et al.*, 1992). If these assumptions are reasonable, then N_2 fixation at Station ALOHA would equate to 3–4% of the total N demand for the microorganisms that inhabit that ecosystem, similar to all previous studies. However, when compared to estimates of new production (140–256 mmol N m^{-2} year^{-1}) or to N exports by sinking particles and migrant zooplankton (159–203 mmol N m^{-2} year^{-1}), N_2 fixation appears to be a significant (40–60%) source of new N.

A key to the renewed interest in N_2 fixation was a better appreciation for the quantitative role of export production and a shift in research focus from gross to net and export pelagic production. While it had been known for at least two decades that N_2 fixation could supply ~2–5% of the daily N quota of the microbial assemblage, it was only more recently realized that this was a *large* percentage of the total new N for systems like the North Pacific trades biome which export from the euphotic zone <10% of their daily organic matter production, rather than being a *negligible* source. Not since the discovery of marine N_2 fixation in 1961 (Dugdale *et al.*, 1961) had there been so much interest in this key ecosystem process, and it quickly emerged as one of the primary research foci of the HOT program.

The initial HOT investigations of marine N_2 fixation had a deliberate focus on the large filamentous, colony-forming *Trichodesmium*. This was, in part, because it forms spectacular near-surface accumulations (sometimes called "blooms") in warm subtropical and tropical seawaters worldwide, especially during periods of extreme calm (Capone *et al.*, 1997). The encounter with the 1989 *Trichodesmium* bloom near Station ALOHA led to three basic questions that, to date, remain only partially resolved (Letelier, 1994): (1) What are the mechanisms that lead to aperiodic, enhanced *Trichodesmium* biomass near the sea surface (*in situ* growth vs. physical aggregation/accumulation)?, (2) What are the fates of that *Trichodesmium* biomass?, and (3) What roles does *Trichodesmium* play in upper water column biogeochemistry and ecology? A unique aspect of this research prospectus was that it was embedded in the systematic time-series study with comprehensive ancillary data sets to place observations of *Trichodesmium* into a broader ecological context. If *Trichodesmium*, and perhaps other diazotrophic microorganisms, constitute a significant source of new N for the North Pacific trades biome, this would lead to changes in the dynamics of the N and P cycles unless there was an alternate source of P to sustain the balanced growth of the plankton assemblage. Therefore, detailed ecosystem study of the N-cycle, N_2 fixation specifically, is incomplete unless comprehensive analysis of the P-cycle is also achieved. For this reason, N_2 fixation studies at Station ALOHA were designed within a more comprehensive framework of C, P and associated bioelemental cycles. Two conceptual models were initially devised to account for the spatial N and P decoupling: (1) the upward P-flux model and (2) *Trichodesmium* P-transport model (Karl *et al.*, 1992). Alternatively, changes in the N and P cell quotas, specifically a reduction in P under P-limited growth, could decouple N and P cycles.

In the upward P-flux model, P derived from the upward transport of low density, low N:P content organic matter (e.g., lipid–P enriched organic matter; Yayanos and

Nevenzel, 1978) is taken up and assimilated by *Trichodesmium* at or near the ocean's surface. In the *Trichodesmium* P-transport model, unique physiological attributes, including the ability to store "ballast" carbohydrate, under conditions of P-limited/light-saturated metabolism near the surface, and polyphosphate under conditions of carbohydrate-based/P-saturated metabolism near or below the base of the euphotic zone, combined with their ability to alter buoyancy by gas vacuole formation, provides the mechanism for the vertical transport of *Trichodesmium* colonies based on P status (Karl *et al.*, 1992). Both models, as well as the atmospheric deposition of low N:P particles (Karl and Tien, 1997) or meridional advection of labile DOP without similar flux of labile DON (Abell *et al.*, 2000), would lead to an eventual decoupling of N and P dynamics and selection for N_2 fixing microorganisms. The observation of high frequency variations in the soluble reactive P pools in the surface waters near Station ALOHA (Karl and Tien, 1997) is consistent with the existence of one or more of these (or some other) dynamic processes at this site.

Letelier and Karl (1998) field tested the *Trichodesmium* P-transport model, or "P-shuttle hypothesis" by collecting and analyzing "rising" and "sinking" colonies at a reference depth of 100 m. The lowest N:P ratio was found in rising *Trichodesmium* colonies, which is consistent with the prediction of the *Trichodesmium* P-shuttle hypothesis. Sinking colonies were also shown to actively assimilate phosphate equivalent to 35–57% of their total cellular P content in the dark during the first 12–24 h of post-collection incubation; this result is also consistent with the hypothesis (Letelier and Karl, 1998). A subsequent analysis of the *Trichodesmium* shuttle hypothesis suggested that while limited vertical excursions in the upper 70 m of the water column are possible, deeper migrations appear unlikely unless respiration rates decrease significantly (Villareal and Carpenter, 2003). However, significant differences in the N:P ratios of sinking (N:P = 87:1) vs. ascending (N:P = 43.5:1) colonies at their study site in the western Gulf of Mexico were consistent with the P-shuttle model predictions. More recently, White *et al.* (2006a) have conducted laboratory experiments to examine the ability of *Trichodesmium* spp. to alter its C–N–P stoichiometry in response to variable P in the growth medium, and have incorporated these results into a numerical model (White *et al.*, 2006b). Their results indicate that *Trichodesmium* is capable of P-sparing under P-depleted conditions. The bulk C–N–P elemental composition of the cells was $C_{585\pm56}$: $N_{90\pm10}$:P_1, approximately six times more C and N, relative to P, than the Redfield prediction. When exposed to high external P concentrations, luxury uptake of P was observed and the stoichiometry changed significantly, approaching the Redfield ratio ($C_{96\pm8}$:$N_{16\pm1}$:P_1). They also showed that *Trichodesmium* can survive in dark, P-replete medium for periods of 3–6 days, after which they cannot recover (White *et al.*, 2006a). These data on elemental plasticity and dark survival help to constrain the temporal scale of vertical migration. Based on these and other laboratory and field observations, White *et al.* (2006b) concluded that *Trichodesmium* migrations represent as much as 10% of the P-based export flux at Station ALOHA.

Soon after the HOT time-series began, Letelier and Karl (1996) confirmed that a key N_2 fixing species, *Trichodesmium*, exists *in situ* as both free trichomes (single filaments) and in the more commonly reported colonial trichome morphologies (fusiform or "tuft" and spherical or "puff"), at Station ALOHA. Based on approximately monthly sampling between October 1989 and December 1992, the average
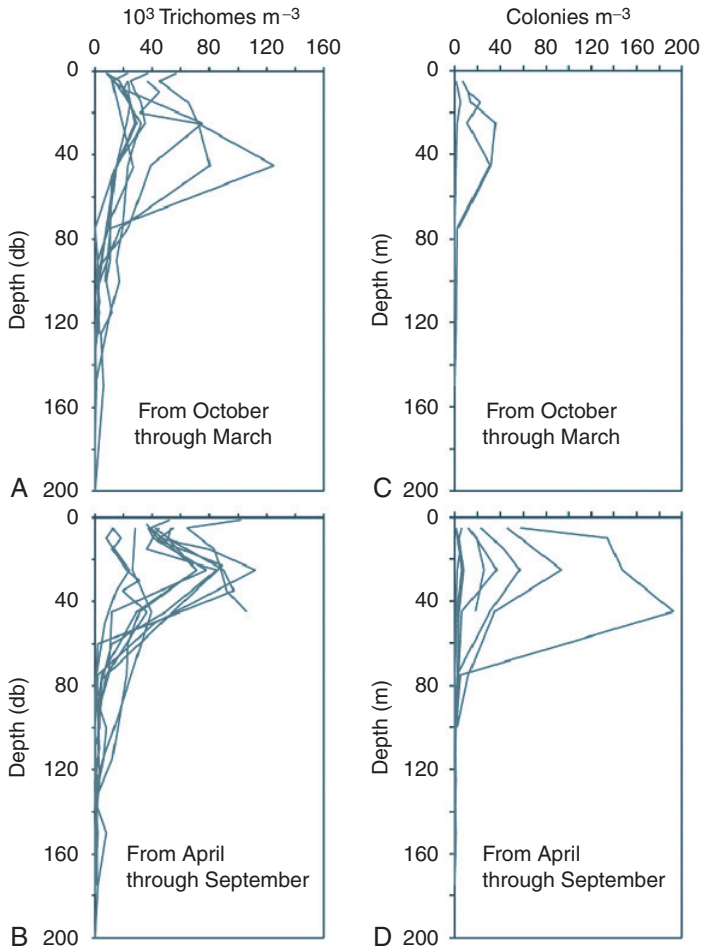
Figure 16.14 *Trichodesmium* spp. vertical distribution of single trichomes (A, C) and colonies (B, D) at Station ALOHA between October 1989 and December 1992. From Letelier and Karl (1996).

abundance of free trichomes in the upper 45 m of the water column was reported to be temporally variable ranging from 1.1 to 7.4×10^4 trichomes m^{-3}, and from 0.02 to 1.4×10^2 colonies m^{-3} (Fig. 16.14). Each colony consisted of an average of 182 filaments, and collectively colonies accounted for approximately 12% of the total biomass of *Trichodesmium* (Letelier and Karl, 1996). While the existence of free trichomes had been reported previously in the North Pacific Ocean (Marumo and Asaoka, 1974; Marumo and Nagasawa, 1976), very little attention had been paid to them because it was thought that only colonial forms of *Trichodesmium* could fix N$_2$ due to the perceived need to sustain local sub-oxic habitats within colonies (Carpenter and Price, 1976, and related papers; but also see Saino and Hattori, 1982 and Letelier and Karl, 1998 for evidence of aerobic N$_2$ fixation in single *Trichodesmium* filaments). Letelier and Karl (1996) also reported a systematic seasonal

variation in *Trichodesmium*, with higher concentrations of both free trichomes and colonies in the spring–summer period (April to September) compared to the fall–winter period (October to March). It was also reported (Letelier and Karl, 1996) that hand–sorted *Trichodesmium* filaments and colonies from the North Pacific trades biome habitat had molar C:N ratios that were indistinguishable from the Redfield ratio of 6.6, but had N:P ratios that were 250–300% higher, approaching 50:1 (compared to the Redfield N:P stoichiometry of 16:1). This P–sparing is predicted for diazotrophs growing under conditions of P limitation. Measurements of *Trichodesmium* production made during March 1990 and August 1991, based on uptake of $^{14}C\text{-}HCO_3^-$ and measured C:N stoichiometry, indicated that it may account for approximately 30% of total ecosystem new production (Letelier and Karl, 1996). The authors cautioned that this estimate should be viewed as a lower bound because these experiments were conducted under non–bloom conditions and also did not consider the potential extracellular release of NH_4^+/DON which has been observed for *Trichodesmium* during active N_2 fixation (Capone *et al.*, 1994; Glibert and Bronk, 1994; Mulholland *et al.*, 2004). This comprehensive time–series study of *Trichodesmium* (Letelier and Karl, 1996), the first of its kind in the North Pacific trades biome, lent support to the N_2 fixation–based, new production hypothesis.

The Station ALOHA investigation led eventually to a more general N-P alternation hypothesis for control of plankton rate processes, with attribution of enhanced rates of N_2 fixation to El Niño induced climate variability (Karl, 1999; Karl *et al.*, 1995; Karl *et al.*, 1997). The conceptual model (Fig. 16.15) suggested that decreased upper ocean mixing and changes in the intensity of water circulation led to enhanced nutrient limitation, increased abundance and activity of N_2-fixing microorganisms and a shift from a primarily N–limited to a primarily P–limited habitat with attendant changes in total, new and export production and nutrient cycling pathways and rates. A number of associated physical, biological and biogeochemical habitat properties and processes displayed significant change during the 1991–1992 El Niño period, and these were all consistent with enhanced N_2 fixation activity despite the lack of any direct rate measurements. A synthesis of seven years of continuous monthly measurements based on several independent data sets including (1) *Trichodesmium* abundances and estimates of their potential rates of N_2 fixation, (2) assessments of the molar N:P stoichiometries of surface ocean dissolved and particulate matter pools and a development of a 1-D model to calculate N and P mass balances, (3) seasonal variations in the natural ^{15}N isotopic abundances of particulate matter exported to the deep sea and collected in bottom–moored sediment traps, and (4) observations on the secular changes in dissolved phosphate, DOP and DON pools during the period of enhanced N_2 fixation, all strengthened the hypothesis that N_2 fixation is a significant source of new N in the North Pacific trades biome (Dore *et al.*, 2002; Karl *et al.*, 1997). From these analyses the authors concluded that N_2 fixation may contribute up to half of the N required to sustain total annual export production at Station ALOHA, a previously neglected source of new N. They further speculated that enhanced input of new N by this mechanism might result from relaxation of upper ocean mixing, the direct opposite of that derived from existing conceptual models of oceanic ecosystems (Karl *et al.*, 1997), which has profound implications for the potential impact of natural or
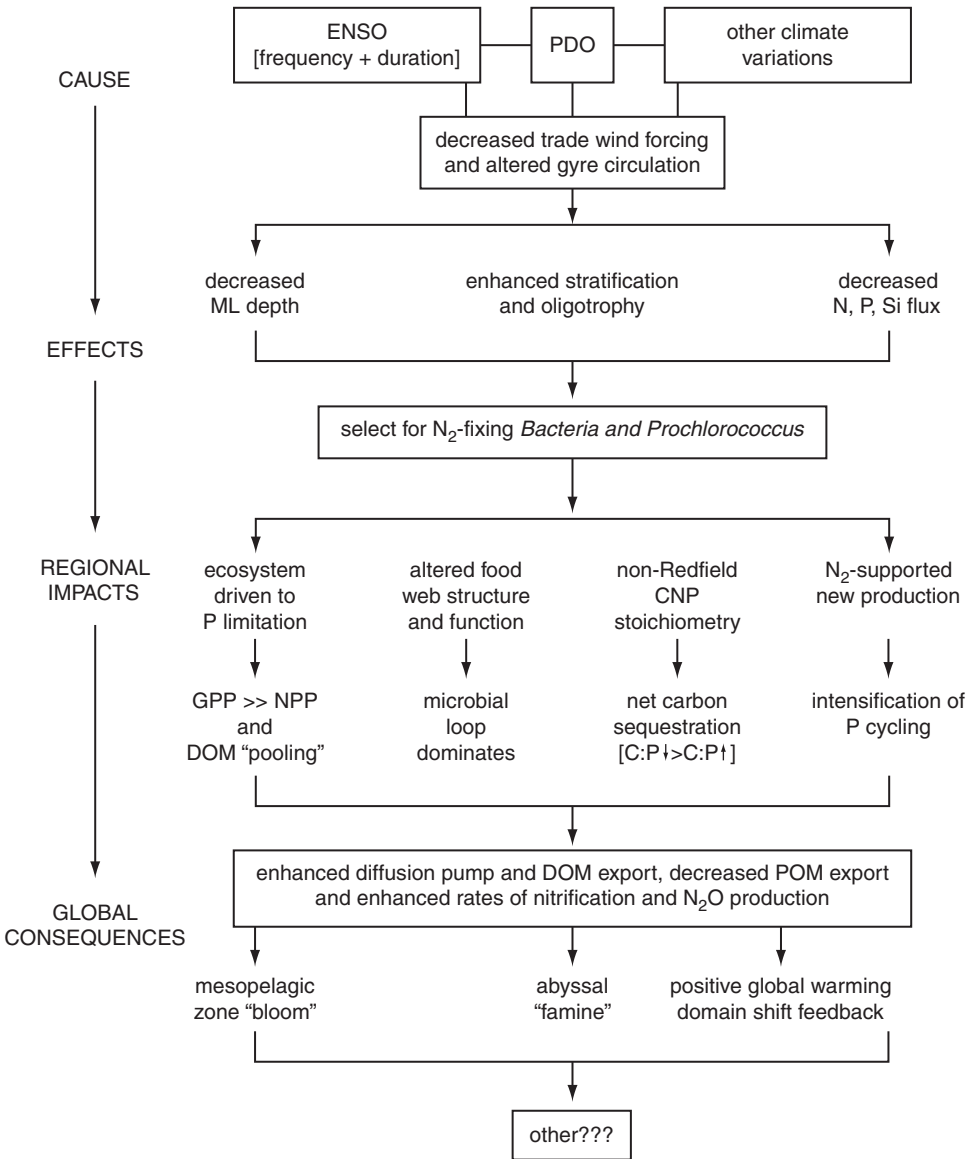
Figure 16.15 Hypothetical view of the effects of climate variability on ecosystem structure and function in the NPSG based, in part, on results obtained during the decade-long HOT research program. Changes in the stratification of the surface ocean have affected nutrient and trace element budgets and have selected for N_2-fixing bacteria and *Prochlorococcus* resulting in a domain shift from predominantly *Eukarya* to predominantly *Bacteria*. Numerous biological consequences have been observed and others are expected. From Karl (1999).

human-induced environmental change. Finally, the sources of P and Fe needed to sustain the continued production of new N supported by N_2 fixation were acknowledged as key unresolved issues (Karl, 2002).

As interest in the role of N_2 fixation was growing in the HOT program, it also emerged as a potentially important ecosystem process at the Bermuda Atlantic Time-series Study (BATS) site in the oligotrophic North Atlantic Ocean (Sargasso Sea) near the location where Dugdale *et al.* (1961) had first reported active marine N_2 fixation. The evidence was based on the observation of anomalously high nitrate:phosphate ratios in the mesopelagic zone, with a relative excess of N termed $N\star$ ($N\star$ = excess nitrate = $[NO_3^-] - 16 \times [PO_4^{-3}]$; Michaels *et al.*, 1996), and with an observed dissolved inorganic C drawdown in the summer in the apparent absence of nitrate (Bates *et al.*, 1996; Michaels *et al.*, 1994). Local N_2 fixation could explain both "mysteries" (Karl *et al.*, 2003; Michaels *et al.*, 2000), so it appeared that N_2 fixation might have been systematically underappreciated in both ocean basins. Because the HOT and BATS programs were part of the Joint Global Ocean Flux Study (JGOFS) program, there was community interest in N_2 fixation rates and controls, and various ecosystem models soon emerged to account for N_2 fixation as a potentially important source of new N for the oligotrophic regions of the global ocean (e.g., Hood *et al.*, 2001 for *Trichodesmium* at the BATS site in the North Atlantic Ocean, Fennel *et al.*, 2002 for *Trichodesmium* at Station ALOHA in the North Pacific trades biome, and Goebel *et al.*, 2007 for three types of diazotrophs also at Station ALOHA). The Fennel *et al.* (2002) model employed a mechanistic parameterization of N_2 fixation based on known or hypothesized physiological responses of *Trichodesmium* to physical conditions of the environment. The model also allowed for variable N:P stoichiometry and shifts from N- to P-control of plankton communities. The biological model was coupled to a modified Price *et al.* (1986) 1-D physical model of the upper ocean that simulated vertical profiles of temperature, salinity, evolution of the mixed layer in response to wind stress and surface heat fluxes, and mixing. The Station ALOHA simulation captured many of the key features of the environment including the vertical structure of and seasonal changes in chlorophyll a, a seasonal cycle and interannual variations in *Trichodesmium* biomass and particulate matter export, and the hypothesized N_2 fixation-driven alternation between N- and P-limitation (Fennel *et al.*, 2002).

Several research teams were also investigating basin-scale and even global-scale consequences of N_2 fixation. Gruber and Sarmiento (1997) used variations in the N:P stoichiometry of the dissolved nutrient pools (with a modified $N\star$ parameter) to estimate rates of N_2 fixation in the tropical and subtropical North Atlantic Ocean and Mediterranean Sea. They derived an estimate of 28 Tg N_2 fixed year^{-1} for these regions and suggested the global ocean rate might be as high as 110 Tg N year^{-1} (Gruber and Sarmiento, 1997). Deutsch *et al.* (2001) applied a similar $N\star$ model to the Pacific Ocean. Based on their primary assumption of N steady-state (N_2 fixation equals denitrification on the basin scale) they concluded that N_2 fixation north of $32°S$ is 59 ± 14 Tg N year^{-1}, with intensification in the western boundaries of the gyres near regional sources of Fe from atmospheric dust deposition (Deutsch *et al.*, 2001). Finally, Lee *et al.* (2002) investigated and mapped regions of the global ocean where DIC concentrations appear to be drawn down in the absence of measurable nitrate. They hypothesized, as Michaels *et al.* (1996) and others had before them, that

N_2 fixation is the mechanism for organic matter production (DIC removal) in these oligotrophic regions. Based on their analysis, the Pacific Ocean ($40°S-40°N$) had the greatest N_2 fixation-supported carbon production (0.5 Pg C $year^{-1}$), equivalent to approximately 63% of the global ocean estimate. The regional distribution within the Pacific basin again showed enhanced rates near known sources of Fe, primarily from dust deposition, re-emphasizing the probable role of Fe as a control on N_2 fixation in the global ocean.

No sooner were the new *Trichodesmium*-centric models up and running when a major discovery was reported, namely the existence of a diverse assemblage of N_2 fixing microorganisms in the North Pacific trades biome, including unicellular cyanobacteria (Zehr *et al.*, 1998, 2000, 2001). Prior to these reports, *Trichodesmium* and, to a lesser extent, the endosymbiotic associations of the N_2 fixing cyanobacterium *Richelia* with several different diatom species, had become the primary foci of marine N_2 fixation research (Capone *et al.*, 1997; Carpenter *et al.*, 1992). Nitrogenase activity by phototrophic unicells in the N-starved North Pacific trades biome might have been predicted from an evolutionary-ecological perspective, but this process was not seriously considered because it was generally thought that high O_2 concentrations would preclude diazotrophic growth of unicells in this habitat. An indication of the ecological potential of unicellular diazotrophs came when RNA was extracted to determine which organisms were expressing the *nifH* gene under *in situ* conditions. Nitrogenase gene transcripts (messenger RNA) attributable to organisms other than *Trichodesmium* and *Richelia* were detected in all samples that were analyzed from the upper portion of the euphotic zone near Station ALOHA (Zehr *et al.*, 2001); little or no *nifH* transcription was observed at depths of 150 m, and greater. This pattern of transcription could be a result of energy (light) limitation or the presence of a sufficient supply of fixed N at depth, or both. Nitrogenase gene fragments from microorganisms in the pico/nano (0.2–10 μm) size class were amplified, cloned and sequenced to establish their phylogenetic relationships to each other and to other known N_2 fixing microorganisms (Fig. 16.17). Results indicated the presence of two different cyanobacteria (termed Group A and Group B) whose *nifH* sequences were most similar to *Crocosphaera* a unicellular cyanobacterium that has been isolated from marine environments but not previously considered to be important in the sea or to the marine N-cycle in general. The ability of these unicells to fix N_2 under *in situ* conditions was suggested by experimental determination of $^{15}N_2$ assimilation into the 0.2–10 μm size fraction (Zehr *et al.*, 2001). N_2 fixation from a sample collected at Station ALOHA (25 m) in July 2000 was 10–16 pmol N L^{-1} h^{-1}, suggesting that the role of small (unicells) N_2 fixers may have been systematically underestimated and underappreciated, if not totally ignored. With the publication of these new field data from Zehr and colleagues, excitement in N_2 fixation research at Station ALOHA was at an all time high; a redirection of the research agenda, to include the possibility of N_2 fixation by the novel unicellular cyanobacterial populations, was now mandatory.

Dore *et al.* (2002) were the first to report euphotic zone depth-integrated measurements of the relative contributions of "small" (<10 μm) and "large" diazotrophs to total N_2 fixation in the North Pacific trades biome. Based on $^{15}N_2$ assimilation rate measurements from HOT cruises conducted in November 2000

and June 2001, they reported a significant contribution by <10 μm cells, ranging from 46 to 50% of the total integrated rate over the upper 0–100 m of the water column and up to 100% of the total rate for individual samples (Dore *et al.*, 2002). Because the whole water was collected using standard CTD-rosette bottle methods, it is likely that the sampling protocol selected against the very large *Trichodesmium* colonies and *Rhizosolenia* aggregates, if present, while quantitatively sampling the unicellular cyanobacteria, diazotrophic proteobacteria and free *Trichodesmium* trichomes, so in some ways this sampling/experimental design may have been biased towards detection and relative importance of the small diazotrophs. Furthermore, a full quantitative assessment could not be achieved without proper accounting of the N_2 fixation that occurs during the sometimes massive, but stochastic blooms of the larger diazotrophic assemblages which are undersampled even with the approximately monthly HOT program sampling frequency. It came as no surprise then, that the measured rates of N_2 fixation were considerably less than those estimated using a model based on the measured sediment trap derived PN export flux and its stable N isotopic composition, a value which they estimate to be 40 mmol N m^{-2} $year^{-1}$ (Dore *et al.*, 2002). Results from the latter data sets indicated that N_2 fixation accounted for 48% of the new N over the 11-year period (1990–2000) with considerable interannual variation (minimum 36% in 1992, maximum 69% in 1999; also see Fig. 16.16). They concluded that, although discrete $^{15}N_2$ bottle incubations are valuable for elucidating patterns and size distribution of N_2 fixation with time and, perhaps, for evaluating nutrient controls of *in situ* N_2 fixation, they are inadequate for assessing time- and space-integrated rates of N_2 fixation in the biome as a whole. Nevertheless, the results of Dore *et al.* (2002), and several subsequent studies (Grabowski *et al.*, 2008; Zehr *et al.*, 2007; Montoya *et al.*, 2004), documented an active population of <10 μm N_2 fixing microorganisms that had not previously been considered in North Pacific trades biome N budgets (see Table 16.5).

Church *et al.* (2005a) used the emerging information about *nifH* diversity and the probable role of unicellular cyanobacteria to design specific oligonucleotide primers and probes that facilitated an enumeration of the various diazotrophs using quantitative polymerase chain reaction (QPCR) of *nifH* phylotypes at Station ALOHA. Initial targets for this analysis included *Trichodesmium*, the unicellular cyanobacterial Groups A (closely related to *Cyanothece*) and B (closely related to *Crocosphaera/Synechocystis*; Fig. 16.17), and a novel "Cluster III" bacterial group that was phylogenetically similar to strict anaerobes. Analysis of total particulate DNA, and specific size fractions thereof, provided a quantitative estimation of the abundances of the various diazotroph groups, at least in terms of *nifH* genes. As with previous studies of cell enumeration and N_2 fixation rates, the vertical distributions of *nifH* genes were most abundant in the near-surface waters (approximately 2×10^5 *nifH* copies per liter at 25 m), but decreased by several orders of magnitude throughout the euphotic zone (Fig. 16.18A). Unicellular cyanobacteria (sum of Groups A plus B plus Cluster III) *nifH* gene copies exceeded *Trichodesmium* gene copies by 1–2 orders of magnitude. The Cluster III *nifH* phylotype was most dominant in the lower euphotic zone (>100 m). The use of QPCR to enumerate *nifH* gene abundances and to establish diazotroph population structure and dynamics is a novel technique that relies upon several as yet untested assumptions regarding uniform
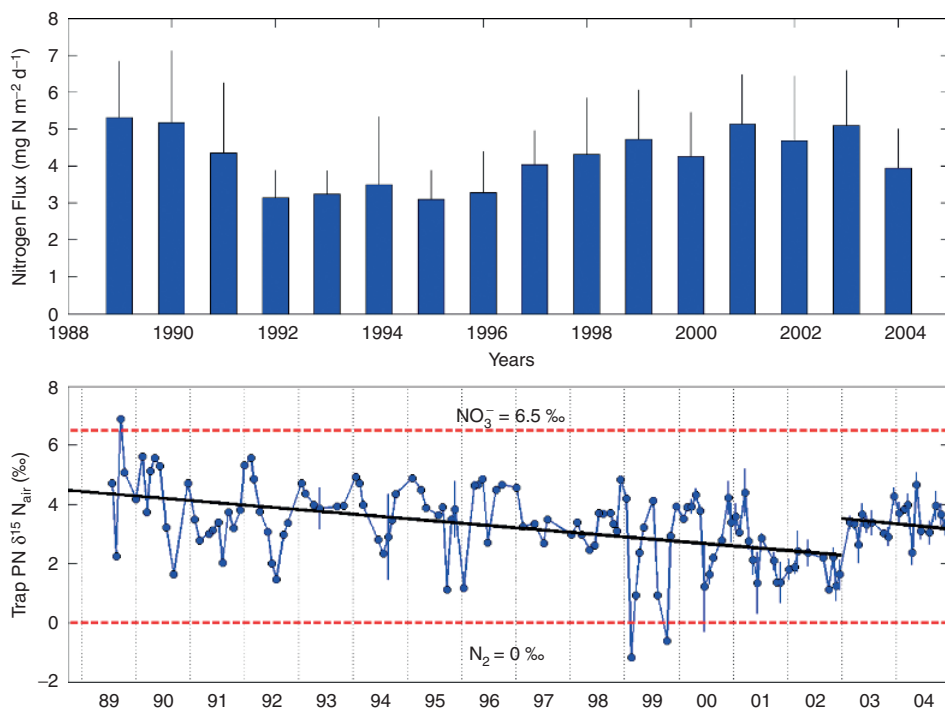
Figure 16.16 Time series of particulate nitrogen (PN) export and isotopic composition determined from 150-m floating sediment trap collections at Station ALOHA. (Top) PN flux (μmol N $m^{-2} d^{-1}$). Error bars represent $\pm$SE of three to six individual trap measurements. (Bottom) Stable nitrogen isotopic composition ($\delta^{15}N$) of trap-collected material ($^o/_{oo}$ vs. air N_2). Error bars, where shown, represent $\pm$SE of two to three individual trap measurements. Dotted lines represent the isotopic compositions of exported PN expected when supported entirely by nitrate ($6.5^o/_{oo}$) or entirely by dinitrogen gas ($0 ^o/_{oo}$). The solid line indicates a decreasing trend determined by linear regression. The trend break at the end of 2002 remains to be explained. Updated and revised from Dore *et al.* (2002).

DNA extraction and amplification efficiencies, and identification of the number of gene copies per cell among the disparate diazotroph groups. However, these gene-based techniques provide a quantitative approach to the important question of diazotroph diversity. Their initial field results opened a new window of observation for the study of N_2 fixation in the North Pacific trades biome.

In a follow-on study, Church *et al.* (2005b) examined both the presence and expression of specific N_2 fixing microorganisms at Station ALOHA. Temporal patterns of nitrogenase expression were estimated by reverse-transcribed QPCR (RT-QPCR) of the phylotype-specific *nifH* gene transcripts. Their results revealed unexpected and complex, but highly ordered, diel patterns with certain phylotypes exhibiting maximum expression around mid-day and others around midnight (Fig. 16.18B). The concentrations of selected *nifH* cDNA copies (e.g., Heterocyst-1 and Group B cyanobacteria) varied by more than 3–4 orders of magnitude over a 6-h period (Church *et al.*, 2005b). While the authors did not confirm a quantitative

Table 16.5 Size Distribution of N$_2$ Fixation in the Upper Portion of the Water Column (0–75 m) at Station ALOHA

Cruise (Date)	Depth (m)	Wholerate[a]	<10 μm rate[a]	>10 μm rate[a]	Contribution of <10 μm
HOT–165	5	0.30	0.35	<DL[b]	100
(Nov 2004)	25	0.27 ± 0.01	0.13 ± 0.02	0.14 ± 0.02	48
	45	0.31 ± 0.04	0.19 ± 0.19	0.12 ± 0.19	61
	75	0.07 ± 0.02	0.04 ± 0.02	0.03 ± 0.03	57
HOT–167	5	0.78 ± 0.08	0.50 ± 0.01	0.28 ± 0.08	64
(Feb 2005)	25	0.93 ± 0.33	0.46 ± 0.04	0.47 ± 0.33	49
	45	0.76 ± 0.07	0.52 ± 0.06	0.24 ± 0.09	68
	75	0.16 ± 0.04	0.05 ± 0.03	0.11 ± 0.05	31
HOT–168	5	2.66 ± 0.61	1.91 ± 0.46	0.75 ± 0.76	72
(Mar 2005)	25	1.78 ± 0.27	1.48 ± 0.25	0.30 ± 0.37	83
	45	1.17 ± 0.05	1.90 ± 0.02	0.27 ± 0.05	77
	75	0.15 ± 0.08	0.03 ± 0.01	0.07 ± 0.08	53

[a] Rates are expressed as mean ± 1 standard deviation ($n = 3$) when replicated.
[b] DL = detection limit, which in this study was 0.03 μmol m^{-3} day^{-1}.
Rates were measured using ^{15}N$_2$ tracer, and size-fractionated following a 24-h incubation. N$_2$ fixation rates (μmol N m^{-3} day^{-1}) for whole water, <10 μm, and >10 μm (calculated by difference) are shown, as well as percent contribution by small (<10 μm) diazotrophs to the total.
Source: Grabowski *et al.* (2008).
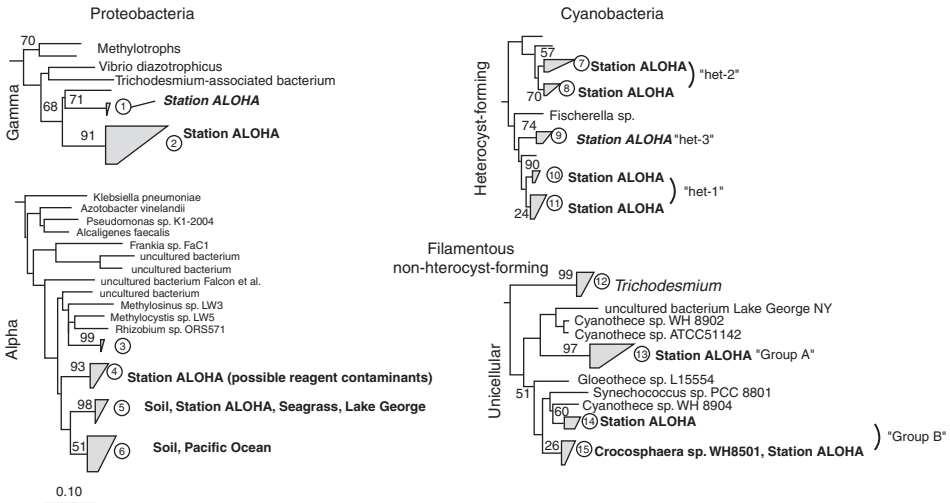
Figure 16.17 Phylogenetic tree showing major *nifH* DNA sequence phylotypes found at Station ALOHA. Cyanobacterial lineages include three heterocyst-forming groups that are associated with diatoms, free-living filamentous nonheterocyst forming *Trichodesmium*, and the two unicellular cyanobacterial groups (Groups A and B). Bootstrap values of 100 replicates are shown at nodes. Scale bars = 0.1 substitutions per site. From Zehr *et al.* (2007).

coupling between *nifH* gene transcription and *in situ* rates of N$_2$ fixation, the former is ultimately required for the latter and, at least for *Trichodesmium*, the diel transcription pattern with highest activity during daylight hours (Church *et al.*, 2005b) is identical to diel N$_2$ fixation patterns that have been reported for *Trichodesmium* cultures (Letelier and Karl, 1998). There are at least two important implications of this work. First, and foremost, different *nifH*-containing phylotypes appear to have different responses to daily fluctuations in irradiance for physiological reasons that are not entirely clear at the present time. These distinct responses may have implications for *in situ* growth and removal processes and, ultimately, for energy flow and food web dynamics. Previous field observations presented conflicting data on the relationships between light and N$_2$ fixation, and on the rates of N$_2$ fixation in the dark. In retrospect, these variable results may have derived, in part, from variations in the *nifH*-containing phylotypes in the microbial assemblages under investigation, or in the design and duration of the incubation experiments. Second is the potential problem of sampling, measurement and scaling. If certain phylotypes alter their *nifH* transcription by 3–4 orders of magnitude over diel time scales, it may be difficult to constrain *in situ* rates of N$_2$ fixation from short-term (1–2 h for C$_2$H$_2$ reduction) assays or to reconcile the results of short- (C$_2$H$_2$) or even longer-term (^{15}N$_2$) incubation studies with geochemical indicators of N$_2$ fixation (e.g., Dore *et al.*, 2002). Accurate scaling from hourly to daily to annual rates of N$_2$ fixation, and from discrete water samples to basin scales clearly has not yet been achieved.
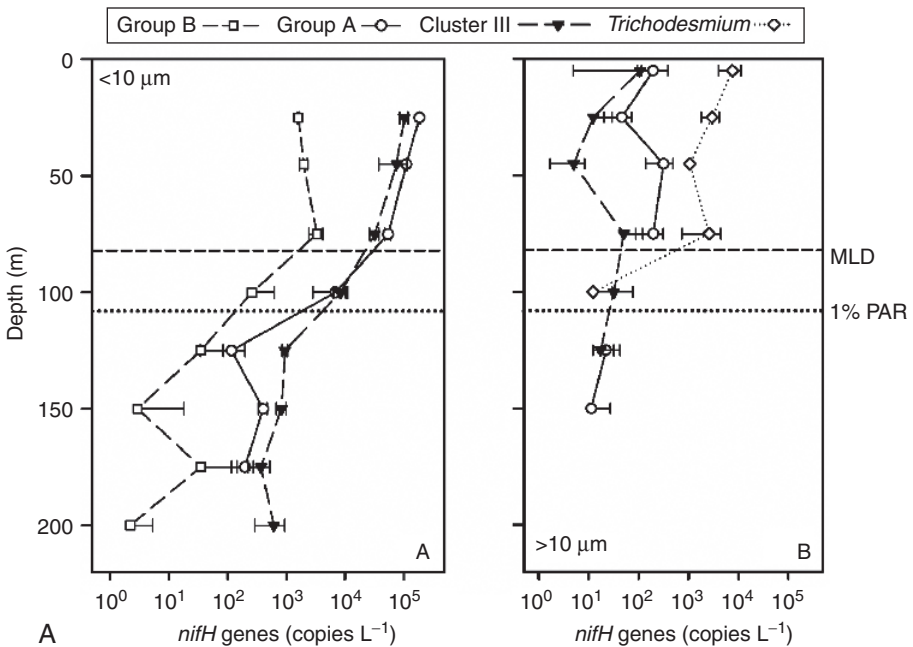
Figure 16.18 Distribution, abundance and temporal dynamics of N$_2$ fixing bacteria at Station ALOHA. (A) Vertical profiles of <10 μm (left) and >10 μm (right) *nifH* phylotypes in December 2002 relative to upper mixed-layer depth (dashed line) and 1% surface radiance isopleth (dotted line). Error bars are ±1 SD of triplicate QPCR (45 cycles) reactions. From Church *et al.* (2005a).

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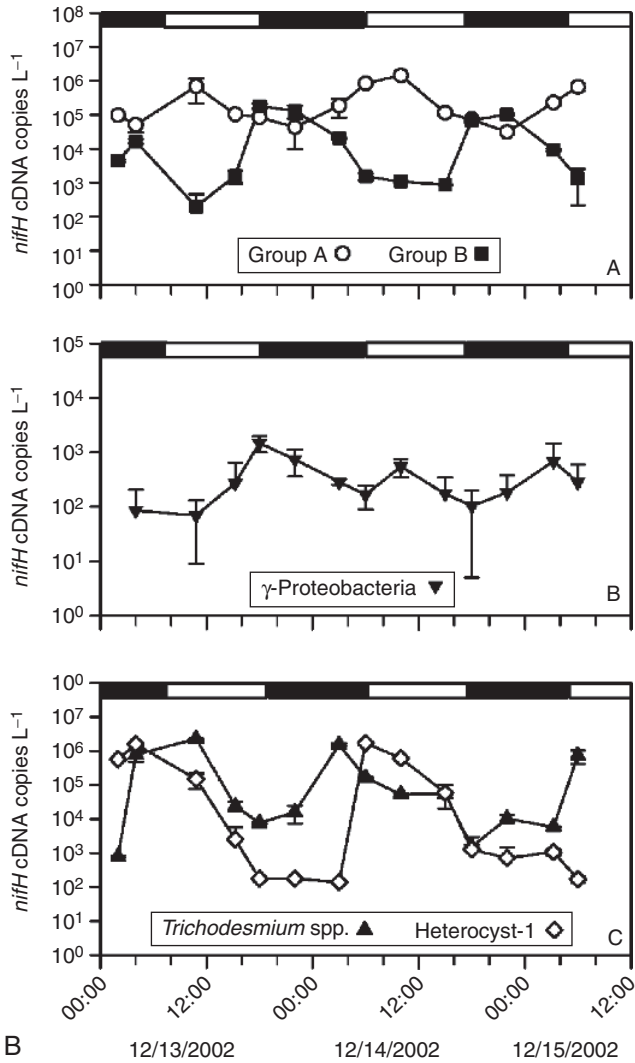
Figure 16.18 cont'd (B) Temporal patterns of *nifH* transcription (*nifH* cDNA copies per liter) by five unique phylotypes at a reference depth of 25 m in December 2002. Error bars are ± 1 SD of mean cDNA concentrations from triplicate QPCR reactions. From Church *et al.* (2005b).

The current paradigm for N_2 fixation in the North Pacific trades biome is one where at least two independent microbial assemblages and ecosystem processes contribute to new production, namely the "background state" wherein a relatively low but relatively constant rate of new N import is supported by the combined activities of pico- and nano-diazotrophs, and the aperiodic "bloom state" wherein large filamentous, colonial and aggregate forming diazotrophs (*Trichodesmium* and/or endosymbiont-containing diatoms) dominate the new N-cycle. Despite the fact that the latter may be more "noteworthy" than the former, and have received a disproportionate

amount of research effort to date (Capone *et al.*, 1997, 1998, 2005), it is not possible to provide an accurate accounting of the relative importance of the two pathways because of inadequate sampling of the blooms in time and in space (White *et al.*, 2007; Dore *et al.*, 2008). Both pathways are important to the N economy of the sea and both are worthy of additional investigation. Because size matters among the planktonic assemblages of the open sea, the short- and perhaps long-term fates of the new N delivered by these two independent pathways are likely to be very different and may require refined models that have an explicit representation of diazotrophy by the two alternate pathways (Fig. 16.19). This remains one of the contemporary challenges in marine N-cycle research.
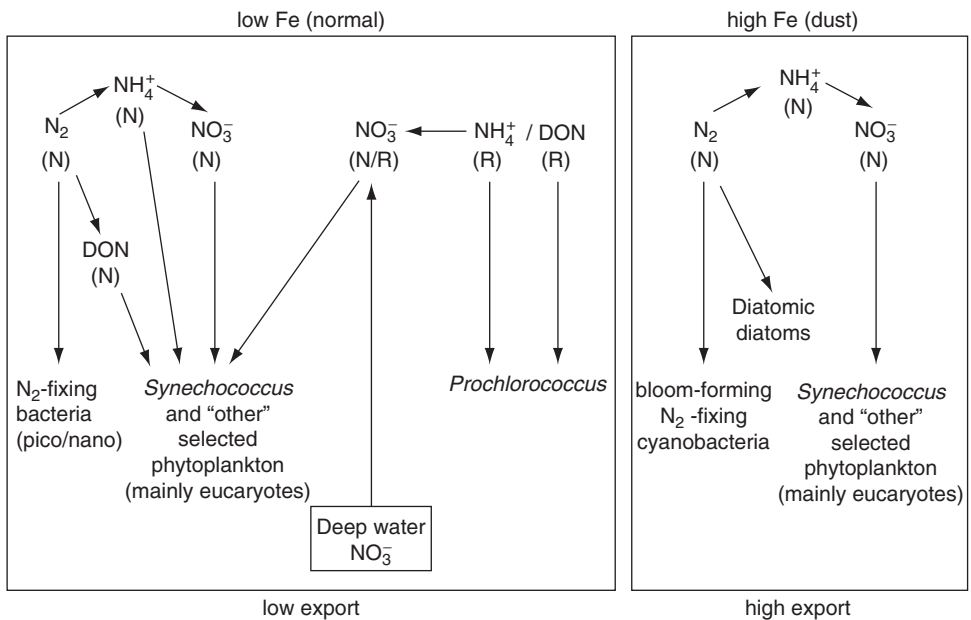
Figure 16.19 Conceptual view of a revised version of the new vs. regenerated nutrient paradigm for primary production in the trades biome. On the left is the background or normal state (low Fe) where allochthonous inputs of N are dominated by upward diffusive flux of NO_3^- from below with a variable subsidy from N_2 fixation by small, unicellular cyanobacteria. N resource partitioning among the dominant phototrophs is a key to species co-existence, especially for *Synechococcus* and *Prochlorococcus*. The (N) and (R) designations refer to new and regenerated N, respectively. A fundamental difference between this view and the one originally proposed by Dugdale and Goering (1967) is the novel sources of "new" N ($NH_4^+/DON/NO_3^-$) from N_2 fixation and subsequent nitrification. In this revised view, much of the NH_4^+ is "new" N and much of the NO_3^- is "recycled" N which makes a straightforward accounting very difficult. Following a dust deposition event (right) there is a selection for rapidly growing, bloom-forming N_2-fixing cyanobacteria (including the "diatomic diatoms", the large diatoms with endosymbiotic N_2 fixing cyanobacteria) and a transient shift in the source of new N to a N_2 fixation dominated system. Following bloom termination there is a large export event that is a key to net carbon sequestration. From Karl (2002).

3.4. Aperiodic delivery of nutrients and consequences for ecosystem metabolism

Long-term time-series studies are ideally suited for investigations of subtle habitat changes, stochastic events and complex interdependent ecological phenomena that affect oceanic biogeochemical cycles, especially N dynamics. Despite their acknowledged importance, oceanic time-series investigations are rare. In the North Pacific trades biome, four time-series programs have been initiated over the past four decades but only one, HOT, is ongoing (Karl and Lukas, 1996). The Climax series ran for nearly two decades, from approximately 1968 to 1985. Of the 22 research cruises that were completed during the 17-year observation period, four were in 1973, three in 1985, two each in 1971, 1972, 1974, 1976, and 1983 and one each in 1968, 1969, 1977, 1982, and none in 1970, 1975, 1978, 1979, 1981, and 1984. Two shorter time-series programs, Gollum and VERTEX, lasted less than two years with thirteen, 2-day cruises on approximately monthly intervals and seven, 7-day cruises on approximately 3-month intervals, respectively (Karl and Lukas, 1996). None of these time-series programs supported a cruise frequency sufficient to observe either the high frequency event scale phenomena (days to weeks) or the lower frequency (subdecadal) regime shifts that are now considered crucial for a comprehensive understanding of the marine N-cycle (Karl *et al.*, 2001b). Furthermore, none of these programs seriously considered material exchange with the atmosphere, a process that is emerging as potentially important for the delivery of fixed N to open ocean ecosystems.

Prospero and Savoie (1989) reported total atmospheric concentrations of 0.3–0.4 µg NO_3^- m^{-3} for the North Pacific trades biome. Seasonal and regional variability in atmospheric NO_3^- was directly related to dust concentrations with maxima in spring. These continental sources of dust, and associated excess N, are predominantly anthropogenic and, therefore, highly susceptible to changes associated with industrial emissions and land use practices (Galloway *et al.*, 2004). Prospero and Savoie (1989) estimated that approximately 1.0 Tg (Tg = 10^{12} g) of NO_3^--N derived from continental sources is deposited annually into the North Pacific Ocean lying outside the coastal and equatorial regions. If we assume that approximately 50% of the total is delivered to the North Pacific trades biome (15°N–35°N and 135°E–135°W, an area of approximately 2×10^7 km^2) then the mean atmosphere to ocean flux of NO_3^- in this region would be 25 mg N m^{-2} year^{-1}, which is lower by approximately an order of magnitude than the fixed N delivery rate reported by Duce (1986), based on direct measurements of wet and dry deposition at a station near Hawaii.

In addition to NO_3^-, delivery to the surface ocean of atmospheric NH_4^+ is also potentially important though poorly documented for the North Pacific trades biome at the present time. Clarke and Porter (1993) reported significant NH_4^+ concentrations in aerosols coincident with local enrichments in near-surface chlorophyll, suggesting a biogenic source or control. Estimates of the sea-to-air flux of NH_4^+ in the equatorial Pacific biome were 10 µmol N m^{-2} day^{-1}, a value that is comparable to the downward flux of PON from the euphotic zone. Presumably, high rates of net primary production and coupled ammonification leads to changes

in local pH and increases in pNH_3 resulting in a net flux of NH_3 out of the surface ocean. Atmospheric NH_3 eventually reacts to form NH_4^+-enriched aerosols that partly reflux back into the more oligotrophic regions of the North Pacific trades biome north of 5°N latitude (Clarke and Porter, 1993). This translocation of fixed N, to our knowledge, has not been considered in the North Pacific basin-scale marine N-cycle nor in the ecology of the trades biome.

During the ADIOS I expedition in March–April 1986, DiTullio and Laws (1991) observed a dust deposition event that was coincident with, and perhaps caused by, a significant low pressure disturbance at 26°N, 155′W in the North Pacific trades biome. This stochastic event resulted in a 72% increase in submicron-sized autotrophic N-assimilation, and a change in the f-ratio from 0.1 before the storm to 0.28 after the dust deposition event. Turbulent mixing of NO_3^- from below the euphotic zone was determined not to be the source of the new N required to support this PON production pulse. Rather this picoplankton bloom was triggered by the simultaneous delivery of NO_3^- and iron from the atmosphere (DiTullio and Laws, 1991). Although they observed near-surface water (0–40 m) enrichments of NO_3^-, no NO_3^- uptake rates were reported. The enhanced total N assimilation could have resulted from NO_3^- deposition, as they suggested, or alternatively could have been a consequence of dust-derived NH_4^+ (with local nitrification accounting for the NO_3^- enrichments) or iron-stimulated N_2 fixation (again with local nitrification to explain the NO_3^- enrichments). Karl et al. (1992) previously reported elevated NO_3^- concentrations during a Trichodesmium bloom event near Station ALOHA suggesting a coupled N_2 fixation-nitrification pathway (i.e., $N_2 \rightarrow NH_4^+ \rightarrow NO_3^-$). Because Prochlorococcus, the dominant phytoplankter in these waters, may not be able to assimilate NO_3^- (Moore et al., 2002; Rocap et al., 2003; but see Casey et al., 2007), the conceptual model presented by DiTullio and Laws (1991) may be in need of revision. The delivery of iron rather than N per se may have been the ultimate cause of the enhanced rates of production and export.

Since late 1988, the approximately monthly HOT program cruises have successfully established robust climatologies for many of the basic physical, chemical and biochemical parameters in the region of Station ALOHA, but more needs to be done. The use of remote instrumentation deployed on Earth-orbiting satellites, geostationary deep-sea moorings, Lagranian drifters and autonomous gliders and vehicles have supplemented the mostly ship-based observations, especially over the past few years of HOT. It has recently been hypothesized that high frequency, aperiodic net autotrophic "bloom" events sustain what otherwise appears to be net heterotrophy in the North Pacific trades biome (Karl et al., 2003; Williams et al., 2004), and perhaps elsewhere. Neither the cause, nor the full ecological consequences of these stochastic events are known at the present time. Net autotrophic events, similar to those seen in moored ocean observing platform data sets (Emerson et al., 2002; Karl et al., 2003), can be reproduced in shipboard perturbation experiments by allochthonous nutrient additions (McAndrew et al., 2006), so stochastic additions of critical growth-limiting nutrients from deep waters below or from the atmosphere above could result in short-term "greening" of the gyre.

In the North Pacific trades biome, the N:P ratios of dissolved inorganic nutrients (i.e., NO_3^-, PO_4^{3-}) and total dissolved nutrients (i.e., the sum of inorganic plus organic TDN:TDP) are significantly different from each other and from the mean assimilation ratio required for net biomass production (Karl *et al.*, 2001a). Whereas the NO_3^-:PO_4^{3-} ratios in the upper 300 m of the water column (and especially the upper 0–100 m where dissolved organic nutrients comprise a significant proportion of the total inventory; Fig. 16.20A), are well below 16N:1P and in the upper water column well below 1N:1P, the corresponding TDN:TDP ratios are well above, averaging approximately 20–25N:1P. While it is generally assumed that NO_3^- and PO_4^{3-} are available to most plankton, the bioavailability of the DON and DOP pools is unknown due to the fact that they remain poorly characterized at the present time. There are systematic and opposing depth-dependent changes in nutrient concentrations and bulk stoichiometry. The greatest changes are observed at, or
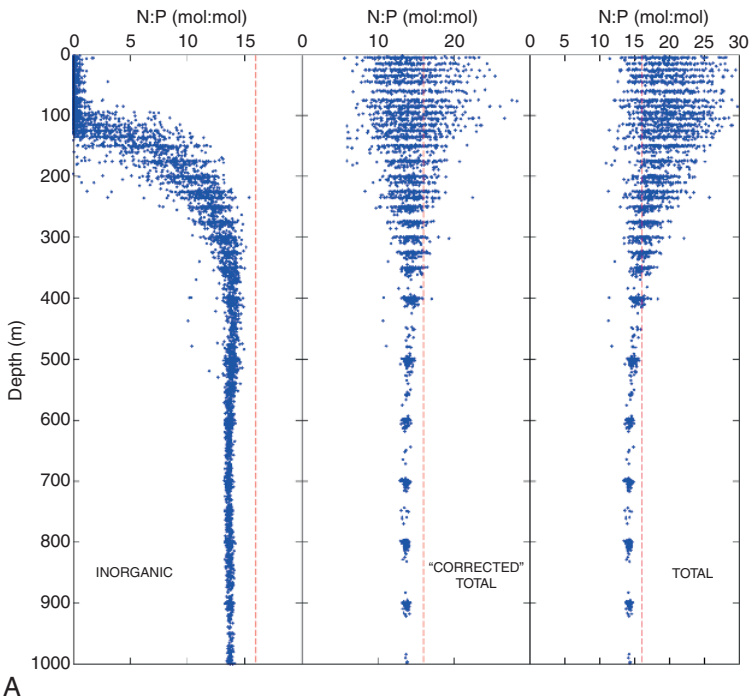
Figure 16.20 Nitrogen–to–phosphorus (N:P) ratios at Station ALOHA. (A) Depth profiles of N:P for inorganic (NO_3^-:PO_4^{3-}) and total (TDN:TDP) pools showing fundamentally different depth trends relative to the 16N:1P Redfield ratio, which is shown as a vertical dashed line in each plot. The elevated N:P (>16) for the total dissolved pool, especially in the upper 100 m of the water column, indicates an excess of N relative to the P requirements for biomass production, if all DON and DOP are biologically available. The middle plot shows the depth dependence for the stoichiometric relationships if the DON and DOP pools are "corrected" for residual deep water concentrations (DON = 2.23 μM and DOP = 0.04 μM, respectively) to remove the contribution of the recalcitrant pools. After this correction, the near-surface N:P appears to converge near the Redfield ratio with a broader envelope of values near the surface. This stoichiometry of the DOM pool may be an important factor in the selection for, or against, N_2 fixing microorganisms. From Karl *et al.* (2001a).

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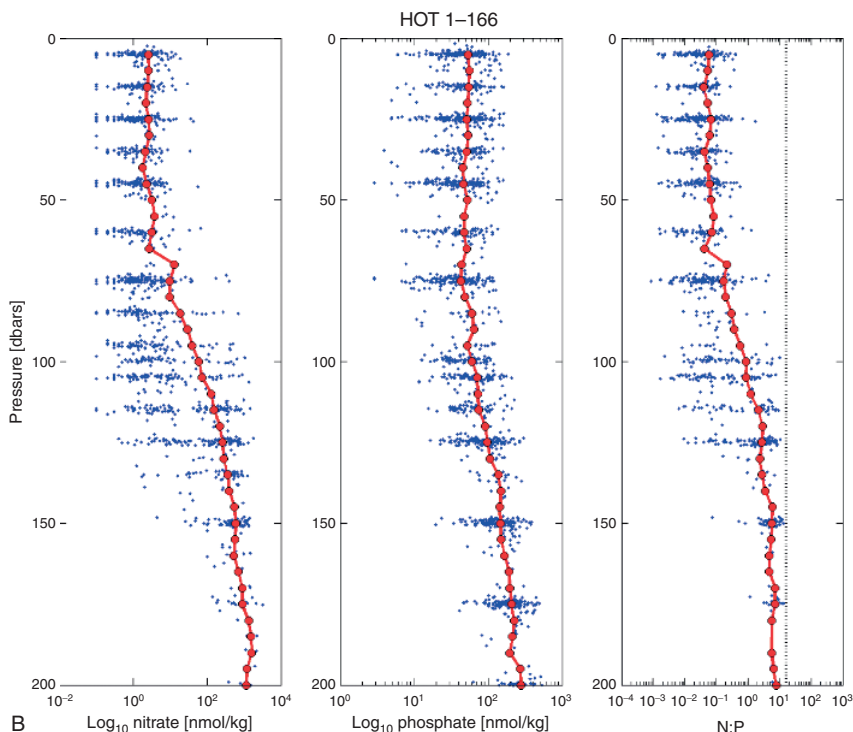
Figure 16.20 cont'd (B) Near-surface distributions (log₁₀ concentrations) of NO_3^- and PO_4^{3-} and the NO_3^-:PO_4^{3-} ratios at Station ALOHA for the period 1989–2004. The large solid circles are the mean values observed and the smaller data points indicate all observations. The dotted vertical line in the plot on the far right is the Redfield reference ratio of 16N:1P.

just below, the water depth where net nutrient delivery occurs mainly by the process of turbulent diffusion. Phototrophic microorganisms that inhabit the zone between the energy-limited/nutrient-sufficient deeper water and energy-sufficient/nutrient-limited shallower waters are among the first to assimilate and reduce NO_3^-, thereby re-setting the N-cycle.

Much has been written on phytoplankton bloom phenomena, mostly in the context of the vernal light–nutrient dynamics (e.g., Sverdrup's critical-depth model; Sverdrup, 1953), and to a lesser extent about fall blooms resulting from density destratification-induced nutrient injections. However, North Pacific trades biome blooms—when they occur—appear mostly in late summer or early fall when the water column is well stratified and mean light levels are declining, not increasing. These blooms have been grossly undersampled, except by ocean color satellites that have consistently observed them, especially in the eastern portion of the gyre (Wilson, 2003; Wilson et al., 2008; Dore et al., 2008). These are significant events; some open ocean blooms cover more than 350,000 km² and last 4 months (Wilson, 2003). Various potential mechanisms have been proposed, including nutrient injection by local atmospheric disturbance, breaking internal waves, nutricline shoaling from passing Rossby waves, cyclonic eddy pumping of nutrient-enriched deep water, atmospheric deposition of Fe and/or P (e.g., Cipollini et al., 2001; DiTullio and Laws,

1991; Leonard *et al.*, 2001; Letelier *et al.*, 2000; McGowan and Hayward, 1978; Sakamoto *et al.*, 2004; Wilson, 2003), and the possibility of vertically migrating phytoplankton (Villareal *et al.*, 1999), as previously mentioned.

Regardless of the mechanism(s) involved, it is almost certain that nutrient loading is a necessary prerequisite to biomass accumulation because the background nutrient state is not sufficient to allow for the net accumulation of plankton biomass. The possible exception to this rule may be the physical accumulation, near the surface, of an otherwise dispersed assemblage of chlorophyll-containing microorganisms, but a physical accumulation of plankton would preclude net growth of the aggregated cells in the nutrient-depleted near-surface habitat. The response of the microbial assemblage to aperiodic nutrient injections will depend on both their frequency and duration, and may result in either a local enrichment of species that are already abundant (e.g., *Synechococcus*; Glover *et al.*, 1988) or a shift in the species composition of the community, especially a selection for large rapidly growing diatoms and other eukaryotes (Cullen *et al.*, 2002). Indeed, it has recently been reported that eddy-induced upwelling of nutrients near Hawaii stimulated a diatom bloom but led to the selective export of particulate silica to depth relative to carbon and nitrogen for reasons yet unexplained (Benitez-Nelson *et al.*, 2007). This overprinting of the microbial food web by the classical diatom-copepod-fish food chain has significant consequences for energy transduction and the export of organic matter.

The trajectory of processes in the perturbed ecosystem state depends critically on the physical delivery mechanism and whether N, P and Fe are co-delivered or not (Karl, 2002). If, for example, Fe and P are deposited by atmospheric dust then the system will most likely select for diazotrophs near the ocean's surface. Excess N_2 fixation could lead to the accumulation of NH_4^+, bioavailable DON (e.g., amino acids) and, via nitrification, NO_3^- (Karl *et al.*, 1992). These new sources of fixed N could trigger a secondary bloom of non-diazotrophic phototrophs. Alternatively, if deep water is the source of the nutrients, then biological processes near the top of the nutricline (~100 m) may be the first to respond. For example, Goldman (1993) has shown that very large phytoplankton cells, particularly diatoms, normally found in low abundances are among the most successful competitors at low light levels following the episodic injection of new nutrients from beneath the euphotic zone. These large, rare, potentially rapidly growing species have few predators in the background ecosystem state, so their biomass can accumulate rapidly following the introduction of new nutrients. With time, these cells can aggregate into very large masses (Alldredge and Gotschalk, 1989; Carpenter *et al.*, 1977) and either sink or be grazed by larger predators normally feeding at a much higher trophic position (Goldman, 1993). In their "wake," these subsurface diatom blooms would leave behind a new production oxygen signal, an inorganic carbon deficit and any residual non-limiting nutrients. Because the $NO_3^-:PO_4^{3-}$ ratio of the upwelled waters at Station ALOHA is much lower than the 16N:1P assimilation ratio in diatoms, excess P would tend to accumulate. This selective retention would lead to the upward diffusion of P, relative to N, and would eventually decouple N–P dynamics. Selective P retention would lead to a habitat that is conducive for the growth and proliferation of diazotrophs, and to a secondary N_2–based new production bloom in the well-lit regions of the euphotic zone (Fig. 16.20B). The N_2 fixing assemblages would import new N and eventually alter the N:P stoichiometry of sinking particulate matter,

in favor of N (i.e., molar N:P ratio of exported POM = 25–40 compared to the expected Redfield ratio of 16N:1P; Karl *et al.*, 2001a). Over time, the subeuphotic zone remineralization of this high N:P sinking flux would be expected to increase the $NO_3^-:PO_4^{3-}$ ratio at the top of the nutricline to an extent that subsequent upwelling events of these regenerated nutrients might result in fundamentally different successional patterns for the planktonic assemblages (Karl, 2002).

Wiegert and Penas-Lado (1995) compared the effects of upwelled pulses of nutrients to a constant supply of an equivalent annual flux. In their simulation of an open ocean pelagic community, nutrient pulsing produced a rich dynamical behavior and complex trophic structure that was not present under constant nutrient supply. Furthermore, reduced mixing can also lead to complex behavior and ''advection-diffusion instability'' for phytoplankton assemblages located in the deep chlorophyll maximum zone at Station ALOHA (Huisman *et al.*, 2006). Based on model results, changes in mixing rate which could result from greenhouse gas-induced warming of the global ocean can generate oscillations and chaos in phytoplankton biomass and species composition, thereby impacting food web structure, primary production and export. Thus aperiodic fertilization of the North Pacific trades biome can be expected to create boom and bust cycles that, depending upon their frequency and duration, may not be adequately sampled even by field-intensive time-series programs like HOT (Gaines and Denny, 1993; Katz *et al.*, 2005).

4. Epilogue

A major, but currently underappreciated, feature of the marine N-cycle is that it is solar-powered. Energy, ultimately derived from sunlight, is used to reduce all partially or fully reduced forms of N (e.g., NO_3^-, NO_2^-, N_2) to the level of NH_4^+ either directly by phototrophic microorganisms or indirectly via heterotrophs that depend upon the phototrophs for a continued supply of energy in the form of organic carbon. Upon death by grazing, viral lysis or autolysis, and during the remineralization of the POM, reduced dissolved N compounds including both organics (e.g., proteins and nucleic acids and their respective monomeric constituents, urea, vitamins) and NH_4^+ are released to the surrounding waters. A large proportion of the total flux of reduced N fuels the next round of combined photo- and heterotrophy, especially in near-surface waters. By utilizing NH_4^+/DON rather than a more oxidized form of N, an organism conserves energy and conducts a more efficient metabolism which would be of selective value in an energy-limited habitat like the open sea. Eventually, reduced N is oxidized back to N_2/N_2O or NO_3^-. During this coupled ammonification/nitrification process the remaining bioavailable energy is extracted from the reduced N by specialized *Bacteria* and *Archaea* that can sustain chemolithoautotrophic growth in the absence of energy in the form of sunlight or reduced carbon compounds.

Based on the cumulative data base of N pools and fluxes in the North Pacific trades biome, but relying largely on the HOT program accomplishments over the past two decades, a quantitative N budget is beginning to emerge. This contemporary view should be considered a ''work in progress'' and may change as new discoveries are made and new methodologies are developed and employed. In particular, high

frequency and, perhaps, unattended measurements of key N-cycle components will be necessary to ensure that time-integrated fluxes properly account for stochastic or seasonally-phased phenomena. The physical and biogeochemical consequences of climate variability and human–induced change may be especially difficult to observe and interpret due to the possibility of non–linear, complex interactions. Models, be they conceptual, statistical or numerical simulations, will be absolutely required for addressing the challenges ahead (Rothstein *et al.*, 2006).

In his review on N_2 fixation published more than 30 years ago, W. D. P. Stewart (1973) declared, ''the days of making gross extrapolations of rates of N_2 fixation based on a few spoonfuls of soil or a bucket of lake water are past. What is urgently required now are detailed studies based on well-established ecological principles where sampling error and distribution in space and time (including both diurnal and seasonal variations) are investigated.'' This suggestion was then, and still is today, sage advice. Future research on the marine N-cycle should consider the following: (1) we only see what we see, hence our current level of understanding is incomplete and we should expect new discoveries in both the near- and long-term, (2) the marine N-cycle is a solar-powered, time-variable, non steady-state, climate sensitive array of mostly microbiological processes, (3) microbial diversity and changes in community structure (e.g., selection for or against N_2-fixing micro-organisms or *Prochlorococcus*) can have profound effects on the marine N-cycle, (4) the marine N-cycle is closely coupled to the availability and flow of other elements through pelagic ecosystems and must be studied in the full context of other bioelemental cycles, especially C, P and Fe, and (5) humans have already begun to influence the global N-cycle primarily by the production and mobilization of excess fixed N through industrial processes. This accumulation of fixed N leads to a catalytic ''N cascade'' with yet to be determined ecological consequences (Galloway *et al.*, 2003). Over time, these anthropogenic impacts will begin to be detected even in the most remote oceanic habitats like the North Pacific trades biome. Furthermore, because of the close balance between N, P and potentially Fe limitation in the trades biome, the ecosystems supported in these regions, which collectively comprise the largest portion of our planet, may be particularly susceptible to this ''N cascade.''

Finally, the establishment of Station ALOHA as an oceanic outpost for the comprehensive study of microbial biogeochemistry has promoted the collaboration of scientists who otherwise do not frequently interact. This field-intensive research program will, hopefully, continue to provide access to the North Pacific trades biome to further enhance our knowledge of the marine N-cycle for years to come.

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Coastal Upwelling

Frances Wilkerson *and* Richard C. Dugdale

Contents

1. Introduction

Although contemporary research has focused on the flux of biogenic elements between the upper and deep ocean, or ocean and atmosphere with an emphasis on open ocean systems (e.g., JGOFS studies 1996, Murray, 1995; 1996; Smith, 1999; Smith *et al.*, 2000), it has become apparent that the ocean margins play an important role in global ocean flux. A significant proportion of the global production occurs in the coastal ocean (Chavez and Toggweiler, 1995; Walsh, 1991) where wind-driven coastal upwelling results in surface waters rich in nutrients including nitrate (NO_3). As a consequence, phytoplankton in this well lit, eutrophic environment assimilate

the dissolved inorganic nitrogen (DIN) supplied, photosynthesize and grow. Eventually they sink out or are eaten and end up in fecal pellets. This biological pump acts to export carbon to deep water and lowers the pCO_2 in surface waters (Volk and Hoffert, 1985).

The pump's efficiency depends on the fraction of carbon fixation that escapes recycling within the mixed layer (Berger *et al.*, 1988), i.e., the amount of new production (Dugdale and Goering, 1967), which is supported by nutrient influx (typically NO_3) to the euphotic zone. Eastern boundary ocean margins, fueled by episodes of nutrient enrichment due to coastal upwelling, have high rates of new production (e.g., Dugdale and Wilkerson, 1989a; Dugdale, 1985; MacIsaac *et al.*, 1985). The aim of this chapter is to describe the nitrogen concentrations and the nitrogen assimilation by the resident phytoplankton communities that result in these regions being ecosystems crucial to the flux of nitrogen in the sea. Our focus will be on the supply of inorganic nitrogen as NO_3 in these areas and the new production processes that result from autotrophic cells rather than heterotrophic organisms that also mediate the nitrogen cycle, through regenerated production. Other chapters in this volume deal with nitrogen fixation processes (Chapter 4 by Carpenter and Capone, this volume), dissolved organic nitrogen as a nitrogen source (Chapter 7 by Mulholland and Lomas, this volume) and regeneration processes (Chapter 8 by Bronk and Steinberg, this volume).

In the original volume of ''Nitrogen in the Marine Environment'' the chapter concerning Nitrogen in Upwelling Ecosystems (Codispoti, 1983) reviewed the physical and chemical processes in upwelling areas that lead to high nitrogen supply rates and uptake rates. They did not discuss phytoplankton physiological aspects or ecosystem models involving nitrogen cycling in upwelling areas. Since then, there have been many more field studies of upwelling regions that have continued to describe distributions of nitrogenous nutrients and their uptake by phytoplankton. These have resulted in numerous review or synthesis volumes specific to coastal upwelling such as Bas *et al.* (1985) reporting the presentations given at ''The International Symposium on the Most Important Upwelling Areas off Western Africa'' and Payne *et al.* (1992) summarizing the ''Benguela Trophic Functioning Symposium.'' A Dahlem Workshop ''Upwelling in the Ocean'' discussed both coastal and equatorial upwelling on modern and ancient time scales and resulted in Summerhayes *et al.* (1995). Dedicated special issues of scientific journals have been produced that include a number of associated papers from a specific upwelling region or program. For example, the Lagrangian studies of the Iberian upwelling system (Joint and Wassmann, 2001, Progress in Oceanography), the Coastal Ocean Advances in Shelf Transport (COAST) program off the Oregon coast (Barth and Wheeler, 2005), and the Wind Events and Shelf Transport (WEST) study of the Bodega upwelling area (Largier *et al.*, 2006). Included within these are studies concerning the nitrogen cycle such as nitrogen assimilation measurements using Eulerian and Lagrangian approaches, experiments with shipboard enclosures and simulated upwelling studies in the laboratory.

Our views of how and which phytoplankton in coastal upwelling areas respond to the high supply rates of nitrogen (mostly in the form of NO_3) have progressed from the original chapter (Codispoti, 1983). Information included in this update

include descriptions of cell size-fractionated nitrogen uptake, DIN uptake kinetics, the interaction of other upwelled nutrients with DIN uptake, and how fluxes of nitrogen in upwelling systems can be modeled with this new information. However, many of the new data obtained since the original chapter, collected in coastal upwelling areas that were previously unstudied, confirm the earlier observations in Codispoti (1983) and serve to illustrate the common features of coastal upwelling systems. Since 1983 there have also been many technological advances that have been applied to the study of nitrogen flux and we review some preliminary studies pertinent to our knowledge of nitrogen in upwelling areas made using remote sensing and molecular genetics approaches. First the supply of nitrogen in coastal upwelling areas will be reviewed and compared among different sites. Then the available data on the uptake of nitrogen and new production rates in different upwelling areas will be summarized. The major phytoplankton groups contributing to these levels of new production will be discussed, followed by our view of how these phytoplankton (typically diatoms) are physiologically adapted to injections of NO_3 that accompany upwelling favorable winds. Some simulation models of new production in these ecosystems, along with some preliminary molecular studies appropriate for studying nitrogen flux in upwelling areas will be outlined, concluding with a look to future studies and approaches that might be addressed.

For sake of space we have limited ourselves to coastal upwelling areas and will not discuss equatorial upwelling. However equatorial upwelling is an important source of NO_3 in this high nutrient low chlorophyll region (see Chapter 37 by Kudela, this volume) and equatorial NO_3 flux and new production have been the focus of many field studies (e.g., WEC 88, JGOFS EqPac) as summarized in Barber (1992), Chavez and Smith (1995), Murray (1995, 1996), Murray *et al.* (1997), Turk *et al.* (2001a,b), and others. We cannot review everything that has happened since 1983 in all coastal upwelling regions, but have tried to provide a representative review of the major studies that have involved nitrogen flux measurements.

2. Inputs and Concentrations of Dissolved Nitrogen

2.1. Source of dissolved inorganic nitrogen

The surface waters of most of the ocean, in the absence of upwelling, are stripped of the nutrients used by phytoplankton, including dissolved inorganic nitrogen as NO_3, nitrite (NO_2) and ammonium (NH_4). Below a certain depth (the nutricline or ∼nitracline), NO_3 concentrations are elevated as the result of oxidation of organic material of biological origin containing nitrogen. This occurs in a series of steps, i.e., nitrification which converts NH_4 to NO_2 and then to NO_3 (Chapter 5 by Ward, this volume) resulting in low or undetectable concentrations of NH_4 and NO_2 in the sub–nutricline waters. When NH_4 and NO_2 do occur near the nutricline, it is the result of the other transformations in progress. They may be intermediates of either nitrification, assimilatory NO_3 reduction (NO_3 to NO_2 to NH_4), or denitrification (NO_3 to NO_2 to N_2) (Chapter 6 by Devol, this volume). Oxidation of organic N is complete at relatively shallow depths, *ca.* 500 m in the North

Pacific leading to a relatively constant concentration of NO_3 below that depth. The large reservoir of nitrogen, in the form of NO_3, is unavailable for primary production until uplifted into the euphotic zone by some mechanism, the most effective of which is upwelling, the vertical advection of deep water from below the nutricline. Along eastern boundary coasts the upwelling is wind driven and surface water is advected equatorward and replaced by sub-surface water resulting in high concentrations of NO_3 (and silicate, $Si(OH)_4$) over the coastal shelf (Fig. 17.1). This example from the upwelling center off Bodega Bay, California shows the uplifted NO_3 isopleths (paralleled by the $Si(OH)_4$ isopleths), with surface NO_3 reaching over 20 μM.

Vertical profiles of NO_3 may be modified in regions of low oxygen, or anoxia that can occur in conjunction with highly productive coastal upwelling systems, e.g., the low oxygen regions north and south of the equator in the Pacific (Codispoti, 1983; Codispoti *et al.*, 1986). Denitrification can result in a region of low NO_3 accompanying the subsurface low oxygen zone. In the upwelling region of 15°S, Peru, besides low NO_3 at depth there can be a maximum in NO_2 as a result of NO_3 reduction and low oxygen concentrations. Complete NO_3 reduction will result in a profile with zero NO_3, zero NO_2 and a maximum in NH_4 from the oxidation of organic material (Dugdale *et al.*, 1977). H_2S will usually be detectable in such fully denitrified water as bacteria use sulfate as electron donors for denitrification. In the highly productive Benguela upwelling system additional conversion of inorganic N to N_2 has been shown to occur through the anammox reaction (Kuypers *et al.*, 2005). The anammox process occurs under anaerobic conditions by bacterial mediated combination of NO_2 with NH_4. The reaction was first suggested by Richards (1965) and first shown directly to occur in wastewater bioreactors (Mulder *et al.*, 1995). The anammox reaction uses NH_4 produced during oxidation of organic matter required for denitrification. As a result, when the anammox reaction is combined with denitrification, the total production of N_2 from inorganic nitrogen is increased by as much as 29% compared to only denitrification (Dalsgaard *et al.*, 2003).

2.2. Characteristic NO_3 concentrations in different upwelling regions

Upwelled water in coastal systems is usually derived from maximum depths of 100–200 m (Hutchings *et al.*, 1995). The maximum depth of upwelling is a function of the wind stress and the degree of stratification so it will vary in time and space. The maximum NO_3 concentrations achieved during strong upwelling events are important for two reasons: (1) to determine the rate of specific NO_3 uptake (i.e., growth rate as nitrogen), and (2) to determine the total new production (NO_3 uptake) that can be realized from the particular event. The first effect is on algal physiology, the second on biomass accumulation or production.

The NO_3 content of upwelling water is strongly influenced by the location of a system (Codispoti *et al.*, 1982) in the global oceanic conveyer belt that transports deep water through the ocean basins (Broecker and Peng, 1982, their Fig. 1.12). From the North Atlantic to the Pacific and Indian Oceans through the Antarctic Circumpolar Current, there are increasing nutrients, from the fallout of surface productivity.
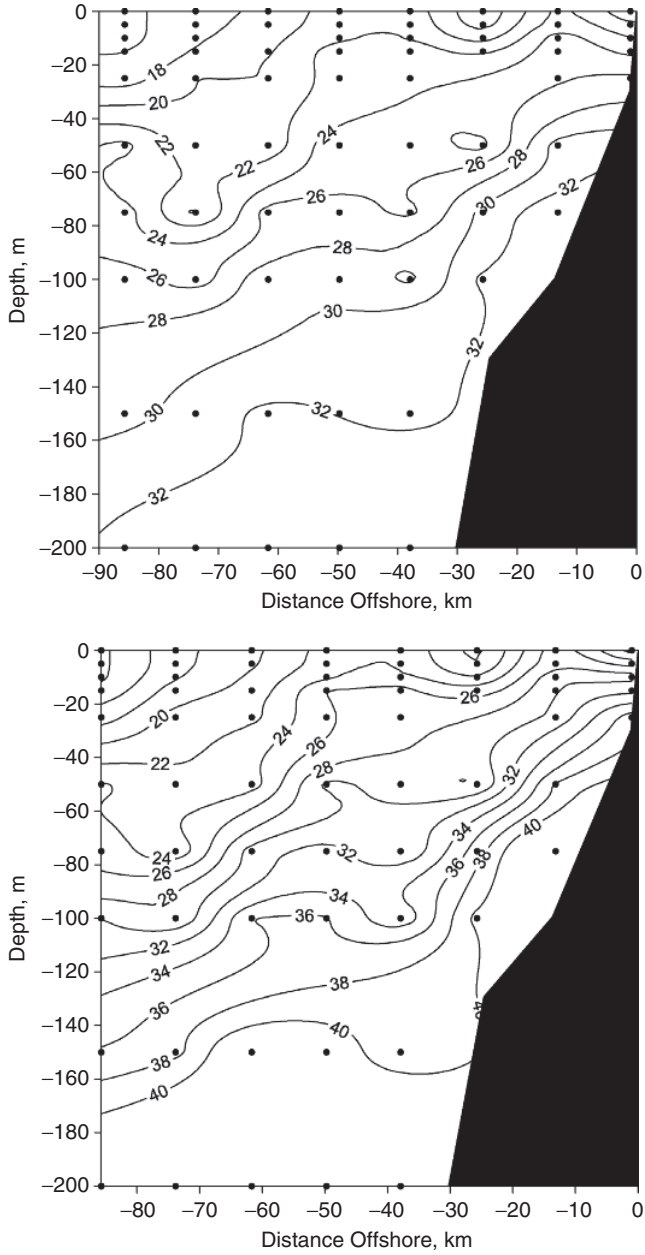
Figure 17.1 Distribution of nitrate (upper panel) and silicate (lower panel) (μM) at the upwelling area off Bodega Bay, CA during WEST 2000 cruise.

This oceanic conveyer belt is essentially a counter–current concentration system in which the Atlantic Ocean is continually stripped of nutrients that eventually find their way to the North Pacific and Indian Ocean. The conveyor belt suggests that newly upwelled waters in the Atlantic Ocean will have lower surface nutrients than in Pacific upwelled waters. Maximum NO_3 concentrations observed during ocean-ographic cruises to selected upwelling systems (Table 17.1) support this with the Pacific systems having the highest maximum concentrations, 32–49 µM for the northern hemisphere sites (note, the Baja California values represent weak, spring upwelling conditions). The Indian Ocean (Somali Coast) concentration is lower, 25.6 µM. The South Atlantic Benguela system has a concentration, 24.35 µM, about the same as the Indian Ocean value. The North Atlantic site, Cap Blanc, northwest Africa has a value that is much lower, 12.0 µM. This series of high Pacific, lower Indian Ocean and South Atlantic and low North Atlantic concentrations (Table 17.1) are the result of the characteristic nutrient concentrations of these basins brought about by the effect of the oceanic conveyer belt system (Broecker and Peng, 1982).

In addition there is a local influence on nutrient concentrations. The local source of upwelled water in coastal upwelling centers is in part from the poleward undercurrents that underlie virtually every coastal upwelling system. The original concentration of the water entrained in the undercurrent is enhanced by the counter–current exchange process (Dugdale, 1972; Hart and Currie, 1960) in which the poleward undercurrent receives and processes detrital material from the equatorward surface waters containing high algal material produced from the upwelled nutrients (Barber and Smith, 1981).

Upwelled NO_3 concentrations are usually reduced during El Niño conditions (e.g. Corwith and Wheeler, 2002), by as much as 50% in the upwelling areas of Peru (e.g., data for 1976) and Baja California (e.g., in 1972) (Wilkerson et al., 1987). Point Conception, California in the El Niño year 1983 experienced about a 30%

Table 17.1 Maximum surface nitrate and silicate concentrations from selected upwelling systems

Upwelling area	Nitrate µM	Silicate µM	Reference
Aleutian Islands	30.00	70.00	Hood and Kelley (1976)
Oregon	48.70		Kokkinakis and Wheeler (1987)
Bodega Bay, CA	35.10	47.30	Unpublished CoOP WEST data
Point Conception, CA	32.52	32.74	Wilkerson et al. (1987)
Monterey Bay, CA	24.81	33.04	Wilkerson et al. (2000)
Baja California	13.28	16.69	Wilkerson et al. 1987
15°S, Peru	26.68	31.25	Wilkerson et al. (1987)
Somali Coast	25.60	21.10	Toon et al. (2000)
Benguela	24.35	<50.00	Probyn (1985); Shannon and O'Toole (1999)
Cap Blanc, NW Africa	12.00	7.00	Dugdale (1985)

reduction (Dugdale, 1985; Wilkerson *et al.*, 1987). NO_3 concentrations in Monterey Bay, California were also reduced during the 1992 El Niño to about 30% of 1993 values (Chavez, 1996) and even more during the 1998 El Niño (Chavez *et al.*, 2002).

In coastal upwelling systems, maximum silicate $(Si(OH)_4)$ concentrations are generally equal or greater than NO_3 concentrations (Table 17.1, Jones *et al.*, 1983). Diatoms, the major producers in upwelling systems (reviewed in Section 17.4 and Brink *et al.*, 1995), require silica for construction of their shells in about a 1:1 ratio of N:Si (Brzezinski, 1985). Generally, in coastal upwelling regions, the supply of Si is adequate for diatoms so NO_3 will run out first during biomass production. However, in the Peru system variation in the ratio of N:Si brought about by intensive denitrification, may result in alternating N and Si limitation of diatom production (Dugdale, 1983).

2.3. Dissolved organic nitrogen

DON concentrations of 4–8 µM have been observed in the relatively weak upwelling area off the northwest coast of Spain (Álvarez-Salgado *et al.*, 1999). Hill and Wheeler (2002) reported values of ~7 µM DON in upwelled waters off the Oregon coast. These are similar DON concentrations to the open ocean as summarized Table 17.3 of Hill and Wheeler (2002). Some of this DON is upwelled; e.g., water at 50 m depth off Oregon had a mean value of 5.98 (Hill and Wheeler, 2002). Another source is from production by phytoplankton as they partition newly assimilated NO_3 into particulate nitrogen and DON that is released (Bronk *et al.*, 2006; Wetz and Wheeler, 2003).

3. DISSOLVED INORGANIC NITROGEN UPTAKE IN UPWELLING AREAS

3.1. NO_3 uptake and new production

The incorporation of new inputs of nitrogen into phytoplankton biomass—i.e., NO_3 advected from depth (e.g., Dugdale and Goering, 1967) as occurs in coastal upwelling regions or from atmospheric inputs through rain (e.g., Paerl, 1985; 1997; Chapter 9 by Seitzinger and Harrison, this volume) or through nitrogen fixation (Chapter 4 by Carpenter and Capone, this volume) has been termed new production to differentiate from regenerated productivity that is based upon nitrogen that has come from a recycled source. Typically the regenerated source is NH_4 resulting from meso or micro zooplankton grazing. The concept of new production was used to describe the potential loss rates—i.e., the export of nitrogen as sinking particles (Eppley and Peterson, 1979) or loss to upper trophic levels and ultimately fishery yields (Dugdale and Goering, 1970). New production in upwelling areas can be compared using the percent new production or *f*-ratio. This is typically calculated from the uptake of NO_3 into the particulate nitrogen pool, compared to the uptake of NO_3 plus regenerated N sources, usually NH_4 and sometimes including urea and other DON. It is also used as a way to parse total productivity, i.e., carbon fixation usually measured with the radio-isotope ^{14}C, into total and new fractions. New

production plays a major role in the biological pump as it determines the amount of carbon (and nitrogen) that can be pumped from atmosphere to the deep ocean. Regions with high f-ratios (i.e., >0.5) are areas with an efficient biological pump (Eppley and Peterson, 1979). There may be other sources of nitrogen that may at first glance appear to be regenerated nutrients but are also new to the system (e.g., NH_4 runoff, Dugdale and Wilkerson, 1988; Paerl, 1997). Consequently the term new production and the calculation of f-ratio should be defined carefully when used (see discussion in Chapter 7 by Mullholland and Lomas, this volume). A re-evaluation of new production measurements was made to include DON produced during NO_3 uptake (Bronk $et\ al.$, 1994; Bronk and Ward, 2005). This diverted new production will contribute to the flux of particles (export production) but is not represented in the f-ratio measured as ^{15}N uptake into the particulate N pool. Bronk and Ward (2005) suggested that the DON pool acts as an intermediate between DIN assimilation and the net formation of particles for export and so will influence the export production of the system.

Typically new and regenerated production rates have been measured using tracers—mostly the stable isotope ^{15}N and mass spectrometry. The different sources of nitrogen for phytoplankton can be labeled and incubated with the phytoplankton to measure uptake of the different forms of DIN or DON (e.g., Wilkerson and Grunseich, 1990). In this chapter we will limit ourselves to DIN. Having the uptake rates for $^{15}NO_3$ and $^{15}NH_4$, estimates of percent new production and f-ratio can be made. Phytoplankton incorporation of ^{15}N labeled compounds was first carried out in the Sargasso Sea by Dugdale and Goering (1967) and with the advent of mass spectrometers designed specifically for tracer use of stable isotopes, this approach has now become a standard oceanographic technique (e.g., Dugdale and Wilkerson, 1986; JGOFS, 1996). Other approaches have used the radio-isotope ^{13}N that has a very short half life of 10 min (e.g., Collos $et\ al.$, 1992; Zehr and Falkowski, 1988; Zehr $et\ al.$, 1988). One limitation with this is that studies must be carried out in close proximity to a cyclotron that can supply the isotope for the incubations. Another approach is to use ^{14}C labeled methyl-ammonium, an analog of NH_4 to measure regenerated production (Balch, 1986; Wheeler, 1980). Alternatively NO_3 and NH_4 depletion can be followed in incubations but these do not provide direct incorporation of N and so have their limitations (Dugdale and Wilkerson, 1986; Price $et\ al.$, 1985).

The uptake of NO_3 into the particulate N pool can be expressed as a transport rate, with a volume specific unit, ρNO_3 as $\mu mol\ N\ l^{-1}\ h^{-1}$ (or integrated throughout the water column, to get $\mu mol\ m^{-2}\ h^{-1}$) or as a biomass specific rate V usually based upon the particulate nitrogen (PON) of the sample or the chlorophyll content of the sample (Dugdale and Wilkerson, 1986). Dickson and Wheeler (1995a) proposed that V_{Chl} was a better measure in the Oregon upwelling system, although chlorophyll per cell will change with photoadaptation. Kudela (1995) and Kudela and Dugdale (2000) compared specific uptake rates for NO_3 normalized to PON and to chlorophyll in the Monterey Bay upwelling area and found no difference except during winter when detrital N diluted the values of VNO_3 normalized to PON.

Coastal upwelling areas show high NO_3 uptake rates, with maximal rates of ρNO_3 on the order of $0.5\ \mu mol\ l^{-1}\ h^{-1}$ (Table 17.2). The only data available for

Table 17.2 Examples of published maximum nitrate uptake rates (surface) in coastal upwelling areas

Upwelling Area	ρNO_3, $\mu mol\, l^{-1} h^{-1}$	$V NO_3$, h^{-1}	mean f-ratio	Reference
Oregon	1.29	0.08	0.86	Dickson and Wheeler (1995a); Kokkinakis and Wheeler (1987)
Monterey Bay, CA	0.55	0.08	0.86	Kudela (1995)
Bodega Bay, CA	0.46	0.08	0.80	Dugdale et al. (2006)
Point Conception, CA	0.21	0.096	0.57★	Wilkerson et al. (1987) Dugdale and Wilkerson (1991, 1992)
Baja California		0.045	0.78	Wilkerson et al. (1987) Dugdale and Wilkerson (1991, 1992)
15°S, Peru	0.57	0.122	0.82	Wilkerson et al. (1987) Dugdale and Wilkerson (1991, 1992)
Benguela	0.55		0.71	Probyn (1985, 1992)
Cap Blanc	0.36	0.04	0.7	Wilkerson et al. (1987) Dugdale and Wilkerson (1991, 1992)

★ Measured during 1983—El Niño year.

Table 17.3 Percent nitrate uptake by large-sized phytoplankton in upwelling areas

Upwelling Area	% Uptake by larger cells	Definition of larger cells, cell diameter = μm	ρNO_3, $\mu mol\, l^{-1}\, h^{-1}$ of total unscreened cells	Reference
Bodega Bay, CA	73	>5	0.44	CoOP–West Data 2001
Monterey Bay, CA	87	>5	0.54	Wilkerson et al. (2000)
Monterey Bay, CA	81	>5	0.17	Franck et al. (2005)
NW Indian Ocean	90	>5		Owens et al. (1993)
Benguela	64	>10	0.42	Probyn (1985)
Westland, New Zealand	66	<20	1.97	Chang et al. (1992)
Westland, New Zealand	81	<20	1.19	Chang et al. (1995)

Point Conception, California was obtained during an extreme El Niño year (1983) when nutrients (and uptake) were lower than more normal years (Wilkerson *et al.*, 1987). Accompanying the high transport rates are high specific uptake rates (VNO$_3$ based upon PON) up to 0.1 h^{-1} [µmol l^{-1} h^{-1} (µmol l^{-1})$^{-1}$], that can be approximated to a maximal growth rate of the phytoplankton of 0.1 h^{-1}. High *f*-ratios are also characteristic of coastal upwelling areas, with values of >0.7 (Table 17.1), except for the low Point Conception value which was likely due to the El Niño conditions. These uptake rates translate to a rapid ability to drawdown ambient nutrients. For example in the Bodega Bay, California upwelling area (Fig. 17.2), ambient upwelled NO$_3$ of 23 µM was drawn down to zero within four days, VNO$_3$ reached 0.05 h^{-1}, with an accompanying accumulation of biomass of 14 µg l^{-1} chlorophyll (Dugdale *et al.*, 2006; Wilkerson *et al.*, 2006).

Light is required for NO$_3$ uptake so the high rates of uptake in upwelling areas are limited to the upper euphotic zone near the surface (Fig. 17.3) and during the daytime (Cochlan *et al.*, 1991a), although subphotic uptake has been described in the Benguela upwelling (Probyn *et al.*, 1996). The response of NO$_3$ uptake to ambient irradiance in natural phytoplankton was originally described for the upwelling systems of Peru (MacIsaac and Dugdale, 1972; MacIsaac *et al.*, 1985; Wilkerson and Grunseich, 1990), northwest Africa (MacIsaac *et al.*, 1974; Nelson and Conway, 1979) and Baja (MacIsaac, 1978; Nelson and Conway, 1979), summarized in Wilkerson and Grunseich (1990), using a Michaelis-Menten hyperbola to obtain uptake parameters. More recently it has been described by Cochlan *et al.* (1991b) for the frontal high NO$_3$ regions of the Strait of Georgia. Kudela *et al.* (1997), working in the upwelling area of Monterey Bay, California confirmed the use of a hyperbola to describe the response, but indicated that it should be modified for dark uptake of NO$_3$ that can occur. Rarely is there photoinhibition of NO$_3$ uptake (Kudela *et al.*, 1997). In their study the uptake parameter, K_{LT}—the half saturation constant for light—similar to the derived photosynthetic parameter I_K, ranged from 35.7 to 123.5 µmol m^{-2} s^{-1}, (high to low sensitivity to light) as upwelling progressed, showing physiological adaptation from the low light conditions of the source water to the high light expected in a stratified water column.

3.2. NH$_4$ uptake and regenerated production

In upwelling regions, NH$_4$ is usually close to undetectable in deep water, having been oxidized to NO$_3$ by bacterial activity. It may be present in low concentrations in euphotic waters, a result of either regeneration through grazing or bacterial activity, or advected from outside the upwelling center. However, NH$_4$ and other products of grazing, e.g., urea and creatine (Whitledge, 1981; 1982, Whitledge and Packard, 1971) appear in the surface regions as upwelled water ages and is depleted of NO$_3$. Zooplankton and nekton are major sources of NH$_4$ and urea in upwelling systems. Whitledge (1978; 1981) measured equal amounts of NH$_4$ excretion by both in the northwest Africa upwelling system, the sum about equal to the uptake by phytoplankton. There was a higher rate of urea excretion by the nekton than by the zooplankton. The smallest size fraction of zooplankton included was 102–223 µm. The Peru upwelling system at 15°S was unique in that anchovy excretion provided
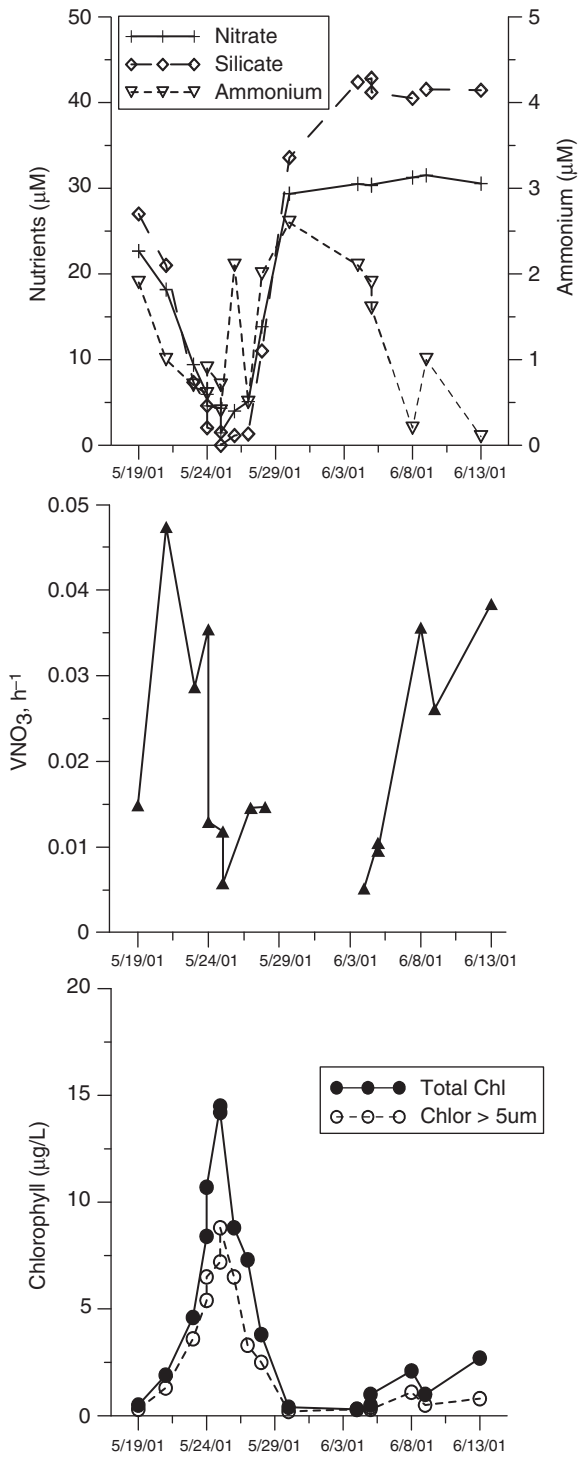
Figure 17.2 Time series of surface nutrients, nitrate uptake and chlorophyll at a shelf station at the upwelling area off Bodega Bay, CA during WEST 2001. From Wilkerson *et al.* (2006) and Dugdale *et al.* (2006).
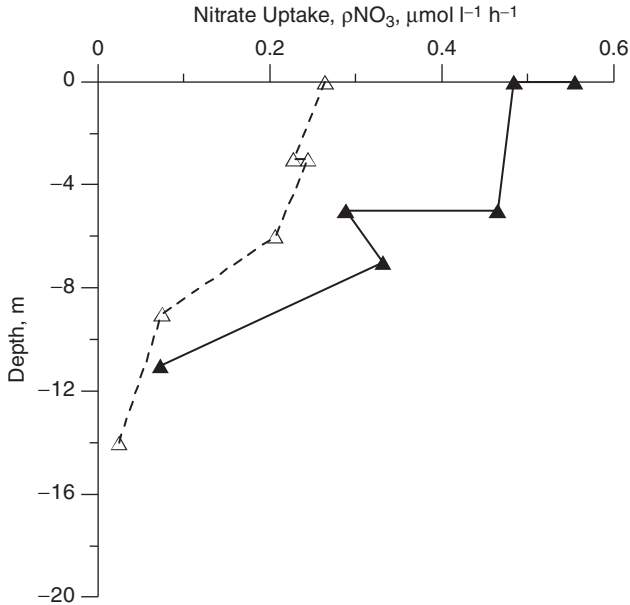
Figure 17.3 Two vertical profiles of nitrate uptake at the upwelling area off Bodega Bay, CA during May (open triangles) and June (closed triangles) during WEST 2001. From Dugdale *et al.* (2006).

the major source of NH_4, creatine and urea (Whitledge and Packard, 1971). They calculated that the daily NH_4 uptake requirement of the phytoplankton would be met by excretion of a school of anchovy in two hours. The unique property of anchovy is that as nekton they occupy a position near shore and regenerate nitrogen there, so acting as a nutrient trap to maintain upwelled nitrogen close to the coast rather than exported away.

NH$_4$ is preferentially used by the smaller phytoplankton (e.g., Probyn, 1985). In aged upwelled water in the Benguela upwelling system in the presence of 5 μM NO_3 the picoplankton (<2 μm) and nanoplankton (2–20 μm) took up mostly NH_4 (Probyn *et al.*, 1990). The larger fraction (>20 μm cell size) utilized NO_3 more under the same conditions. Urea was also detected in the aged water and was taken up by all size fractions. In the upwelling area of Monterey Bay urea uptake did not contribute significantly to total N uptake (Kudela 1995) and NH_4 uptake was less than NO_3 uptake during early and mid upwelling but dominated N assimilation in older upwelled water (Kudela *et al.*, 1997). For the upwelling area off Oregon, Dickson and Wheeler (1995b) reported the lowest rates of NH_4 uptake during an active upwelling event and the fastest rates in aged upwelled water. Regeneration rates for both NH_4 and urea were about equal to the measured uptake rates in the Benguela upwelling system (Probyn *et al.*, 1990). In the upwelling area of northwest Spain, NH_4 did not accumulate suggesting a daily time scale coupling between re-generation and uptake (Varela *et al.*, 2003). Their measurements of NH_4 and DON regeneration by microplankton were minimal during active upwelling and maximal

during relaxation (Varela *et al.*, 2003). Microheterotrophs may play a role in upwelling nitrogen cycles. Sherr and Sherr (1994) noted that microzooplankton (protists) routinely consume 25–100% of daily phytoplankton production, even in diatom dominated upwelling blooms. Sherr *et al.* (2005) described a high abundance of ciliates and heterotrophic dinoflagellates in the Oregon upwelling region that would contribute to the regeneration of nitrogen.

4. Phytoplankton Functional Groups and Nitrogen Assimilation

The composition and size structure of the phytoplankton assemblage in the coastal upwelling ecosystems play an important part in determining the amount of NO_3 assimilation (new production) that occurs (Hutchings *et al.*, 1995; Michaels and Silver, 1988; Probyn, 1992; Chavez, 1996). In most coastal upwelling areas the larger sized cells dominate the chlorophyll biomass (Brink *et al.*, 1995; Estrada and Blasco, 1985; Wilkerson *et al.*, 2006). Stolte *et al.* (1994) describe how larger phytoplankton cells only become dominant when NO_3 is the major nitrogen source available and NH_4 is typically low or at detection levels, as in coastal upwelling ecosystems. The larger net (or micro-) plankton size fractions of the phytoplankton community are more effective than the smaller size classes of the microbial web at using recently upwelled NO_3 (Brink *et al.*, 1995; Chang *et al.*, 1992, 1995; Probyn, 1985, 1992; Probyn *et al.*, 1990; Varela *et al.*, 1991; Wilkerson *et al.*, 2000) and are the major new producers. Size fractionated uptake measurements made using ^{15}N in a variety of upwelling regions using different size fractions show that greater than 65% and up to 90% of the total NO_3 uptake by the phytoplankton community can be attributed to the larger phytoplankton (Table 17.3).

Taxonomic studies show that these larger cells in upwelling waters are chain-forming and colonial diatoms (e.g., Chavez *et al.*, 1991; Estrada and Blasco, 1985; Lassiter, 2003; Lassiter *et al.*, 2006) with individual cell diameters of typically 5–30 µm (Estrada and Blasco, 1985). Margalef (1978) was one of the first to observe that the diatom genera *Chaetoceros, Thalassiosira, Rhizosolenia* and *Nitzschia* dominated in coastal upwelling regions and are apparently well suited to both the high turbulence and high nutrient conditions there. Diatoms have been described as the dominant marine phytoplankton species to occur when external ''new'' nitrogen enters the euphotic zone (Malone, 1980; Bode *et al.*, 1997). Diatom physiology is well adapted to exploit increased nutrient availability (Hutchings *et al.*, 1995; Sarthou *et al.*, 2005) as occurs in wind driven upwelling. Phytoplankton cells in upwelling situations have greater intracellular pools of NO_3 than those from non-upwelling situations (Bode *et al.*, 1997). They have large vacuoles (e.g., Antia *et al.*, 1963) that may take up to 90% of the total cell volume in diatoms >5 µm in diameter (Smayda, 1970) and are capable of storing large amounts of NO_3 (Collos *et al.*, 1997; Lomas and Glibert 2000). Diatoms have fast division rates (Furnas, 1991) and a favorable respiration to photosynthesis ratio (Harris, 1978), enabling photosynthetic carbon to be used in growth rather than completely used in respiration. They are also well adapted to

thrive in cold waters as occurs in recently upwelled water. Lomas and Glibert (1999a, 2000) describe how diatoms in colder water have higher uptake capacity for NO_3, and lower optimal temperatures for nitrate reduction enzymes than flagellates (Lomas and Glibert, 1999b).

Consequently rates of new production in many coastal upwelling areas with high NO_3 concentrations are dominated by NO_3 assimilation by diatoms (Franck et al., 2005; Wilkerson et al., 2000), resulting in short diatom-based food chains (e.g., Ryther, 1969). The role of diatoms in downward nitrogen flux (from surface to deep ocean) is also important in these areas where they are the key players in biological export (e.g., Pilskaln et al., 1996). However in some upwelling areas, when the water conditions are less turbulent, dinoflagellates may also use upwelled NO_3 sweeping the euphotic zone clear of NO_3 as they migrate vertically daily. Such conditions were encountered in the upwelling region of the Baja California coast during weak upwelling (MacIsaac, 1978; Walsh et al., 1974). Dominance of the dinoflagellates over the diatoms in this situation was apparent from the high $Si(OH)_4$ concentrations with near zero NO_3 concentrations (Huntsman et al., 1981). In 1977, at 15°S, Peru, there was dominance by patches/blooms of the motile symbiotic ciliate Mesodinium (=Myrionecta) rubrum that were able to exploit the high DIN conditions (Wilkerson and Grunseich, 1990). In some upwelling areas with lower levels of NO_3, such as the Iberian upwelling system (Joint et al., 2001b) picoplankton may play the most important role in NO_3 uptake (Joint et al., 2001a) and consequently new production in these areas has less potential for sedimentation (Joint and Wassmann, 2001) and downward flux of N.

5. PHYSIOLOGICAL ADAPTATION OF NITROGEN ASSIMILATION

The sequence of events that occurs when upwelled phytoplankton respond to the favorable irradiance and nutrient conditions has been described as a conveyor belt (Fig. 17.4; Dugdale et al., 1990; MacIsaac et al., 1974, 1985; Wilkerson and Dugdale, 1987) or cycle of events. Along this conveyor, with sufficient residence time and relaxed winds, phytoplankton cells go through a series of physiological changes (Bode et al., 1997). The productivity cycle begins with upwelling of low levels of seed stocks of phytoplankton cells that have minimal phytoplankton rate processes (Slawyk et al., 1997). The upwelled surface waters are cold with high nutrient concentrations and low phytoplankton biomass (chlorophyll). Then as the water is advected downstream, with relaxed wind conditions, the phytoplankton increase their physiological processes in response to the high light and nutrients. First nutrient uptake mechanisms are turned on and then photosynthesis. This up-regulation or acceleration of rate processes (Fig. 17.4) has been termed shift-up (MacIsaac et al., 1974) and used to model plankton responses to upwelling conditions by Dugdale et al. (1997), Botsford et al. (2003), and Zimmerman et al. (1987). As the cells continue along the conveyor or cycle they reach balanced growth as uptake of C and N approach the Redfield ratio (Kudela et al., 1997). At this stage (often near an upwelling frontal area) chlorophyll biomass begins to accumulate due to the high uptake rates and the surface nutrient
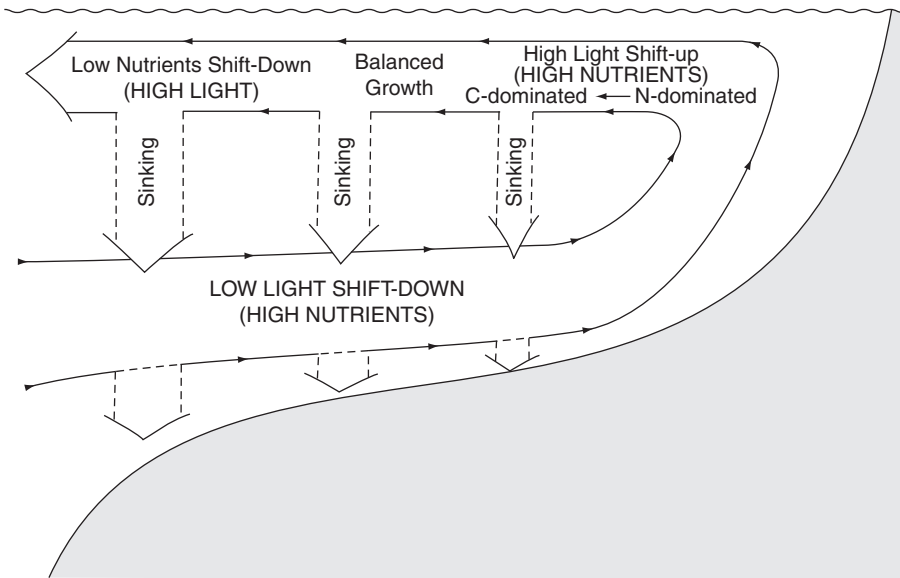
Figure 17.4 Conceptual cycle (conveyor belt) of phytoplankton nitrogen and carbon processes in an upwelling center. From Wilkerson and Dugdale (1987).

concentrations are lower due to phytoplankton drawdown. As the cells continue downstream the larger phytoplankton cells do not have sufficient nutrients, their rate processes down-regulate or shift down. Zooplankton grazing in this region responds to the increased chlorophyll biomass (e.g., Smith and Whitledge, 1977), with regeneration of NH_4 that feeds a small celled phytoplankton community and a microbial loop (Chang et al., 1992; 1995; Kudela et al., 1997; Painting et al., 1993; Probyn et al., 1990; Wilkerson et al., 2000). Sinking algal cells or fecal pellets carry the C and N below the euphotic zone. Any living cells will act as seed stock for the next cycle or conveyor when the winds are upwelling favorable.

Field studies show this cycle (conveyor) for cells to go through high light shift-up to balanced growth and finally to low nutrient shift-down (Fig. 17.4) to take between 5–7 days (e.g., MacIsaac et al., 1985 in Peru; Dugdale and Wilkerson, 1989b in Point Conception, California, Wilkerson et al., 2006 for Bodega, California). These temporal changes described above as chronological changes since upwelling, are translated into spatial changes as newly upwelled water is advected offshore or downstream. The greatest bloom development and spatial extent occurs when upwelling ceases and there is an optimal period of water column stability due to relaxation of the winds. An alternative explanation to physiological adaptation by the phytoplankton along a conveyor was put forward by Garside (1991) and Dickson and Wheeler (1995), who explained the increase in NO_3 uptake (=depletion) that occurs following upwelling to result from increased biomass of phytoplankton, and that an adjustment of metabolism by the phytoplankton was not necessary. However, Kokkinakis and Wheeler (1987) showed data with specific nitrate uptake VNO_3 (based on chlorophyll, V_{Chl}) increasing daily at high NO_3 concentrations

in the Oregon upwelling system. Shift-up of phytoplankton metabolism was also confirmed by Berges *et al.* (2004) who showed that the physiological indicators of nitrogen and carbon metabolisms (nitrate reductase activity and variable fluorescence, Fv:Fm) responded quickly and with great sensitivity to changes in irradiance in simulated upwelling conditions.

Zimmerman *et al.* (1987) and Dugdale *et al.* (1990) used simple models (see section 7 below) based upon the initial NO_3 concentration in the upwelled water and the acceleration rate (a term used to describe the increased uptake rate of NO_3 by upwelled phytoplankton that is directly related to the available NO_3) to predict the time it would take for upwelled phytoplankton to respond and deplete the upwelled nutrients. They showed that, regardless of how much nutrient is upwelled during the upwelling phase, with a suitable period of relaxation, all upwelled NO_3 was taken up within 72 h, i.e., 3 days. This sets the minimum relaxation period for full utilization of upwelled nutrients: a conceptual framework for understanding the phytoplankton response in upwelling regions. Field studies support this time frame (e.g., Wilkerson *et al.*, 2006).

6. Factors Affecting Nitrogen Assimilation in Coastal Upwelling Areas

6.1. Kinetics of NO_3 and NH_4 uptake

NO_3 and NH_4 uptake by phytoplankton in eutrophic systems were initially shown to follow Michaelis-Menten kinetics yielding a saturation curve (MacIsaac and Dugdale, 1972) according to the equation:

$$VNO_3 = V_{max}NO_3{}^*[NO_3]/K_{S(NO3)} + [NO_3]$$

using NO_3 uptake as the example, where VNO_3 is the biomass specific NO_3 uptake rate, $[NO_3]$ is the NO_3 concentration, and $K_{S(NO3)}$ is the half saturation constant for NO_3 uptake (i.e., the concentration of NO_3 at which $VNO_3 = \frac{1}{2} V_{max}NO_3$.

Early values of K_S obtained using ^{15}N incubations in upwelling areas were on the order of 1 μM for both forms of DIN (MacIsaac and Dugdale, 1969); 0.98 μM for NO_3 in the Costa Rica Dome, 4.21 μM for NO_3 and 1.3 μM for NH_4 in the Aleutian Islands. Subsequent measurements of nutrient uptake have largely confirmed the applicability of the Michaelis–Menten formulation at relatively low substrate concentrations. There have been relatively few measurements of NO_3 uptake kinetics in upwelling systems (Table 17.4), in part because NO_3 concentrations are typically high, at levels that would suggest saturated uptake rates to occur. Also experimentally it is often difficult to get low nutrient water (to make substrate additions to) with sufficiently high phytoplankton biomass to be able to measure uptake rates efficiently. Under weak upwelling conditions off the coast of Portugal with surface NO_3 concentrations of 0 to 1 μM, Slawyk *et al.* (1997) measured $K_{S(NO3)}$ values of 0.17–0.39 μM and $K_{S(NH4)}$ values of 0.08–0.3 μM.

Table 17.4   Examples of Michaelis–Menten parameters of DIN uptake for upwelling areas

Upwelling Area	$K_{S(NO3)}$ µM	$K_{S(NH4)}$ µM	Reference
Aleutian Islands	4.21	1.3	MacIsaac and Dugdale (1969)
Washington coast	0.4–0.6		Dortch and Postel (1989)
Oregon coast	1.26		Dickson and Wheeler (1995b)
Monterey Bay, CA	1.22–4.14		Kudela (1995)
			Kudela and Dugdale (2000)
Pacific coastal upwelling	4.0		Kanda et al. (1985)
Cap Blanc, NW Africa	0.8–1.8		Harrison and Davis (1977)
Benguela	2.02		Probyn et al. (1995)
Costa Rica Dome	0.98		MacIsaac and Dugdale (1969)
Costa Rica Dome	1.42		Franck et al. (2003)
Cap Sines, Portugal	0.17–0.39	0.08–0.3	Slawyk et al. (1997)
Westland, New Zealand	0.2–1.1	0.2–0.5	Chang et al. (1995)

Chang et al. (1995) measured K_S values for NO_3 and NH_4 uptake by different size classes of phytoplankton in Westland, New Zealand. For >100 µm sized cells, $K_{S(NO3)}$ ranged from 0.4–1.1 µM, for <20 µm cells, 0.3–0.8 µM and for <2 µm cells, 0.2–0.5 µM. $K_{S(NH4)}$ for <200 µm cells ranged from 0.4–0.5 µM, for <20 µm cells from 0.3–0.4 µM and for <2 µm cells, 0.2–0.3 µM. K_S values for urea uptake were in the same range as for NH_4. Kudela (1995) reported values of 1.22 and 4.14 µM NO_3 in Monterey Bay. Dickson and Wheeler (1995b) studying the Oregon coastal upwelling system reported a $K_{S(NO3)} = 1.26$ µM. The value of $K_{S(NO3)} = 2.02$ µM for the Benguela upwelling system (Probyn et al., 1995) falls within the range of the Monterey Bay values, both high NO_3 coastal upwelling systems.

Two complications tend to blur this relatively simple view of NO_3 uptake kinetics. The first is the existence of multi-phasic uptake systems in some algae (see Chapter 7 by Mullholand and Lomas, this volume), and the second is the shift-up process (acceleration of uptake in upwelling systems) in which the maximum specific uptake rate, is a function of both time since upwelling and initial NO_3 concentration. Consequently if a series of upwelled water masses with varying initial NO_3 concentrations were followed and the peak VNO_3 determined for each, the plot of $V_{max}NO_3$ versus initial NO_3 would be linear (see Dugdale et al., 1990, Fig. 4b). An example of linear kinetics resulting from this shift-up response in which VNO_3 is determined both by NO_3 and time since upwelling (Fig. 17.5) is shown for nearshore stations at the Bodega Bay upwelling area. The data include both periods of increasing and decreasing NO_3 concentration with time, from just before and after an upwelling event.

The multi-phasic phenomenon was first suggested by Serra et al. (1978) who observed NO_3 uptake in nutrient deficient cultures of the diatom *Skeletonema costatum* to follow Michaelis-Menten kinetics up to 6 µM NO_3 but at higher concentrations, a linear increase in uptake with increased NO_3 concentration.
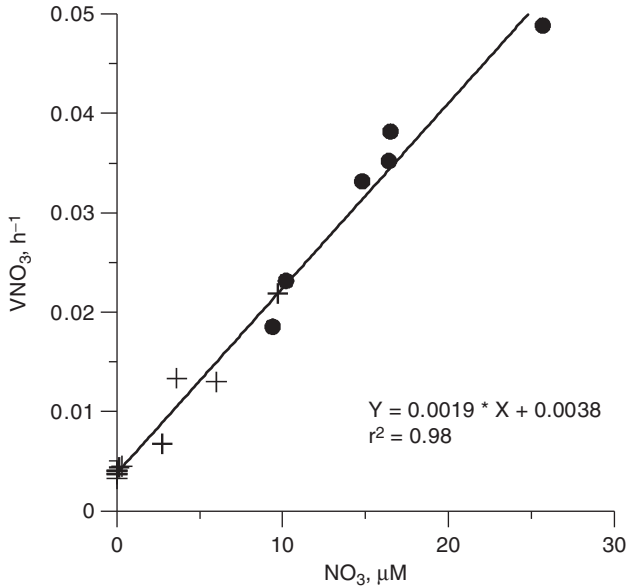
Figure 17.5 Nitrate uptake versus nitrate concentration, at a shelf station at the upwelling area off Bodega Bay, CA during WEST 2000. Circles are stations sampled during upwelling, crosses during decreased upwelling conditions. From Dugdale *et al.* (2006).

They suggested the existence of two uptake systems, a carrier mediated uptake that would result in Michaelis-Menten kinetics, and a linear diffusion mediated system. Later work by Collos *et al.* (1992) showed two plateaus of NO_3 uptake by *S. costatum*, the second at very high NO_3 concentrations (50–100 μM). Since that publication, numerous examples of multi-phasic uptake have been discovered. Collos *et al.* (2005) reviewed K_S values for NO_3 uptake and reported low affinity uptake systems with $K_{S(NO3)}$ values ranging from 18–120 μM among the diatom genera *Rhizosolenia, Skeletonema,* and *Thalassiosira*. A linear system in *S. costatum* appeared above a threshold NO_3 concentration of 30 μM. Collos *et al.* (2005) also reported the existence of multiple thresholds and suggested that coastal upwelling productivity models should incorporate multi-phasic kinetics. Kokkinakis and Wheeler (1987) provide data from the Oregon upwelling system that indicate a threshold for different kinetics beginning at about 20 μM NO_3.

6.2. Interaction of NH_4 and NO_3 uptake

NH_4 may inhibit NO_3 uptake and reduction (e.g., Conway, 1977; Chapter 7 by Mulholland and Lomas, this volume). When NH_4 is present at concentrations of *ca.* >1 μM, NO_3 uptake is often inhibited (Cochlan and Harrison, 1991; Dortch, 1990). Although uncommon, when this occurs in upwelling systems, the phytoplankton first lower the NH_4 concentration by uptake and then access the NO_3; so adding some time delay before the phytoplankton population can take up NO_3 at
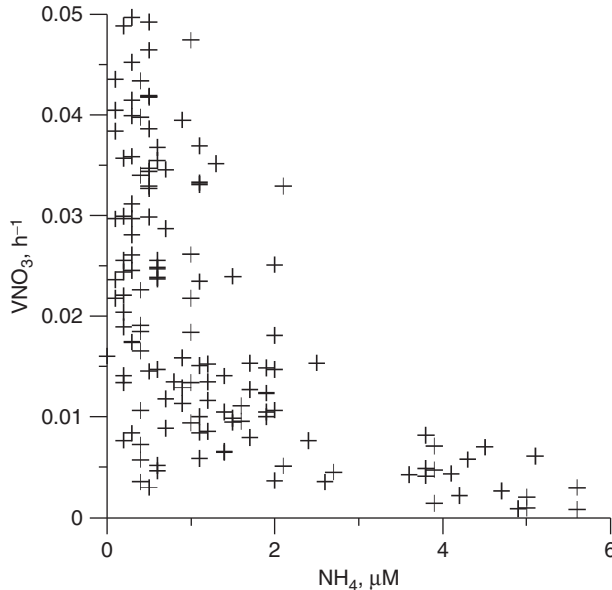
Figure 17.6 Nitrate uptake versus ammonium concentration for all stations sampled at the upwelling area off Bodega Bay, CA during WEST 2001. From Dugdale *et al.* (2006).

increasingly high rates. Upwelling systems therefore typically show a pattern of increasing specific NO_3 uptake with decreasing NH_4 concentrations. An example from recent studies in the upwelling center off Bodega Bay, California is shown in Fig. 17.6 where VNO_3 shows higher uptake when NH_4 concentrations are <2 μM. The elevated NH_4 (up to 6 μM) was contained in cold, high salinity, recently upwelled water and may have been added during contact with sediments. NH_4 inhibition of NO_3 uptake was also observed and modeled in the Peru upwelling center (MacIsaac and Dugdale, 1972; Dugdale and MacIsaac, 1971).

6.3. Interaction of $Si(OH)_4$ and NO_3 uptake

The way in which $Si(OH)_4$ affects NO_3 dynamics is discussed in detail in Chapter 37 by Kudela, this volume. Concentrations of $Si(OH)_4$ in upwelled water are usually sufficient to match NO_3 concentrations and uptake in the 1:1 Si:N ratio required on the average by diatoms (Brzezinski, 1985) provided upwelling is from depths greater than the crossover depth where the ratio of NO_3:$Si(OH)_4 \geq 1$. Under very strong upwelling $Si(OH)_4$ is likely to exceed NO_3 by a considerable amount, e.g., Bodega Bay, Monterey Bay and Aleutian Islands (Table 17.1). In low oxygen regions where denitrification has reduced the NO_3 content of upwelling water and $Si(OH)_4$ concentration has been augmented by the undercurrent in contact with the sediments, e.g., at 15°S Peru, the limiting nutrient may switch back and forth between NO_3 and $Si(OH)_4$ (Dugdale and Goering, 1970). Since diatoms are the major consumers of NO_3 in upwelling systems, low $Si(OH)_4$ concentrations relative to NO_3 become the primary limiting nutrient, controlling $Si(OH)_4$ uptake and NO_3

uptake as well (Dugdale and Goering 1970). White and Dugdale (1997) measured $Si(OH)_4$ and NO_3 uptake simultaneously in the Monterey Bay upwelling during 1992 and 1993 and showed that VNO_3 was stimulated by the addition of $Si(OH)_4$ in 20-L shipboard enclosure experiments. However Kudela and Dugdale (2000) determined that although $Si(OH)_4$ additions increased VNO_3 they did not result in accumulation of biomass. They described this upwelling region to be limited in the Liebig sense on NO_3 availability during most of the year and $Si(OH)_4$ to be regulating but not limiting. $Si(OH)_4$ concentrations in the surface water can become limiting when grazing or sinking occurs over a sufficiently long time due to the slower regeneration of $Si(OH)_4$ at the surface compared to N, the biologically active silicate pump (Dugdale *et al.*, 1995; Ragueneau *et al.*, 2002).

6.4. Interaction of iron and NO_3 uptake

The enhancement of diatom populations by addition of Fe to some oceanic regions with low Fe concentrations are well known. Some enclosure or grow out experiments carried out in coastal upwelling systems (e.g., Big Sur, California and some parts of the Peru coast) show similar effects, (Bruland *et al.*, 2005; Hutchins and Bruland, 1998; Hutchins *et al.*, 2002). Enhancement of NO_3 uptake with the addition of Fe is typically observed in these grow–out experiments in which NO_3 disappearance is followed (Chapter 38 by Hutchins and Fu, this volume). Some direct measurements in the field using ^{15}N incorporation have been made by Franck *et al.* (2005) who reported that upwelling areas with higher ambient Fe (i.e., Monterey Bay) had more $^{15}NO_3$ uptake by the larger phytoplankton (diatoms) than areas with less Fe (i.e., the eastern tropical Pacific). Studies of the effects of Fe on NO_3 uptake kinetics in upwelling areas are rare. Franck *et al* (2003) measured the effect of Fe on the kinetics of NO_3 and $Si(OH)_4$ uptake in water sampled from central California and showed a direct effect of Fe on V_{max} for both uptake systems (i.e., an effect on transporter synthesis). In the Pacific equatorial upwelling system, a co-limitation of new production by $Si(OH)_4$ and Fe was inferred since Si uptake kinetics were shown in the field to follow Michaelis–Menten (Leynaert *et al.*, 2001) and culture studies revealed Fe modification downward of the Michaelis–Menten hyperbolae (Leynaert *et al.*, 2004). More data on the effect of Fe on NO_3 uptake kinetics would be useful in understanding situations where co-limitation of NO_3 and Fe are likely to occur. If the effect of Fe is found consistently to affect the V_{max} for NO_3 uptake and is a general property of trace metal action (Dugdale, 1967) the problem of modeling co-limitation of primary and trace elements would be greatly simplified.

7. MODELING NITROGEN PRODUCTIVITY IN UPWELLING SYSTEMS

7.1. Ecosystem models of nitrogen flux

New production models in upwelling systems usually are constructed with nitrogen as the currency. Earliest simple models evolved from studies of the Peru upwelling system. For example, Dugdale and MacIsaac (1971) used a relationship between

specific NO_3 uptake (VNO_3) and NH_4 concentration to predict N-based growth rates. Walsh and Dugdale (1971) and Walsh (1975) employed a series of box-models trending offshore in simulating nitrogen flow in the 15°S, Peru upwelling plume, as was also used by Botsford *et al.* (2003) for the Bodega upwelling area. Wroblewski (1977) coupled a numerical model of upwelling circulation with one of NO_3 assimilation by phytoplankton to model nitrogen dynamics in the Oregon coastal upwelling region. Many models of nitrogen (and carbon) flux in coastal upwelling now include at least two forms of DIN (i.e., NO_3 and NH_4) (e.g., Ianson and Allen, 2002) usually incorporated through a Michaelis-Menten function and sometimes the inhibition of NO_3 uptake by NH_4 (e.g., Spitz *et al.*, 2003, 2005) and inclusion of DON (Olivieri and Chavez, 2000). Few include more than one size of autotrophic phytoplankton (Chai *et al.*, 2003).

Measurements made during drifter-following studies and experiments in large shipboard enclosures resulted in the shift-up concept (Fig. 17.4) and a conceptual model of algal physiological responses from upwelling to NO_3 exhaustion (see Section 17.4; MacIsaac *et al.*, 1974, 1985; Wilkerson and Dugdale, 1987). Model simulations by Zimmerman *et al.* (1987) showed the concept of acceleration (shift-up) of VNO_3 to be necessary to predict realistic time courses of NO_3 utilization. Their model gave boundaries where low NO_3 concentrations and high mixed layer depth precluded shift-up and high new production rates, as nutrients or light were insufficient for the up-regulation of physiological responses. The different boundaries measured in upwelling systems were used to estimate the optimal conditions for each.

Dugdale *et al.* (1990) then quantified the nitrogen-based physiological shift-up model of Zimmerman *et al.* (1987) to compare the uptake efficiency/performance of different upwelling systems. Using the initial (maximum) NO_3 concentration characteristic of each system, the maximum NO_3 uptake that could be achieved was calculated, following acceleration of uptake. This enabled the ''r'' or realization of maximal new production to be evaluated and compared amongst upwelling areas.

Until very recently, the resolution of physical models for coastal upwelling was too coarse to be useful for modeling biological processes. However, with increased computer power now available, physical models can resolve the plumes produced by topographical variations along upwelling coasts and are being combined with biologically derived models to predict nitrogen fluxes. For example, the regional NCOM-CCS (Navy Coastal Ocean Model-California Current System) model for the United States West Coast (Shulman *et al.*, 2004); a global circulation model used for ocean prediction now includes a one dimensional biological model (Chai *et al.*, 2003) based mainly upon the biological flux of nitrogen. This biological model was modified from the CoSINE (Carbon, Silicon, Nitrogen Ecosystem) ecosystem model originally developed for the equatorial Pacific upwelling system (Chai *et al.*, 2002; Dugdale *et al.*, 2002) that includes different forms of DIN, Si $(OH)_4$, different sized phytoplankton and interactions of NH_4 and Fe on phytoplankton metabolism. It has also been applied to the California coastal upwelling system (Klein 2003). In the next decade, with increased computing power and more ecophysiological information (e.g., nutrient-nutrient interactions, uptake

versus light relationships, species specific fluxes) available, accurate forecasting of new production and phytoplankton blooms for different eastern boundary upwelling systems will be possible.

7.2. Physical models to estimate new production

Rates of new production have been measured indirectly in upwelling areas from physical estimates of NO_3 fluxes into the euphotic zone (i.e., using the volume of water upwelled and the NO_3 it contains). This was first applied to equatorial upwelling (e.g., Carr et al., 1995; Chavez and Barber, 1987; Lewis et al., 1986), but has also been used in the Peru upwelling region (Chavez and Barber, 1987) and globally for all coastal upwelling systems by Walsh (1991). This approach was also used by Chavez and Toggweiler (1995) to estimate the global fraction of new production that is tied directly to upwelling, by differentiating NO_3 input attributed to wind driven upwelling (coastal) from that supported by vertical mixing or other processes. Their ''crude'' calculations suggest that 11% of global new production is supported directly by coastal upwelling.

7.3. Models to estimate new production from remotely-sensed data

The highly dynamic nature of coastal upwelling, in which the driving meteoro- logical conditions change on short time scales, days and weeks, makes it difficult to sample the system synoptically with ships. One solution is to employ remote sensing and there have been many models developed for estimating primary production in coastal upwelling areas from satellite data (e.g., Bricaud et al., 1987; Carr, 2002; Carr and Kearns, 2003; Gabric et al., 1993). Most depend upon estimation of chlorophyll concentrations at the surface and a series of assumptions about vertical distribution of chlorophyll and incident irradiance. However models of new production have been developed using the relationship of NO_3 and temperature in upwelling areas (e.g., Sathyendranath et al., 1991). An early version (Dugdale et al., 1994) of such a nutrient oriented remote sensing model for an upwelling system used the relationship between remotely sensed sea–surface temperature (from AVHRR imagery) and NO_3 concentrations, combined with a mean value of NO_3 uptake from existing ^{15}N uptake measurements. A similar approach (combined with temperature versus $Si(OH)_4$ relationships) calculated $NO_3:Si(OH)_4$ ratios based upon AVHRR derived surface temperatures and showed that new production in the eastern equatorial Pacific might be limited by $Si(OH)_4$ (Wilkerson and Dugdale, 1996). More complex models were devel- oped for coastal upwelling areas to estimate new production from remotely sensed temperature and ocean color. These employed temperature to estimate nutrient concentrations and time since upwelling, and incorporated the shift–up concept (Dugdale et al., 1990). The first such model was generated for the northwest Africa upwelling center at Cap Blanc (Dugdale et al., 1989) and later applied to the upwelling areas of Monterey Bay (Fig. 17.7; Kudela, 1995; Kudela and Dugdale, 1996) and Point Conception, California (Dugdale et al., 1997). Kudela and Chavez (2000) input temperature and chlorophyll from moorings, rather than from
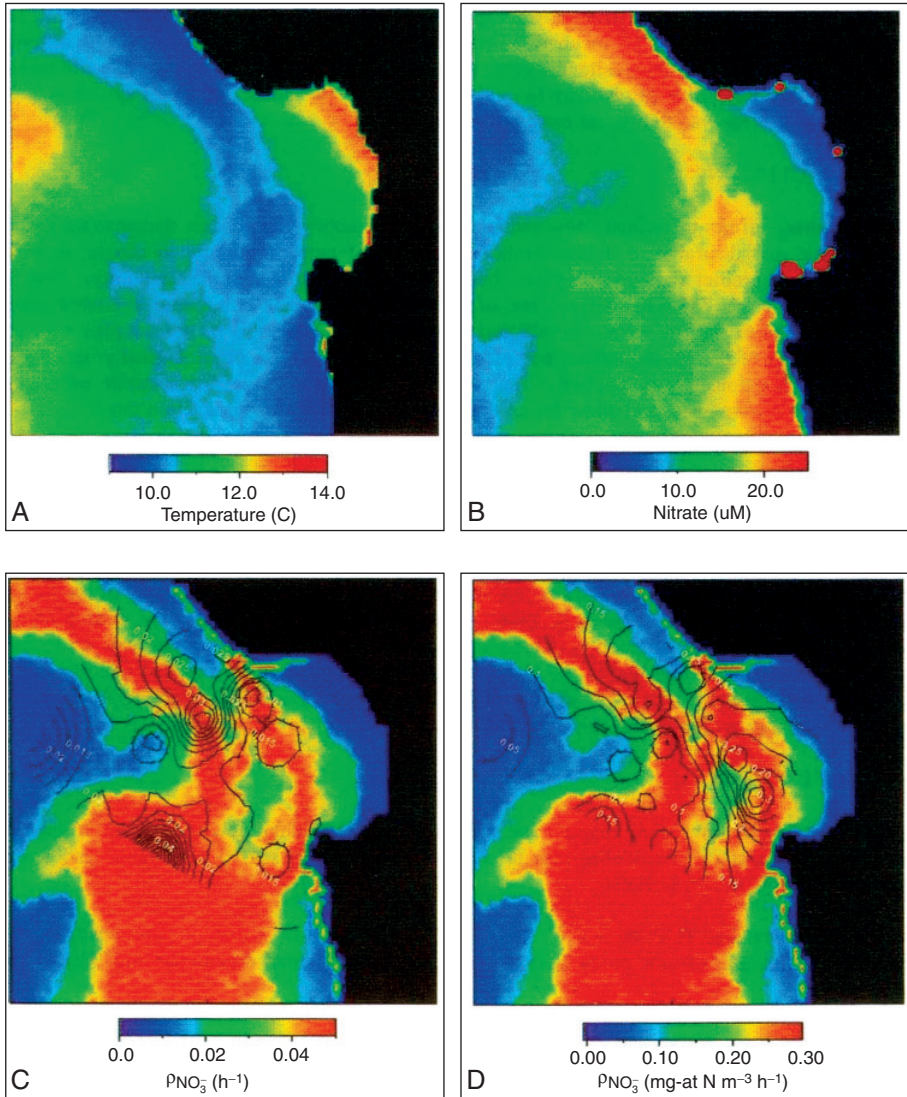
Figure 17.7 New production modeled from remotely sensed sea surface temperature, a 3 day composite for Monterey Bay, California. (A) AVHRR derived temperature, °C, (B) modeled surface NO$_3$, μM, (C) VNO$_3$, h^{-1} and (D) ρNO3, μmol l^{-1} h^{-1} with superimposed contours of shipboard ^{15}N uptake data. From Kudela and Dugdale (1996).

satellites, into the same model to evaluate the spatial impact of the 1992 El Niño on new production in Monterey Bay. Both satellite observations and mooring data were used later (Kudela and Chavez, 2002) to compare new production in Monterey Bay during the 1997–8 El Niño and subsequent La Niña conditions. Models that ignore the phytoplankton nutrient processes in upwelling are unlikely

to be successful, since the new production in upwelling systems will be proportional to the flux of new nutrient to the surface regions, the product of the upwelling rate and the concentration of source nutrients which may vary by factors of three or more (Table 17.1).

8. MOLECULAR APPROACH TO STUDYING NITROGEN ASSIMILATION

The uptake and metabolism of N is controlled by a number of key enzymes, which provide good molecular targets for studies of these processes in natural communities (see Chapter 7 by Mulholland and Lomas, this volume). Recent advances in isolating genes and obtaining entire genome sequences have enabled the development of specific gene probes for different members of the phytoplankton community. Molecular approaches can now be used to examine what components of the phytoplankton population have the capacity to synthesize and make the correct enzymes to assimilate different forms of N in response to environmental factors. Before this, our understanding of nitrogen dynamics in upwelling areas was restricted to measuring bulk changes in water constituents by the entire phytoplankton community (i.e., the particulate fraction that is caught on a filter). Traditional methods, such as tracer techniques (Chapter 31 by Lipschultz, this volume) or measuring enzyme assimilation rates (e.g., Berges, 1997; Berges and Harrison, 1995; Berges *et al.,* 1995; Slawyk *et al.*, 1997; Chapter 32 by Berges and Mulholland, this volume) provide nitrogen uptake/assimilation (productivity) rates, but do not identify the major players involved in mass transformation, or the physiology of the phytoplankton involved. Information on the response and physiology of specific phytoplankton groups, or breaking open the phytoplankton ''black box,'' provides a mechanistic understanding of observed rates of biogeochemical transformations. Molecular genetic techniques are beginning to be applied to the study of nitrogen cycling processes in marine systems (for review see Zehr and Ward, 2002; Chapter 30 by Zehr and Jenkins, this volume), where species- and group-specific information on the physiological/biochemical responses of marine microbes can be obtained (e.g., Cooksey 1998; Zehr and Voytek, 1999).

There has been an explosion of molecular genetic technologies becoming available and constantly being improved in the last decade. In particular QPCR (quantitative polymerase chain reaction) used with reverse transcription, can provide quantitative measurements of gene expression of functional genes involved in nitrogen assimilatory processes by specific microbial cells. Such approaches are being developed to study the molecular basis for biogeochemical changes (e.g., Parker and Armbrust, 2005) and variability in acceleration/adaptation of N uptake and new production rates in simulated upwelling studies. Smith *et al.* (1992) in a laboratory study to simulate upwelling measured the response of the gene for the major NO_3 assimilation enzyme, nitrate reductase (NaR) in light- and N-limited diatom cells of *Skeletonema costatum* when given an increase in light and NO_3 availability. They showed increases in levels of

NaR–specific messenger RNA (i.e., showing increased transcription of the gene for NaR) that coincided with the acceleration (shift-up) in NO_3 assimilation measured with ^{15}N. To be able to use gene expression as a proxy for species-specific flux or productivity, there first needs to be sufficient sequence data and identification of genes involved in N uptake in target microbial cells expected in these upwelling systems. Other chapters (e.g., Chapter 30 by Zehr and Jenkins, this volume; Chapter 7 by Mulholland and Lomas, this volume) describe our knowledge to date for prokaryotic members of the microbial community. However, in coastal upwelling areas, since phytoplankton communities are dominated by the eukaryotic diatoms, sequences are required for genes for N assimilation by this eukaryotic group, and these are limited at the present time (see below and Armbrust *et al.*, 2004).

Diatoms take up NO_3 and NH_4 via membrane transporters and assimilate NO_3 using assimilatory nitrate and nitrite reductase (NaR and NiR), and NH_4 using glutamine synthetase (GS) and glutamate synthase (GOGAT) (see Chapter 7 by Mulholland and Lomas, this volume, for detailed descriptions). Genes for a number of diatom nitrogen transporters have been identified. Hildebrand and Dahlin (2000) and Hildebrand (2005) first described NO_3 and NH_4 transporter genes (*hnat* and *amt*, respectively) in *Cylindrotheca fusiformis*. The whole genome analysis of *Thalassiosira pseudonana* yielded a genome that encodes multiple NO_3 and NH_4 transporters (Armbrust *et al.*, 2004). Few gene and protein sequences for nitrate reductase (NaR) have been well characterized from eukaryotic algae. Song and Ward (2004) characterized the gene for nitrate reductase (*nar*) from the eukaryotic green alga *Dunaliella tertiolecta* and showed it to be positively regulated by the presence of NO_3 rather than negatively regulated by NH_4. Allen *et al.* (2005) have sequenced the complete NaR gene for the pennate diatom *Phaeodactylum tricornutum* and designed PCR primer sets that are able to amplify NaR fragments in other diatom strains (including *Skeletonema costatum, Chaetoceros mulleri, Thalassosira pseudonana, T. oceanica, T. weissflogi, Coscinodiscus granii,* and *Asterionellopsis glacialis*). These primer sets were also used to amplify diatom specific NaR fragments in DNA samples collected in Monterey Bay indicating that nitrate reductase specific DNA of diatoms can be tracked in field studies. This demonstrates that DNA and RNA based methods can target diatom specific nitrogen assimilation genes in coastal upwelling ecosystems. Poulsen and Kroger (2005) also showed that NaR expression was inducible in transgenic *Cylindrotheca fusiformis* when cells were given NO_3. This offers a genetic manipulation approach to understand the relative expression of NaR genes in simulated upwelling conditions.

NH_4 assimilation occurs by the action of GS and GOGAT (Wheeler, 1983; Falkowski, 1983). The source of NH_4 may be externally supplied or internally as a product of NO_3 reduction or photorespiration. Consequently GS activity gives a measure of both regenerated (based on NH_4) and total productivity (based on NO_3 and NH_4) and is commonly measured in studies of algal physiology (e.g., Slawyk *et al.*, 1997; Slawyk and Rodier, 1986; Rees *et al.*, 1995). Two different isoforms of eukaryotic algal GS proteins have been described based upon their size: GS II that is found in eukaryotes (Chen and Silflow, 1996 in *Chlamydomonas reinhardtii*; Pesole *et al.*, 1991) and GS III which has been identified in diatoms and some other

eukaryotes (Robertson and Alberte, 1996; Robertson and Tartar, 2006; Robertson et al., 1999; 2001), although it was first described in bacteria (as described in Kumada et al., 1993). It has been suggested that one isoform is found in the cytoplasm of the cell and functions to assimilate external source of NH_4 whereas the other is located in the chloroplast (where nitrate reductase is found) and is responsible for assimilating NH_4 that has resulted from NO_3 reduction (reviewed in Anderson, 1999). To establish whether GS II and GS III might have these different functions in diatoms, Takabayashi et al (2005) measured expression of the gene responsible for GS II (*gln II*) in *Skeletonema costatum* cells grown with either elevated NO_3, or NH_4 or both forms of DIN. GS II gene expression increased in cells taking up NO_3. GS II-specific messenger RNA (i.e., transcript abundance of *gln II*, the gene that encodes for the enzyme isoform GS II) and total GS enzyme activity were increased 2–3.5 times above background in cells taking up NO_3 but not cells taking up NH_4. This confirms that GS II is using NH_4 derived from NO_3. The addition of NO_3 results in *gln II* transcription being induced to express the GS II isoform, which catalyzes assimilation of NH_4 that has been made available by reduction of NO_3 and NO_2 by NaR and NiR. Because of this specific role of GSII in diatoms assimilating NH_4 derived from NO_3 assimilation rather than NH_4 taken up directly from the environment, quantification of GS II gene expression (i.e., *gln II* mRNA) promises to be a useful indication (or proxy) of new production by phytoplankton (Takabayashi et al., 2005). In a simulated upwelling study using *Skeletonema costatum* in which cells were shifted into high light, there was a rapid increase in GS II gene expression measured as *gln II* mRNA using quantitative PCR (Barada, 2006). This indicates that the high light shift-up (Fig. 17.4) of physiological response may have a molecular basis.

9. Conclusions and Directions

The original chapter on ''Nitrogen in Upwelling Ecosystems'' written in 1983 by Codispoti described standing stocks and nutrient concentrations with some limited studies of rates of DIN uptake usually using ^{15}N and some measurements of N assimilation enzyme activities. Researchers were beginning to understand the importance of organic forms of DON (e.g., Whitledge, 1981) and had started to develop biological models of N flux to combine with physical simulation models (e.g., Walsh and Dugdale, 1971). Since then focus has been on understanding what components of the phytoplankton community are doing most of the N assimilation and flux, and how other nutrients interact in N flux. Taxonomic studies (e.g., Blasco et al., 1981; Jimenez, 1981; Rojas de Mendiola, 1981) pointed the way to the importance of diatoms and the larger phytoplankton in the coastal upwelling communities. So in the 1990s, studies of N flux were aimed at measuring size fractionated ^{15}N uptake to evaluate new production (or NO_3 flux) by this component (e.g., Franck et al., 2005; Wilkerson et al., 2000). With new molecular genetics techniques, the diatoms can be specifically targeted and we should expect to see many more studies using molecular probes for N assimilation to discern gene expression by the different components of the alga community. This

will let us understand the mechanisms used by diatoms to outcompete the pico–plankton for NO_3, and whether all phytoplankton (e.g., the picoplankton) have the capacity to assimilate all forms of DIN. With the molecular tools, studies of how different parts of the N assimilatory pathway can also be examined such as how or when transporters are switched on following upwelling of cells into the well lit nutrient rich surface waters, compared to the assimilatory enzymes, nitrate reductase and glutamine synthetase.

Although there was awareness of how DIN uptake could be modulated by other nutrients, such as $Si(OH)_4$ (Dugdale *et al.*, 1981) and micronutrients (Dugdale, 1967), the importance of studying these interactions has increased in recent years. Even so, specific experiments designed to measure the interactions are rare. Future research in upwelling will be sure to include laboratory based experiments and ship board enclosure/mesocosm experiments (e.g., Franck *et al.*, 2005; Joint *et al.*, 2002; Kudela and Dugdale, 2000) to study such interactions. The analysis of kinetics of nitrogen uptake has become more complicated since 1983 as evidence for linear uptake at high substrate concentration has accumulated (e.g., Collos *et al.*, 2005) and earlier evidence of multi–phasic uptake mechanisms corroborated. As expressed by Collos *et al.* (2005) more in-depth studies of DIN uptake kinetics that can occur in upwelling systems and what modulates them are required, both at the physiological and molecular level.

Another aspect of N flux in coastal upwelling studies that has ramped up since 1983 has been our ability to model the biological transformations taking place within the upwelling ecosystem, both so N flux can be measured indirectly from some other parameter such as vertical advection or from remotely sensed sea surface temperature, and also for inclusion in global ocean flux models. Most global ocean flux models use global circulation models with embedded ecosystem models. Since a significant proportion of the global marine biological production and natural carbon burial occurs in eastern boundary coastal upwelling areas (Walsh, 1991) due to the active nature of the biological pump in these eutrophic waters, accurate ecosystem models for these regions are required. Future coastal upwelling models will need to incorporate the results of our proposed future directions: i.e., advanced kinetics for N uptake, N flux capabilities of different components of the autotrophic community and the interaction of N flux with other nutrients and micronutrients. Although many advances have been made in understanding and modeling nitrogen fluxes in upwelling systems there are many more avenues to be explored and questions to answer.

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CHAPTER 18

ESTUARIES

Walter R. Boynton *and* W. Michael Kemp

Contents

1. INTRODUCTION

In the first edition of this book, Nixon and Pilson (1983) began their chapter on nitrogen in estuaries by indicating it was an exciting and challenging time to be engaged in such studies. In the two decades since, that assessment continues to apply. In fact, studies of nitrogen (N) in estuarine ecosystems have so greatly expanded, intensified, and diversified that it is a challenge to capture even highlights of progress in this hyperactive area of research.

Why have so much intellectual and other forms of capital been devoted to understanding N in estuarine ecosystems? A case can be made that a central work stimulating N investigations in estuarine systems was Ryther and Dunstan's (1971) paper in Science where they concluded, based on nutrient ratios and bioassay experiments, that N rather than P limited coastal algal growth and eutrophication and that reductions in P inputs to these systems would have little restorative effect. Prior to this, and for many subsequent years in some regions of the world, it was generally believed that P limited primary production, if indeed any essential element was limiting in estuaries. In a limited number of estuaries it was known that relatively large loads of N and P entered these systems. However, light was often thought to be limiting in typically turbid estuarine waters and short water residence times (compared to many lakes) were believed to rapidly transport nutrients from estuaries before biota could make use of these compounds (e.g., Schindler, 1981). A decade later the book Estuaries and Nutrients appeared (Neilson and Cronin, 1981) and for the first time there was a broad examination of nutrients in estuarine ecosystems. Papers included reviews and a few syntheses (e.g., Nixon, 1981), case studies from a limited number of estuarine ecosystems, and a few examples of estuarine responses to nutrient management efforts (Jaworski, 1981; Smith, 1981).

By the early to mid-1980s it became increasingly clear to many government agencies and environmental advocacy groups that something was seriously wrong with some estuarine systems (e.g., D'Elia et al., 2003; Malone et al., 1993). During this time several of the large estuarine restoration programs began in such sites as Long Island Sound, Chesapeake Bay, Tampa Bay and San Francisco Bay. With these programs came ambitious monitoring efforts where nutrient inputs from major rivers were gauged, point sources of nutrients were documented and estuarine nutrient concentrations, as well as other chemical (e.g., dissolved oxygen) and biological (e.g., chlorophyll a) variables, were routinely measured, often at many sites. A real estuarine data avalanche had begun and continues today; however, it also appears that many of these data sets remain severely under-analyzed. Bricker et al. (1999) produced a qualitative evaluation of water quality and habitat conditions in USA estuaries that included some 143 systems, again indicating the broad distribution of activities in these ecosystems. This trend has included sharp increases in the number of reported rate measurements and associated improvements in measurement techniques including measurements for rates of nutrient inputs to estuaries, primary production, denitrification rates, sediment-water exchanges of N, P and C, deposition of organic matter, burial of particulate nutrients, and others.

Given two decades of measurement frenzy since 1984, some have asked, what does all this mean? Are these systems all different or do they share some fundamental similarities? Do they respond to N loads in some understandable fashion, are internal losses of N proportional to loads and are there estuarine features that regulate the magnitude of N export to the coastal ocean? How do nutrients shape the structure and function of estuarine food-webs? There is an emerging, and hopefully accelerating, pattern of estuarine data synthesis taking place and N plays a prominent role in these efforts. Early work along these lines (e.g., Boynton et al., 1982; Nixon, 1981) indicated the importance of N in regulating algal primary production and

biomass, and Nixon substantially expanded comparative estuarine analyses to include aspects of nutrient cycling (Nixon, 1981) and secondary production (Nixon, 1982, 1988, 1992). Monbet (1992) related algal biomass in a large number of European estuaries to N concentrations after classifying sites according to tidal range. More recently, nutrient budgets have been used as a quantitative framework to organize diverse data sets towards testing our understanding of N dynamics at the level of whole ecosystems (e.g., Boynton *et al.*, 1995; Nixon *et al.*, 1996; Wulff *et al.*, 1990).

Degradation of estuarine environments prompted much of the interest in estuarine research and it now appears that nutrient-based eutrophication has become a central estuarine research theme, just as it did in limnology where this theme had the effect of unifying theoretical and applied aspects of that discipline. In recent years several reviews and syntheses have appeared with eutrophication being the core issue and considerations of N playing a very prominent role. Kelly (2001) and Rabalais (2002) reviewed N effects on coastal marine ecosystems, Cloern (2001) proposed several versions of a new eutrophication conceptual model and Smith (2003) reviewed and compared eutrophication responses of primary producers in lake, reservoir, marsh and estuarine systems (see also Chapter 11 by Paerl and Piehler, this volume). While it is clear that much needs to be done, it is equally clear that understanding of N in estuarine ecosystems has improved during the two decades since publication of Nitrogen in the Marine Environment (Nixon and Pilson, 1983).

The goal of this chapter is to provide an overview of advances during the past 20 years in our understanding of N in estuarine ecosystems and to suggest some fruitful directions future research might take. We have taken advantage of recent reviews, added information to several existing syntheses and generated several new summaries and syntheses from the almost overwhelming amounts of estuarine N data available. Three data summaries were developed specifically for this paper and these included N and P concentrations in the water columns of a variety of estuaries (Frank *et al.*, 2007), aquatic denitrification rates (Greene, 2005a), and estuarine sediment-water solute fluxes (Bailey, 2005). In this review we have: (1) examined N concentrations from a wide variety of estuaries to describe distinct spatial-temporal patterns in relation to season and climate, (2) reviewed, compared and expanded reported N-budgets for estuarine systems, (3) compiled extensive data on sediment–water fluxes of N and other solutes and examined regulating factors, (4) summarized and compared annual patterns and rates of phytoplankton primary production in relation to nutrient limitation, and (5) reviewed current concepts concerning interactions between N and both trophic state and secondary production in estuaries.

2. Distribution of Dissolved Nitrogen in Estuarine Waters

During the last twenty years, water column concentrations of dissolved N were probably the most intensively measured feature of N in estuarine environments. For example, about 8000 measurements of each of the common dissolved N species (i.e., NO_2, NO_3, NH_4 and dissolved organic nitrogen) were made per year for the

past twenty years as part of routine monitoring in Chesapeake Bay (Chesapeake Bay Water Quality Monitoring Program, 2004). Similar measurement programs have been gathering N concentration data for a decade or more (e.g., Danish estuaries, Conley *et al.*, 2000; Tampa Bay, Greening and DeGrove, 2001). In this section we review earlier efforts describing N distributions, provide some comparative analyses of N concentrations gleaned from examination of data compiled from many estuaries and conclude with a more detailed description of N distributions in Chesapeake Bay where these compounds have been measured at many sites and under variable climate conditions for several decades.

2.1. Previous examinations of nitrogen distributions

Earlier reviews of N distributions struggled to gather sufficient nutrient data to search for commonalities or differences among estuaries, but the current challenge is to organize the huge number of measurements into some tractable form. Given the enormous increase in the number of observations available, it's appropriate to ask what we have learned. Are there a limited number of N distribution patterns among estuarine systems or is variability in space and time the dominant feature? Estuarine characteristics such as river flow, depth, degree of stratification, water transparency, autotrophic community composition, and water residence time all influence N distributions. Since these characteristics vary widely both within and among estuaries we might expect reactive N-compounds to do the same.

Boynton *et al.* (1982) organized seasonal–scale dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) data from 28 estuarine systems and reported that concentrations of DIN ranged from <1 to 60 µM at the time of the year when phytoplankton production was at a maximum (generally during summer). In addition, DIN: DIP ratios in 75% of these systems were less than 10 during the period of highest production, suggestive of N-limitation, although ratios varied widely during the year, especially in river dominated estuaries. Nixon and Pilson (1983) examined nutrient concentrations in more detail from about a dozen USA estuarine systems. They also noted an extreme range in N concentrations among estuaries (0.1 to >200 µM) and found estuaries where DIN was very depleted in the water column, always abundant, or seasonally variable; the limited data available in the early 1980s may have largely prevented finding clear patterns of N concentration among estuaries.

In a recent and comprehensive review of 81 Danish estuaries it was reported that nutrient loading rate explained about 70% and 55% of the annual variation in total nitrogen (TN) and total phosphorus (TP) concentrations, respectively (Conley *et al.*, 2000). Concentrations of both DIN and TN were highest during winter and lowest during summer. The relative uniformity in annual patterns of N and P concentration in Danish estuaries is consistent with the similarity in their structure, with most being small and shallow, with relatively short water residence times and heavily loaded with agricultural N and P. A large estuarine characterization was completed by Bricker *et al.* (1999) in which 143 estuaries, representing about 90% of USA estuarine surface area, was examined for symptoms of eutrophication. Nitrogen was identified as a major cause of eutrophication, but this effort stopped short of examining data for patterns of N distribution.

Twenty years ago issues concerning dissolved organic nitrogen (DON) were just emerging onto research agendas, measurement techniques were in early stages of development, little was known concerning the various compounds comprising the DON pool, sources and sinks were not well established, and there were only a limited number of measurements available. Much has changed concerning all of these issues, resulting in the development of several important reviews, one from just over a decade ago (Antia et al., 1991) and two more recent works (Berman and Bronk, 2003; Bronk, 2002). Additionally, Burdige and Zheng (1998) developed a summary of DON exchanges between water and sediments in coastal and estuarine systems. We have added several more total dissolved nitrogen (TDN) and DON concentration measurements to those provided by Berman and Bronk (2003; Table 18.1). Several important points are clear. In most estuaries DON ranges from an important (30–40%) to the dominant (>80%) component of the dissolved N pool. DON is operationally defined but is actually a complex mixture of N-compounds with widely differing chemistries, sources and sinks. We echo here the conclusions of Berman and Bronk (2003) that DON is not just a conservative N pool, can have strong source and sink pathways in estuaries and needs more explicit consideration in evaluations of the N economy of estuaries.

2.2. Comparative examination of nitrogen distributions

We re-visited the issue of water column N distributions to see if we could find distinctive seasonal patterns related to estuarine type, location within an estuary and climate variability (i.e., wet, dry, average inflow conditions). We obtained ammonium (referred to hereafter as NH_4), nitrite (NO_2), nitrate (NO_3), and phosphate (PO_4) concentration data from 44 USA estuarine systems. Several locations (e.g., tidal freshwater, oligohaline, mesohaline, polyhaline) were selected in some systems and in a dozen cases we also obtained concentration data during dry, average and wet years (Frank et al., 2007).

Annual $NO_2 + NO_3$ concentrations in surface waters of 44 estuarine sites ranged from 0.05 to 150 µM (Fig. 18.1). Highest concentrations tended to occur in river dominated estuaries and lowest in dry and sub-tropical systems. As expected, concentrations were highest in tidal freshwater or oligohaline locations and decreased seaward. Despite the fact that all data in Fig. 18.1 were collected during average inflow years the range in concentrations was large. This is likely a reflection of the multiple factors, including N input rates, water residence times, and biological uptake, which influence concentrations. In a smaller set of estuaries, $NO_2 + NO_3$ concentrations were available for dry, average and wet years (Fig. 18.2). In Chesapeake Bay there were large differences in concentration between dry and wet years in the polyhaline and mesohaline sites; $NO_2 + NO_3$ was always high in the oligohaline zone because of proximity to the riverine N-source and limited uptake by phytoplanlton in this turbid portion of the system. Systems as different from Chesapeake Bay as Florida and Tomales Bays exhibited similiar but muted patterns. Differences between wet and dry year $NO_2 + NO_3$ concentration were very small or reversed in four of these systems indicating that generalities, even concerning such a common compound as $NO_2 + NO_3$, are hard to find.

Strong seasonal patterns were evident for $NO_2 + NO_3$ and PO_4 concentrations (Fig. 18.3). Nitrite + nitrate concentrations were highest during winter; if winter and

Table 18.1   Concentrations of Total Dissolved Nitrogen (TDN) and Dissolved Organic Nitrogen (DON) from Surface Waters of a Variety of Estuarine Systems

Location	TDN (µM N)	DON (µM N)	% DON	Method	Source
Shinnecock Bay, NY	2–4.9	0.6–4	30–88	PO	Berman and Bronk, 2003
Waquoit Bay, MA	140	40	29	NG	Berman and Bronk, 2003
Chesapeake Bay, mesohaline	34	21	62	PO	Berman and Bronk, 2003
Chesapeake Bay, mesohaline	43	22	51	PO	Berman and Bronk, 2003
Chesapeake Bay, mesohaline	23	22	96	PO	Berman and Bronk, 2003
Chesapeake Bay, polyhaline		16		UV	Berman and Bronk, 2003
Patuxent River, tidal fresh	70	29	41	PO	This study
Patuxent River, oligohaline	76	27	36	PO	This study
Patuxent River, mesohaline	33	24	73	PO	This study
Maryland Cstl. Bay, oligohaline	63	45	72	PO	This study
Maryland Cstl. Bay, polyhaline	40	37	93	PO	This study
Apalachicola Bay, FL	23	15	65	PO	Berman and Bronk, 2003
Delaware estuary		41		PO	Berman and Bronk, 2003
Elbe estuary	72	65	90	PO	Berman and Bronk, 2003
North Inlet, SC	19–35	18–31	87–93	NG	Berman and Bronk, 2003
Tomales Bay, CA		6–13		UV	Berman and Bronk, 2003

Source: Berman and Bronk (2003).
Entries labeled ''this study'' are from the Chesapeake Bay Water Quality Monitoring Program (2004). Measurement methods included: PO, persulfate oxidation; UV, ultraviolet oxidation; NG, not given.

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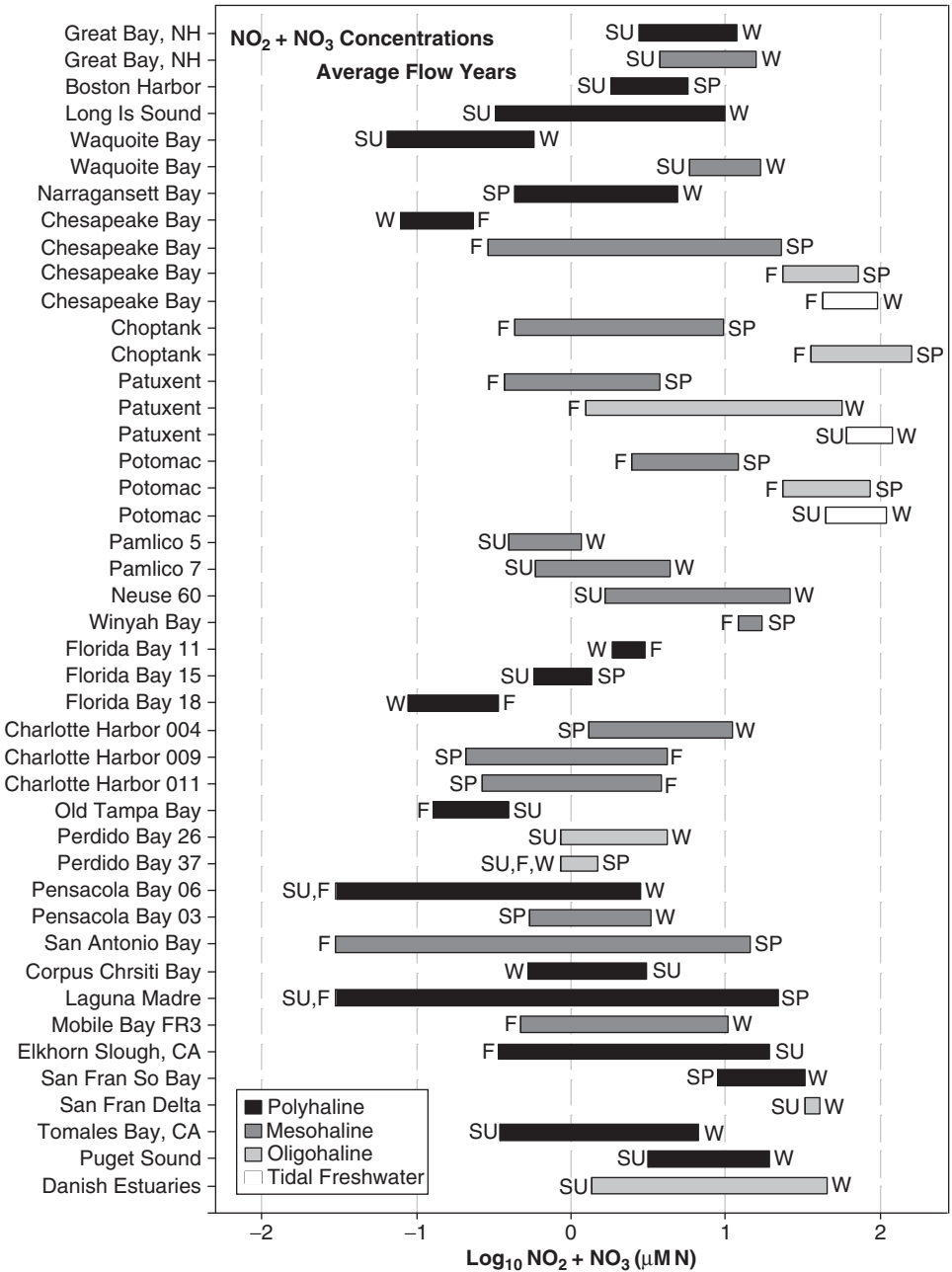
Figure 18.1 Bar graph of maximum and minimum $NO_2 + NO_3$ concentrations measured in a variety of USA estuaries during years of average freshwater inflow conditions. Location of samples from these systems is indicated by salinity regime (tidal freshwater, TF; oligohaline, O; mesohaline, M and polyhaline, P). Season in which maximum and minimum concentrations occurred are also indicated (winter, W; spring, Sp; summer, Su; fall, F). Data were compiled by Frank *et al.* (2007).
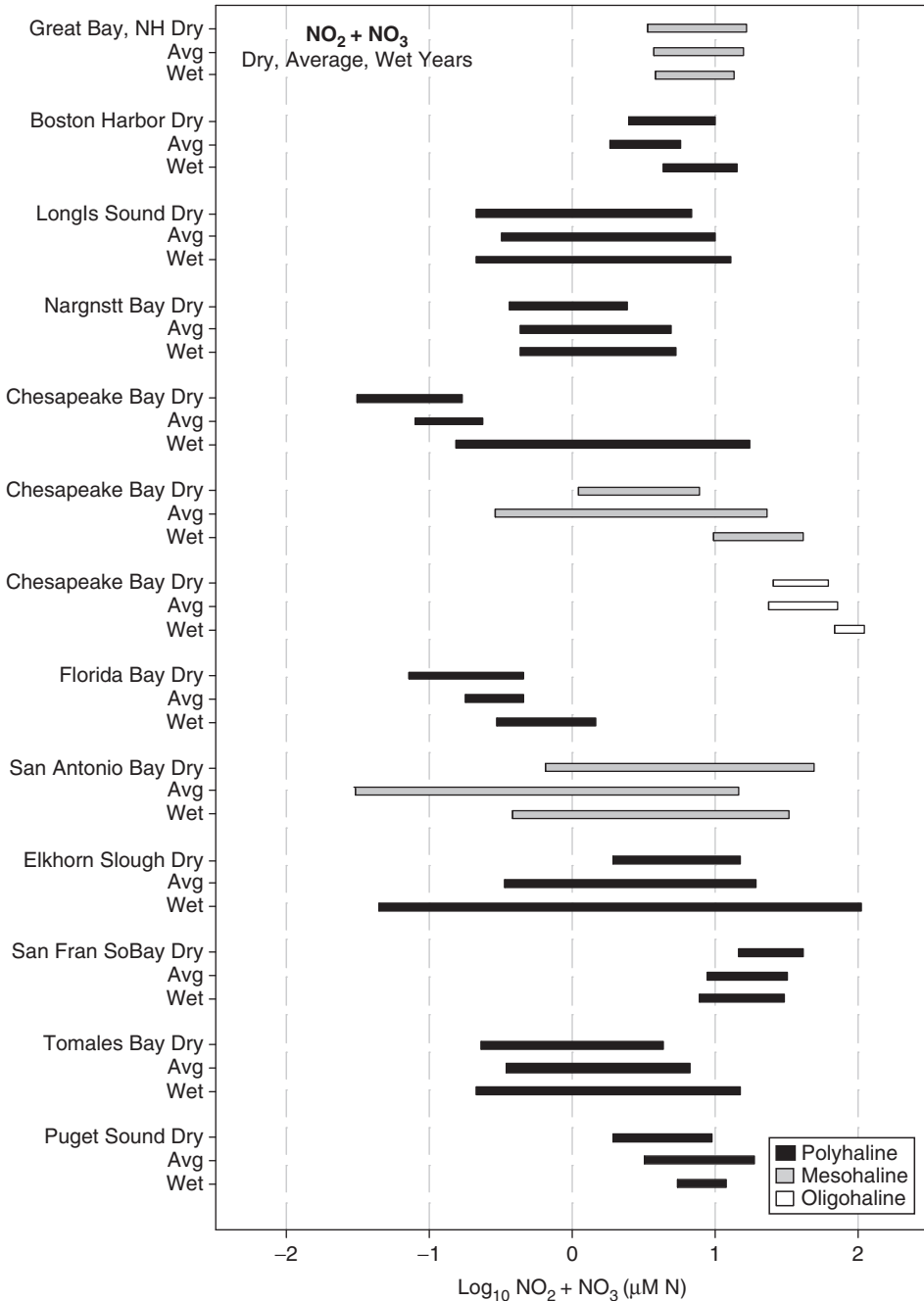
Figure 18.2 Summary of maximum and minimum $NO_2 + NO_3$ concentrations measured in a selection of USA estuaries during wet, average and dry years. Location of samples from these systems is indicated by salinity regime (tidal freshwater, TF; oligohaline, O; mesohaline, M and polyhaline, P). Season in which maximum and minimum concentrations occurred are also indicated (winter, W; spring, Sp; summer, Su; fall, F). Data were compiled by Frank *et al.* (2007).
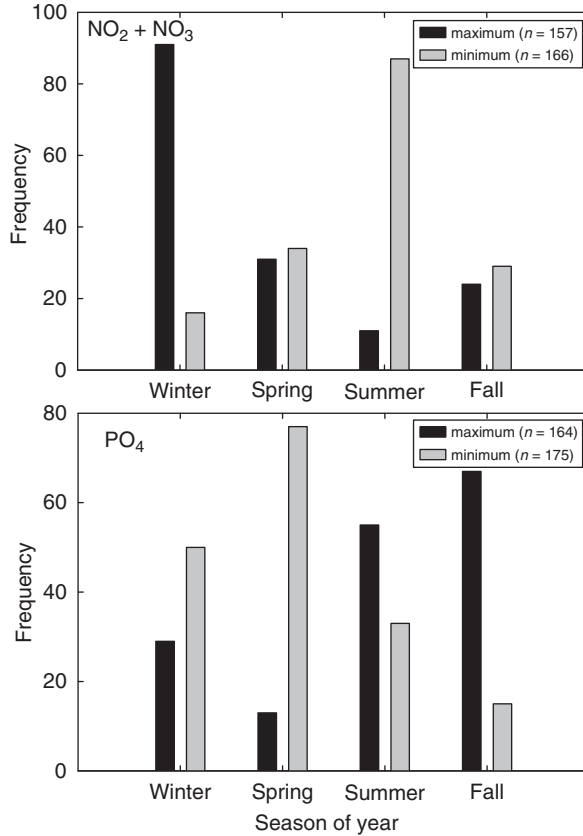
Figure 18.3 Frequency distribution of season in which maximum and minimum $NO_2 + NO_3$ and PO_4 concentrations were observed in a variety of USA estuarine systems. Data were compiled by Frank *et al.* (2007).

spring frequencies of maximum concentration are combined, then 80% of all maximum concentrations occurred during these seasons. Seasonal minimum concentrations mainly occurred during summer. Seasonal maximum PO_4 concentrations generally occurred during summer or fall, opposite the pattern observed for $NO_2 + NO_3$. Seasonal minimum concentrations of PO_4 were most frequently observed during spring, the time of the year that P has been found to limit spring diatom blooms (e.g., Fisher *et al.*, 1999). DIN: DIP ratios were also computed and grouped by categories as a frequency histogram (Fig. 18.4). The most frequently encountered category was less than 10:1 (40%) which suggests potential N-limitation, and almost 60% of all ratios were less than 20:1. The seasonal differences in N (high in winter-spring) and P (high in summer-fall) concentrations certainly play into creating this distribution of ratios. Given that nutrient inputs to many estuarine ecosystems are rich to very rich in DIN relative to DIP, net biogeochemical processes tend to reduce dissolved N concentrations relative to dissolved P concentrations.
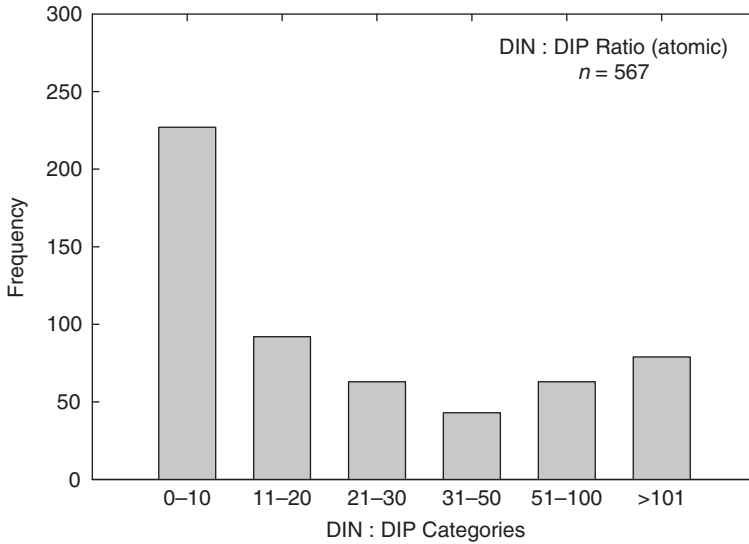
Figure 18.4 Frequency distribution of DIN:DIP ratios computed for a variety of USA estuarine systems. Data were compiled by Frank *et al.* (2007).

2.3. Detailed distributions in Chesapeake bay

While the analyses presented above suggest a wide range of variability in N distributions among estuarine systems, here we examine temporal and spatial patterns for one well-studied estuarine system. An intensive monitoring program in Chesapeake Bay and its tributaries during the past twenty years has experienced a wide range of hydraulic conditions. We summarized N distribution data to illustrate effects of season, location, depth and hydrology.

Monthly surface water nitrate concentrations in three salinity regions (oligohaline, mesohaline and polyhaline) in Chesapeake Bay are displayed as box and whisker plots and as mean values for wet or dry (i.e., high or low river flow) conditions (Fig. 18.5). Strong seasonal patterns are evident throughout the system for wet years and for dry years in all but the polyhaline. Differences between NO_3 concentrations in wet and dry years were pronounced in winter and spring but disappeared during fall. Differences in NO_3 concentrations with river flow in the mesohaline region were similar to the range in algal biomass observed between wet and dry years (Boynton and Kemp, 2000). Peak concentrations occurred in the polyhaline region only a month after the seasonal peaks occurring 300 km farther up the Bay. Harding and Perry (1997) reported the largest changes in algal biomass in the polyhaline zone during a 50 year period of increased nutrient loading, consistent with the NO_3 distribution pattern found between wet and dry years.

Whereas higher nitrate concentrations in surface (compared to bottom) waters reflect NO_3 sources in watershed runoff, higher concentrations of NH_4 in bottom waters derive from recycling processes occurring in the aphotic sediments (Fig. 18.6; Kemp and Boynton, 1992). Seasonal variations in bottom water NH_4
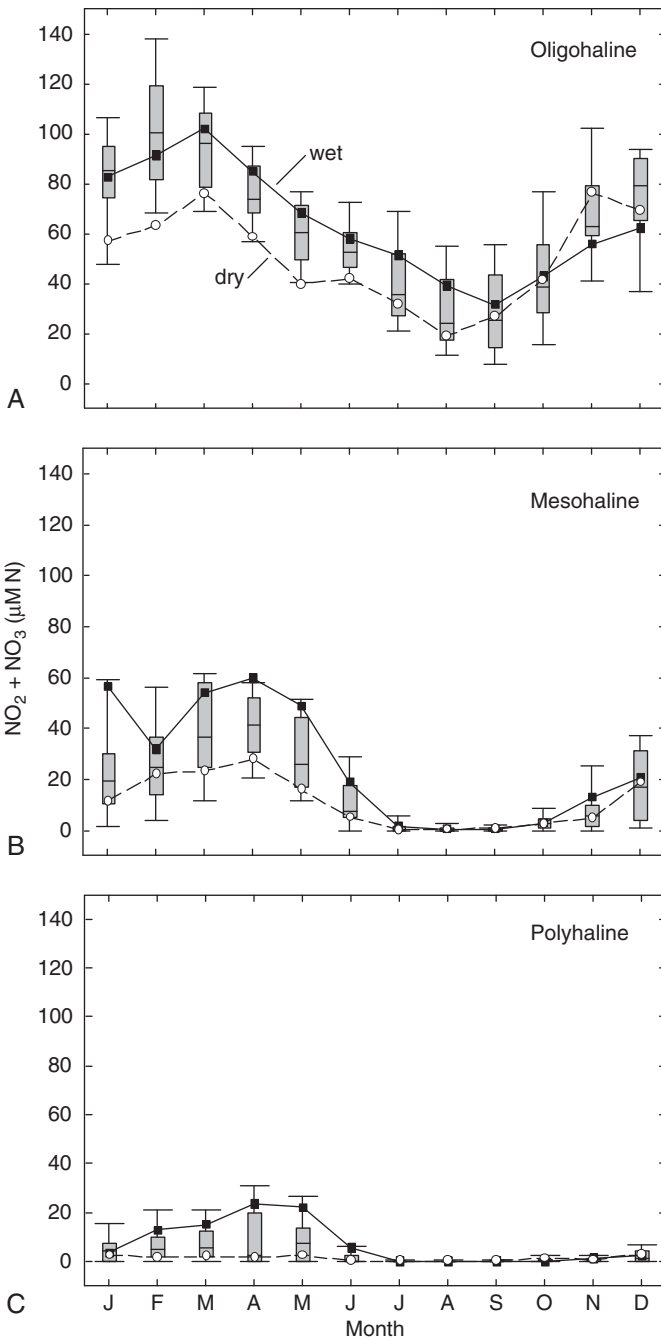
Figure 18.5 Surface water $NO_2 + NO_3$ concentrations measured at stations located in the oligo–haline (A), mesohaline (B), and polyhaline (C) regions of Chesapeake Bay. The box and whisker plots contain all values for each month in the 20 year data set (1985–2004). Dark squares and circles represent $NO_2 + NO_3$ concentrations during all wet and dry years, respectively, in this 20 year data set. Data were from the Chesapeake Bay Water Quality Monitoring Program (2004).

Figure 18.6 Surface and bottom water NH_4 concentrations measured at stations located in the oligohaline (A), mesohaline (B), and polyhaline (C) regions of Chesapeake Bay. Monthly or bi-monthly data in the 20 year data set (1985–2004) were averaged to seasons. Data were from the Chesapeake Bay Water Quality Monitoring Program (2004).

concentrations were most pronounced in the stratified portions of Chesapeake Bay, particularly in the mesohaline and polyhaline area, where deposited phytoplankton blooms provided labile substrate for decomposition processes. Seasonal patterns and vertical differences in NH_4 were not as evident in the oligohaline portion of the Bay, probably because of stronger vertical mixing and less deposition of labile phytode-tritus. During years of very high river discharge, summer peak NH_4 concentrations in bottom waters of the mesohaline, and even the polyhaline Bay, clearly reflected these flow conditions.

Finally, we examined the seasonal distribution of NO_3 along the salinity gradient of the Patuxent River estuary, a tributary of Chesapeake Bay (Fig. 18.7). While these "mixing diagrams" have inherent interpretive limitations (e.g., Nixon and Pilson, 1983), these plots of nitrate concentration versus salinity provide a format to examine variations in non-conservative processing of NO_3 with seasons and hydro-logic conditions. The most consistent feature of these data is the very rapid disap-pearance of NO_3 in low salinity regions during summer under all flow conditions. In contrast, the non-conservative sink behavior of nitrate clearly evident in summer is barely apparent during winter. In addition, concentrations in the tidal freshwater estuarine zone tend to be higher and lower during dry and wet winters, respectively, suggestive of solute dilution with increasing river flow. These seasonally modulated patterns of NO_3 loss in excess of dilution are not as evident for NH_4 or PO_4, both of which exhibited more spatial variability and strong indications of internal sources, probably related to intense remineralization in the sub-pycnoline water column and bottom sediments (e.g., Kemp and Boynton, 1984).

3. Nitrogen Budgets of Estuarine Systems

Although many data have been collected and reported concerning N in estuaries only a limited portion has been used to support development of quantita-tive N-budgets, at the scale of whole ecosystems. Given the early interest in quantifying the sources and fate of N (e.g., Johnstone, 1908), there have been surprisingly few published quantitative estuarine N-budgets. Most of these report rates of N inputs, internal losses and downstream exports for whole estuaries at annual time scales. Nixon *et al.* (1996) have organized annual-scale nutrient budgets for nine estuarine ecosystems.

3.1. Inputs of nitrogen to estuaries

Both the scope and detail of information currently available concerning N inputs to coastal and estuarine waters has changed dramatically since the early work of Meybeck (1982) who reported strong correlations between N concentrations and features of drainage basins (e.g., population density) for 30 rivers. During the late 1980s NOAA organized nutrient load estimates for many estuaries in the USA (e.g., Bricker *et al.*, 1999). More recently, Smith *et al.* (2003) updated the global-scale analysis of Meybeck (1982) using data from 165 rivers to demonstrate that: (1) N and
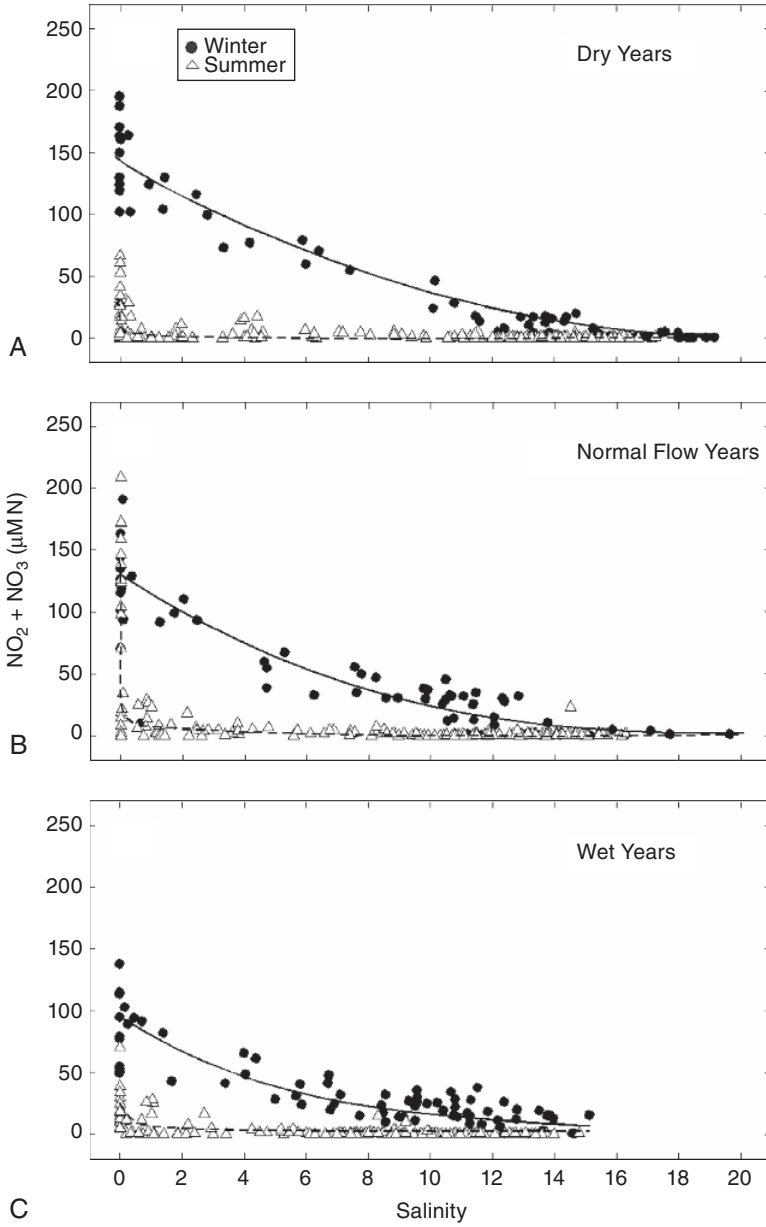
Figure 18.7 Salinity versus concentration plots for $NO_2 + NO_3$ during dry, average and wet years in the Patuxent River estuary. Summer and winter values are shown in each panel. Data were from the Chesapeake Bay Water Quality Monitoring Program (2004).

P loads were statistically related to population density and runoff per unit land area, (2) N and P loads were closely correlated to each other despite different biogeochemistries, (3) loads to coastal waters had increased by a factor of about three since the 1970s. Estimates of future loads to estuaries and the coastal ocean suggest another doubling by 2050 (e.g., Kroeze and Seitzinger, 1998; Chapter 18 by Seitzinger and Harrison, this volume).

Detailed assessments have been reported for anthropogenic N loads to specific river basins and associated riverine N export to adjacent estuaries and coastal waters. Nitrogen exports from a diverse sampling of coastal landscapes appear to be on the order of 20–25% of anthropogenic inputs (Boyer et al., 2002; Castro et al., 2003; Howarth et al., 2002; Van Breemen et al., 2002). A variety of nutrient load models and techniques have also been developed, ranging from those having complex mechanistic structures and goals of high spatial and temporal resolution (Costanza et al., 2002) to simpler, more tractable models designed for use in particular landscapes (e.g., Carmichael et al., 2004; Jordan et al., 2003; Valiela et al., 1997) to very ambitious statistical models used at continental scales (e.g., Smith et al., 1997).

Several recent analyses of nitrogen loads to estuarine systems have been based on direct measurements of loads from riverine and point sources (possible ocean inputs not considered). For example, Conley et al. (2000) reported N-loading rates to 81 Danish estuaries for a 7 year period, Nedwell et al. (2002) reported DIN loads to 93 mainland United Kingdom estuaries and Carmichael et al. (2004) estimated N loads to 15 small Cape Cod estuaries. In addition, estimates of historical N loads suggest 5-fold or larger increases during the last three centuries for both Narragansett Bay (Nixon, 1997) and Chesapeake Bay (Boynton et al., 1995). Recent estimates for Chesapeake (Hagy et al., 2004) and Waquoit Bay (Bowen and Valiela, 2001) suggest more than a doubling of N loads during the previous half-century. Nixon (2003) estimated Nile River nutrient loads to the adjacent Mediterranean sea coast before and after construction of the Aswan High Dam and argued that the loss of nutrients inputs due to damming of the Nile in the 1960s has largely been replaced by anthropogenic inputs associated with run-off of agricultural fertilizers and sewage discharges. Finally, in many estuaries, the adjacent land and atmosphere are the main sources of N, but the adjacent ocean can serve as the main external source of N in some situations. For example, Kelly (1998) estimated that transport of N from the ocean into Boston Harbor provided about twice as much N as land-derived sources. Bricker et al. (1999) noted, but did not quantify, ocean derived N as a major nutrient source along the USA Maine coast during portions of the year.

It appears that estuaries are among the most heavily fertilized systems on the planet. To examine the distribution of N-loading rates among well-studied estuaries, we organized a frequency histogram of N loads (on an estuarine area basis and including only loads from landside, but not oceanic, sources) for 218 estuarine systems (Fig. 18.8). The distribution that emerged indicated that most N-loading rates fell within the range of 6–50 g N m^{-2} year^{-1}, and only about 15% of the systems had loading rates below 5 g N m^{-2} year^{-1}. It is interesting to note that anthropogenic N dosing to major watersheds of the USA ranged from 0.5 to 3.5 g N m^{-2} year^{-1} (Jordan and Weller, 1996) and from 0.9 to about 6 g N m^{-2} year^{-1} for smaller watersheds of USA coastal areas (Castro et al., 2003; Van Breemen et al.,
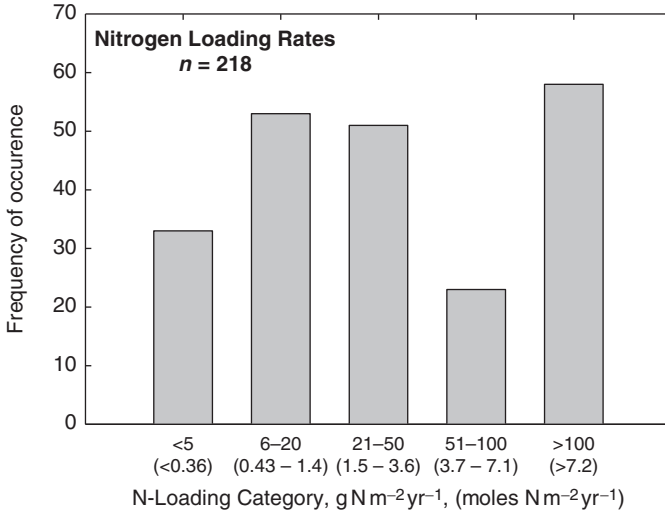
Figure 18.8 Frequency distribution of N-loading rates to a variety of estuarine and coastal marine ecosystems. Data were compiled from Boynton *et al.* (1995), Carmichael *et al.* (2004), Conley *et al.* (2000), Nedwell *et al.* (2002), and Nixon *et al.* (1996).

2002). The anthropogenic rates of N-loading to adjacent estuarine systems are clearly much higher; 37% of the sample exceeded 50 g N m^{-2} year^{-1}, almost an order of magnitude greater than most adjacent land areas.

We also assembled data for 34 estuaries where inputs of both TN and TP (g N or P m^{-2} year^{-1}) were available (Fig. 18.9). There is a very large range in N and P inputs among these estuaries. N loads varied by a factor of almost 200 and P by just over 300; the majority of systems in this sample had N and P loading rates ranging from 5 to 50 and 1 to 10, respectively. Despite the different biogeochemistry of N and P, there was an obvious correlation between loading rates of these elements (Smith *et al.*, 2003). Loading rates for a few systems (e.g., Himmerfjarden, Back River) had especially high N: P ratios because sewage was a major nutrient source and P, but not N, was removed at treatment facilities. In others, elevated N: P ratios were the result of diffuse source inputs that were naturally more enriched in N (mainly NO$_3$) than P. Finally, it is important to note that loading rates alone are not generally sufficient to predict the trophic status (*sensu* Nixon, 1995) of an estuary. For example, both the Potomac River estuary and Narragansett Bay had similar annual N-loading rates but the Potomac exhibited severe eutrophication characteristics while these were far less severe in Narragansett Bay. Several authors have noted that estuarine morphology, water residence times, water column mixing rates, light conditions and biological communities all have potentially strong influences on the impact of loading rates (e.g., Boynton *et al.*, 1996; Valiela *et al.*, 2000; Wulff *et al.*, 1990). A 25 year record of annual TN and TP loads to Chesapeake Bay from the Susquehanna River was added as an inset to Fig. 18.9 to serve as a reminder that inputs to some estuaries exhibit considerable inter-annual variability. In this
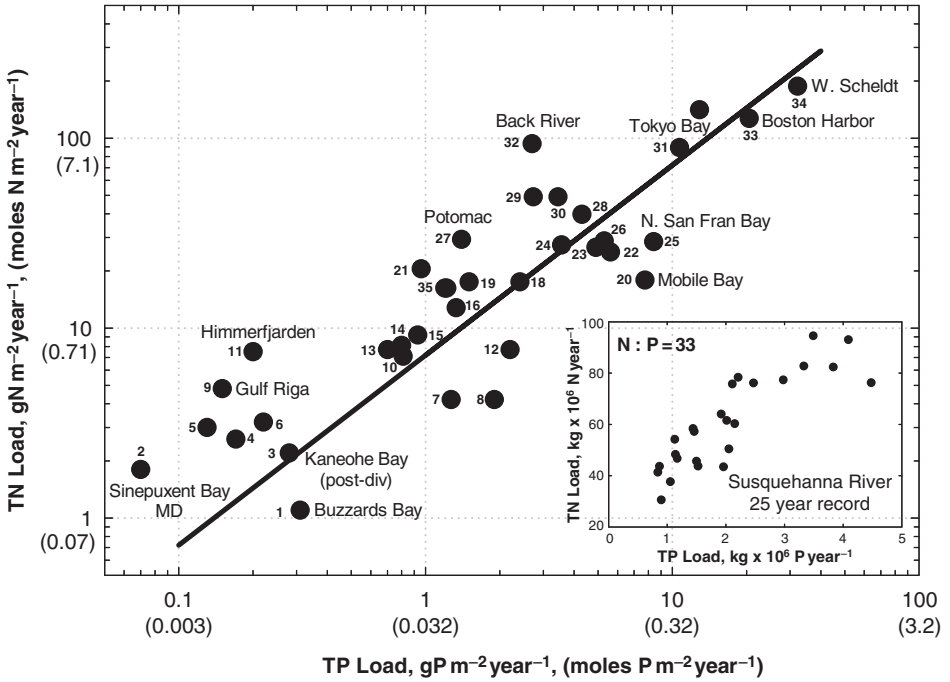
Figure 18.9 A scatter plot of annual TN versus TP loading rates to a selection of coastal, estuarine and lagoon ecosystems. Figure was re-drawn and expanded from Boynton *et al.* (1995). Systems are coded by number: 1 – Buzzards Bay, MA (NOAA/EPA, 1989); 2 – Sinepuxent Bay, MD (Boynton *et al.*, 1992, 1996); 3 – Kaneohe Bay, HI (post-diversion, Smith, 1981); 4 – Isle of Wight Bay, MD (Boynton *et al.*, 1992, 1996); 5 – Baltic Sea (Nixon *et al.*, 1996); 6 – Chincoteague Bay, MD (Boynton *et al.*, 1992, 1996); 7 – Kaneohe Bay, HI (pre-diversion, Smith, 1981); 8 – Narragansett Bay RI (prehistoric; Nixon, 1997); 9 – Gulf of Riga (Yurkovskis *et al.*, 1993); 10 – Albemarle Sound, NC (Nixon *et al.*, 1986); 11 – Himmerfjarden, Sweden (Engqvist, 1996); 12 – Guadaloupe Bay, TX (dry year, Nixon *et al.*, 1996); 13 – Buttermilk Bay, MA (Valiela and Costa, 1988); 14 – Moreton Bay, Australia (Eyre and McKee, 2002); 15 – Seto Inland Sea (Nixon *et al.*, 1986); 16 – Taylorville Creek, MD (Boynton *et al.*, 1992, 1996); 18 – Newport Bay, MD (Boynton *et al.*, 1992, 1996); 19 – N. Adriatic Sea (Degobbis and Gilmartin, 1990); 20 – Mobile Bay, AL (NOAA/EPA, 1989); 21 – Chesapeake Bay, MD (Boynton *et al.*, 1995); 22 – MERL (1x), RI (Nixon *et al.*, 1986); 23 – Delaware Bay, DE (Nixon *et al.*, 1996); 24 – Narragansett Bay, RI (current, Nixon *et al.*, 1996); 25 – N. San Francisco Bay, CA (Hager and Schemel, 1992); 26 – Guadaloupe Bay, TX (wet year, Nixon *et al.*, 1996); 27 – Potomac River estuary, MD (Boynton *et al.*, 1995); 28 – St Martins River, MD (Boynton *et al.*, 1992, 1996); 29 – Apalachicola Bay, FL (NOAA/EPA, 1989; Mortazavi *et al.*, 2000); 30 – Patapsco River Estuary, MD (Stammerjohn *et al.*, 1991); 31 – Tokyo Bay, Japan (Nixon *et al.*, 1986); 32 – Back River, MD (Boynton *et al.*, 1998); 33 – Boston Harbor, MA (pre-sewage diversion, Nixon *et al.*, 1996); 34 – Western Scheldt, Netherlands (Nixon *et al.*, 1996). The solid diagonal line represents the Redfield ratio of TN:TP inputs (weight basis). The inset shows the same variables from a 25 year time series of loading to Chesapeake Bay from the Susquehanna River. The N:P load ratio in the inset is weight based (Chesapeake Bay Water Quality Monitoring Program, 2004).

example, TN and TP loads varied by factors of about 3 and 5, respectively, and were very rich in N relative to P (TN:TP = 33 by weight). There was a strong indication that the TN:TP load ratio decreased during high load years, probably because more sediment, and sediment-bound P, were eroded and transported during wetter than average conditions. Thus, both the quantity and composition of nutrient inputs can vary by substantial amounts due to climate variability.

In general, variations in N-loading rates are reflected in concentrations of N in receiving water bodies. Although many processes act to modify nutrient concentrations at various rates, mean TN concentrations were significantly correlated to TN loading for five sub-systems of Chesapeake Bay averaged over a decadal period and for interannual variations in annual mean values for the Potomac River estuary (Fig. 18.10; Boynton and Kemp, 2000). Conley *et al.* (2000) reported that on an annual basis about 70% on the variation in TN concentration could be explained by variation in TN loads in a large sample of Danish estuaries.

A review of nitrogen sources to estuaries would not be complete without some discussion of nitrogen fixation, an internal source of nitrogen. A comprehensive review of nitrogen fixation in the world oceans is provided by Carpenter and Capone (Section IIIA, this book). Understanding of the magnitude, factors controlling dinitrogen (N_2) fixation rates and the ecological significance of this process in estuarine ecosystems has received considerable attention in the last few decades.
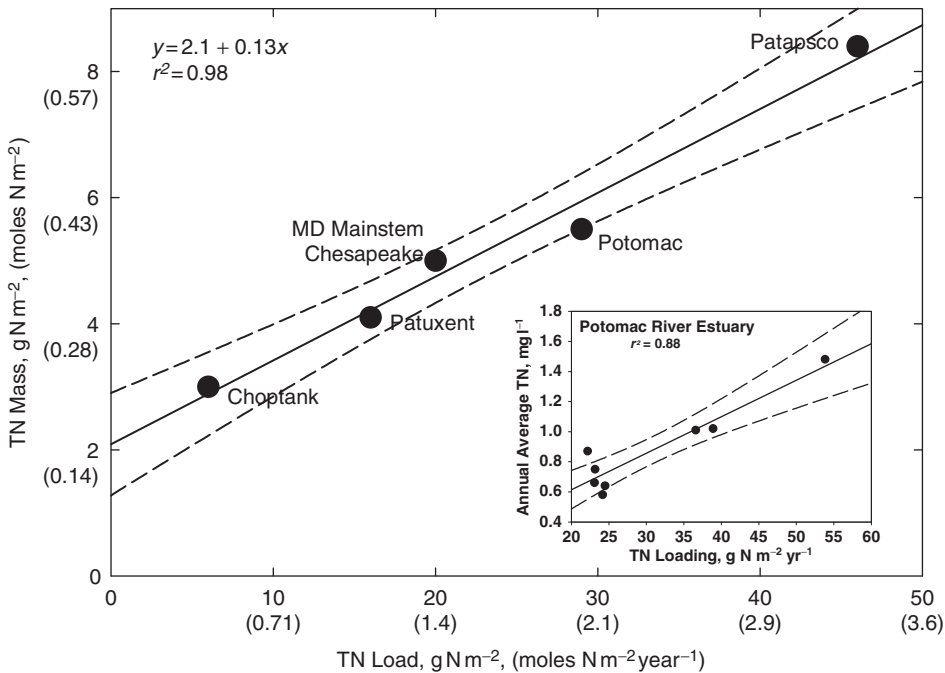
Figure 18.10 Scatter plot of average annual TN mass versus average annual TN loads for a portion of Chesapeake Bay and a selection of Chesapeake Bay tributary rivers. All concentration data were from sampling stations located in the mesohaline regions of the Bay and tributary rivers. Inset shows annual TN concentrations versus TN loads to the Potomac River estuary for an 8 year period. All data were from the Chesapeake Bay Water Quality Monitoring Program (2004).

It appears that in aphotic sediments and in estuarine water columns, N_2 fixation rates tend to be low and not of ecological significance at the level of whole estuarine systems (e.g., Affourtit *et al.*, 2001; Burns *et al.*, 2002; Howarth *et al.*, 1988a, Marino *et al.*, 2002; Paerl *et al.*, 1987). However, in photic sediments of coral reefs (Capone *et al.*, 1992; Koop *et al.*, 2001), salt marshes (Nielsen *et al.*, 2001) and seagrass communities (McGlathery *et al.*, 1998; Welsh, 2000) N_2 fixation rates tend to be higher and of importance to the nitrogen economy of these localized communities (see also Carpenter and Capone, this volume, O'Neil and Capone, this volume; Hopkinson and Giblin, this volume; McGlathery, this volume).

Howarth *et al.* (1988a) reported N_2 fixation rates to be low (<0.2 g N m^{-2} year^{-1}) in bare estuarine sediments, higher in organic-rich estuarine sediments (0.4–1.6 g N m^{-2} year^{-1}) and higher still in cyanobacterial mats (1–76 g N m^{-2} year^{-1}). Herbert (1999) and Welsh (2000) developed excellent summaries of estuarine N_2 fixation rates for a variety of sub-systems and reached similar conclusions. In these more recent summaries, bare sediment rates ranged from 0.03 to 0.65 g N m^{-2} year^{-1}, rates in cyanobacterial mats associated with salt marshes and reefs ranged from about 1–10 g N m^{-2} year^{-1} in temperate areas and were higher in the tropics and rates in seagrass communities ranged from about 0.1 to 6 g N m^{-2} year^{-1} in temperate areas and from 10 to 40 g N m^{-2} year^{-1} in tropical locations. Comparing these rates to loading rates of N from external sources (Figs. 18.8 and 18.9) indicates that N_2 fixation is not a dominant source of ''new nitrogen'' to most estuaries, although it can be an important feature in some estuarine communities, especially in oligotrophic tropical systems (Welsh, 2000).

Understanding of the factors controlling N_2 fixation rates in estuarine water columns and sediments is also evolving. Suppression of water column N_2 fixation rates by iron limitation and sulfate inhibition of molybdenum uptake have been found to be important features in oxic environments and less so in reducing environments (Howarth and Cole, 1985; Howarth *et al.*, 1988b). Paerl *et al.* (1987) reported increased N_2 fixation rates with the addition of labile organics and suggested that additional substrate and the associated creation of anoxic microzones enhanced rates. In a recent series of papers Marino *et al.* (2002, 2003, 2006) found low N_2 fixation rates by planktonic estuarine cyanobacteria under nitrogen limited conditions to be caused by a combination of intrinsic slow growth, grazing by estuarine zooplankton and sulfate- molybdenum interactions. Thus, estuarine N_2 fixation rates much lower than those typically observed in nitrogen limited lakes appear to be the result of both top-down and bottom-up effects.

3.2. Internal losses of nitrogen

Compared to N inputs, the internal loss terms of estuarine N-budgets are not as well documented. In general, internal losses include burial of PN in accreting sediment columns and denitrification in either the water column or sediments (see also Devol, this volume). A few nutrient budgets have estimated N-extraction in the harvest of fish and shellfish biomass and fish migration from estuaries.

Estimates of long-term PN burial (Table 18.2) illustrate a substantial range in rates from very small values (0.05–0.2 g N m^{-2} year^{-1}) in the deepest systems to

Table 18.2 Summary of Long-Term Particulate Nitrogen (PN) Burial Rates for Several
Tidal Marshes and a Selection of Estuarine and Coastal Marine Ecosystems

Ecosystem type	Location	N-burial rate (g N m^{-2} year^{-1})	N-burial rate (mmol N m^{-2} year^{-1})	Reference
Tidal marshes	N. Carolina	1.3–4.1	93–293	Cited in Merrill, 1999
	Louisiana	21	1500	Cited in Merrill, 1999
	N. Carolina	6.9–10	493–714	Cited in Merrill, 1999
	Choptank River	19–27	1367–1929	Cited in Merrill, 1999
	Monie Bay	14	1000	Merrill, 1999
	Patuxent River	21	1500	Merrill, 1999
	Hudson River	2.3–16	164–1143	Merrill, 1999
	Delaware Bay	2.5	179	Cited in Nixon *et al.*, 1996
Estuaries	Chesapeake digohaline	11	786	Boynton *et al.*, 1995
	Chesapeake mesohaline	3.5	250	Boynton *et al.*, 1995
	Patuxent oligohaline	14	1000	Boynton *et al.*, 1995
	Patuxent mesohaline	5	357	Boynton *et al.*, 1995
	Potomac mesohaline	10	714	Boynton *et al.*, 1995
	Choptank mesohaline	1.7	121	Boynton *et al.*, 1995
	Delaware Bay	1.1	79	Cited in Nixon *et al.*, 1996
	Narragansett Bay	3.3	236	Cited in Nixon *et al.*, 1996
	Guadalupe Bay	0.5	36	Cited in Nixon *et al.*, 1996
	Ochlockonee Bay	1.6	114	Cited in Nixon *et al.*, 1996
	Boston harbor	2.6	186	Cited in Nixon *et al.*, 1996
	Scheldt estuary	14	1000	Cited in Nixon *et al.*, 1996
River	Mississippi–Atchafalaya	5.6	400	Milliman and Syvitski, 1992
Deltas	Grijalva–Usumacinta	2.5	179	Milliman and Syvitski, 1992
	Magdelena	58	4143	Milliman and Syvitski, 1992
	Orinoco	4	286	Milliman and Syvitski, 1992

Table 18.2 (*continued*)

Ecosystem type	Location	N-burial rate (g N m^{-2} year^{-1})	N-burial rate (mmol N m^{-2} year^{-1})	Reference
	Amazon	4.6	329	Milliman and Syvitski, 1992
Deeper coastal	Baltic Sea	0.2	14	Larsson *et al.*, 1985
Deeper coastal	Baltic Sea	0.2	14	Larsson *et al.*, 1985
Systems	Laurentian Channel	0.1	7	Muzuka and Hillaire-Marcel, 1999
	Labrador Sea	0.05	4	Muzuka and Hillaire-Marcel, 1999

Methods used to determine PN burial rates varied but all included an estimate of sediment accumulation rate (^{210}Pb, sediment budget, pollen grain analysis) and an estimate of PN concentration at a depth in the sediment column where concentrations were constant with further depth.

very high rates for river deltas and tidal marshes (>20 g N m^{-2} year^{-1}). Although the small values in deep systems suggest processing of PN during transit through deep water columns (Hargrave, 1973; Kemp *et al.*, 1992), high variability in rates among systems with similar depths indicates other influences. In a synthesis of nutrient (N and P) budgets for 9 estuarine ecosystems, burial of PN was a prominent term in only two systems ($>35\%$ of TN inputs buried) while in the others burial accounted for 12% or much less of TN inputs (see Nixon *et al.*, 1996 for nutrient budget details for 9 estuarine ecosystems). The two systems in which burial was important (Patuxent and Potomac River estuaries) are sediment-rich, eutrophic estuaries, with relatively long water residence time (2–4 months). Of the other systems considered (Nixon *et al.*, 1996), some had high loading rates but short residence times (e.g., Boston Harbor), while others had limited sediment supplies (e.g., Narragansett Bay). We suggest that PN burial can be an important internal loss in some sediment-rich systems, but limited measurements make this conclusion speculative.

Denitrification, which represents a potentially important process for removing fixed N from estuaries, occurs predominantly in the upper stratum of sediments where rates tend to be limited by availability of nitrate. Nitrate is also produced via nitrification near the sediment surface which is, in turn, limited by availability of oxygen. A recent review of denitrification rates in aquatic systems included 1757 measurements from 152 sites over 45 years (Greene, 2005a; Fig. 18.11 insert; see also Chapter 18 by Devol, this volume). Since the 1960s at least nine different techniques were used to measure denitrification. The most frequently used method was the "acetylene block" technique in which acetylene is used to inhibit the reduction of N_2O to N_2, with N_2O accumulation used as a proxy for N_2 production. The acetylene technique was introduced in the 1970s and use peaked in the 1990s. There has been a growing interest in direct measurements of N_2 production since
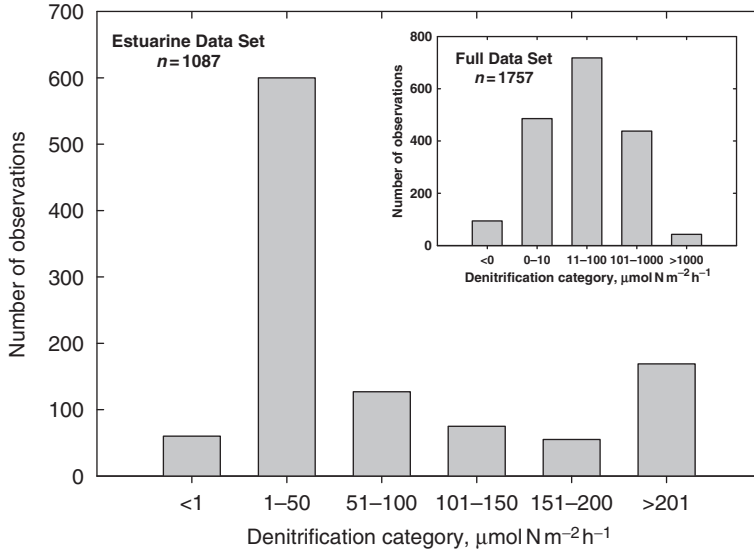
Figure 18.11 Frequency distribution of denitrification measurements from a variety of estuarine ecosystems. The inset is a frequency histogram using denitrification measurements from all aquatic systems. Both figures used data from the denitrification summary developed by Greene (2005a).

the 1980s. In the mid–1990s, isotope pairing and membrane inlet mass spectrometric (MIMS) techniques were developed and the use of the acetylene technique declined substantially.

Most reported denitrification measurements indicated rates between 11 and 100 μmol N m^{-2} h^{-1}, with few rates <1 μmol N m^{-2} h^{-1} and few in excess of 1000 μmol N m^{-2} h^{-1} (Fig. 18.11). In estuarine systems (Fig. 18.11) the most commonly reported rates were between 1 and 50 μmol N m^{-2} h^{-1}, but almost 40% of measurements were larger. Estuaries were also the most intensively measured systems (56% of all measurements), although many estimates were also available for continental shelves, coastal wetlands, lakes, lagoons, inland wetlands and several other environments (Table 18.3). The lower limit of rates in most environments was <1 μmol N m^{-2} h^{-1} while maximum rates ranged from 167 μmol N m^{-2} h^{-1} in seagrass communities to 24,142 μmol N m^{-2} h^{-1} in human engineered systems. Maximum rates above 1000 μmol N m^{-2} h^{-1} were also measured in freshwater creeks, coral reefs, continental shelf and estuarine environments, with maximum estuarine rates reaching nearly 20,000 μmol N m^{-2} h^{-1} in an area near a sewage treatment plant discharge. Mean rates in most systems were between 50 and 250 μmol N m^{-2} h^{-1}. It is useful to note that denitrification rates of 200 μmol N m^{-2} h^{-1} are equal to about 25 g N m^{-2} year^{-1}, a substantial fraction of TN loading rates to many estuaries (Fig. 18.8).

Several earlier papers helped place denitrification, as an internal loss term, into the context of the N economy of estuaries. Seitzinger (1988) summarized available

Table 18.3 Summary of denitrification rates from a variety of aquatic ecosystems. These data were from a summary developed by Greene (2005a)

| System type | Denitrification rate, μmol N m^{-2} h^{-1} | | | | Number of measurements |
	Minimum	Maximum	Mean	Median	
Lakes	0	490	125	100	91
Freshwater creeks	0	1500	266	13	17
Freshwater wetlands	0	330	39	4	52
Human engineered systems	0	24142	1497	8	55
Tidal mudflats	2.1	213	70	30	62
Coastal wetlands	−200	375	96	71	154
Seagrass communities	8.3	167	51	21	7
Coastal lagoons	0	290	19	5	116
Estuaries (sub-tidal)	−93	19616	182	31	1052
Coral reef communities	0	1351	107	19	38
Continental shelf	0	1657	102	13	113

System types are arranged in geographic sequence from land to sea. Total number of measurements = 1757. Negative values indicate that N-fixation rates exceeded denitrification rates.

data from lakes, rivers and estuaries regarding the ecological significance of this process. Important conclusions were that denitrification rates were higher in systems receiving large, anthropogenic nutrient inputs, most of the NO_3 consumed in denitrification apparently came from sediment-based nitrification rather than from the water column and, from a small sampling of estuaries, denitrification rates were proportional to TN loading rates, removing an average of 40% of N inputs. More recently, Nixon *et al.* (1996) computed the proportion of input N removed via denitrification from a larger selection of estuaries and several lakes, with values ranging from 10% to 74%, again indicating the importance of this process.

Although denitrification is an anaerobic process, estuarine rates are often limited by conditions of low bottom water oxygen and organic enrichment of sediments. Resulting low redox conditions, high sulfide concentrations and shallow penetration of oxygen into sediments inhibit nitrification, and consequently denitrification (e.g., Henriksen and Kemp, 1988; Joye and Hollibaugh, 1995; Vanderborght and Billen, 1975). Under these conditions, sediment recycling of N becomes more efficient in that most of the PN deposited to sediments is returned to the water column as NH_4 (Kemp *et al.*, 1990). We identified a limited number of studies in which bottom water dissolved oxygen varied appreciably during the study period and in which denitrification and net sediment–water fluxes of NH_4, NO_2 and NO_3 were also measured. Such data were available from a Danish site (Rysgaard-Petersen *et al.*, 1994) and from several studies conducted in Chesapeake Bay (Kemp *et al.*, 1990 and Cornwell, unpublished data). We developed an index of N recycling efficiency and examined
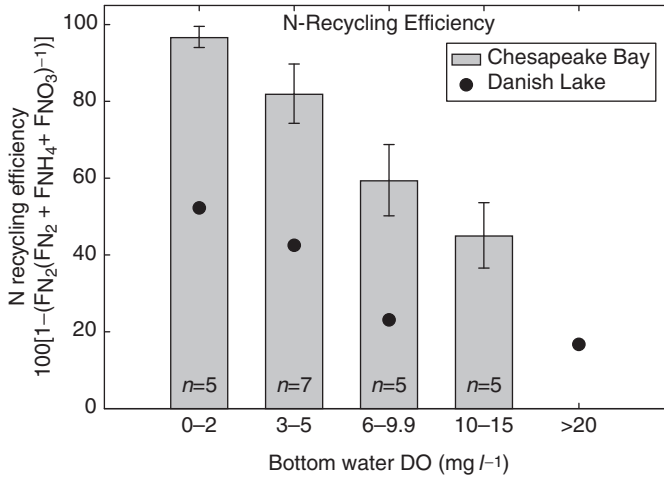
Figure 18.12 Bar graph of N-recycling efficiency as a function of bottom water dissolved oxygen concentration. Bars were based on Chesapeake Bay data reported in Kemp *et al.* (1990) and Cornwell (unpublished data). Solid dots were based on data from Rysgaard-Petersen *et al.* (1994) collected from Vilhelmsborg So, Denmark. Terms in the recycling efficiency calculation (γ-axis) are: F_{N_2} = flux of N_2 from sediments; F_{NH_4} = flux of ammonium from sediments; F_{NO_3} = flux of nitrate from sediments.

this as a function of bottom water dissolved oxygen concentrations (Fig. 18.12). For both systems there was a consistent increase in cycling efficiency as dissolved oxygen concentrations decrease. This trend suggests that estuarine eutrophication can seriously inhibit N removal via coupled nitrification-denitrification. Clearly, more sites need to be examined to see if this is a general pattern of response. However, the increase in N cycling efficiency with low dissolved oxygen conditions is consistent with the relatively low percent N removal via denitrification in Chesapeake Bay and adjacent tributary rivers, all of which have hypoxic or anoxic bottom waters for portions of each year (Boynton *et al.*, 1995; Hagy *et al.*, 2004).

3.3. Export of nitrogen to downstream systems

The final term in typical nutrient budgets is the export or import of materials to or from the adjacent downstream system. While we have indicated that there has been significant progress in understanding N in estuarine systems, there are also areas with pressing needs for improvement. Export of materials at the mouths of estuaries is a case in point. In most N-budgets the export term was estimated by subtracting the summation of internal losses from the full suite of inputs. The main reason for this non-independent approach was simply because more direct estimates of net flux were too difficult to accomplish. The basic problem is that water fluxes at the mouths of estuaries are relatively large, circulation patterns are often complex (e.g., Boicourt, 1983; Chao *et al.*, 1996; Kjerfve and Proehl, 1979) and the differences

in nutrient concentrations across this boundary are often small and variable. Thus, it is extremely difficult to develop accurate estimates of net exchange. It appears that this term will remain uncertain until dependable hydrodynamic models become even more widely available and accessible to those interested in nutrient dynamics.

Despite this difficulty, a recent synthesis of nutrient exchanges between estuaries and coastal waters provides some insights (Nixon *et al.*, 1996). Exports of N from 10 estuarine systems and several lakes ranged from 10% to about 90% of inputs and, as suggested earlier (Boynton *et al.*, 1995), N exports were not well correlated with inputs. The percent of N inputs exported to the coastal ocean was inversely correlated with the log mean residence time of water in the system (Nixon *et al.*, 1996), as has been found for lakes (Kirchner and Dillon, 1975). Adding data for four Chesapeake Bay tributaries to this relationship suggests that other factors (e.g., depth, salinity, nutrient levels) also affect relative export of N (Fig. 18.13). However, despite the widely varying environmental character among these systems, water residence time exerts strong control on the ratio of export to loading. If estuarine communities have sufficient time to process nitrogen, they will, in effect, reduce the N exported via N burial or denitrification. Thus it appears that estuaries can act as either "pipes" where N transport rather than transformation is the dominant process or as "sinks" for N, all as a function of how long water remains in the system.
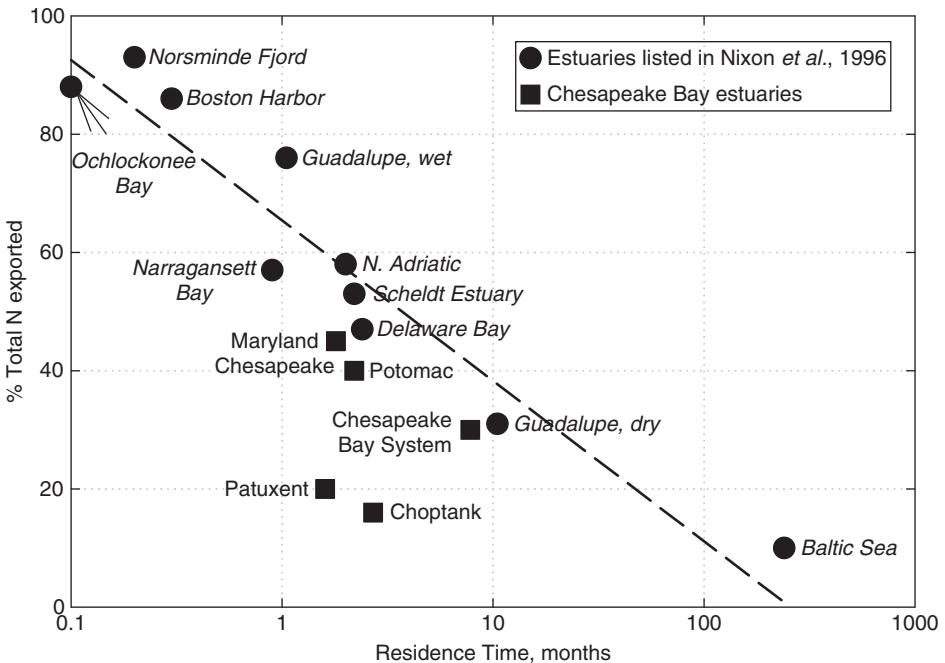
Figure 18.13 Scatter plot of percent N exported versus water residence time for a variety of estuarine ecosystems. Figure was re-drawn from Nixon *et al.* (1996). Patuxent and Choptank Rivers, tributaries of Chesapeake Bay, were not included in the original analysis. Data for those systems were from Boynton *et al.* (1995) and Fisher (unpublished data).

It appears that Chesapeake Bay and its tributary estuaries tend to remove a higher fraction of N loads than expected from the estimated water residence times (Fig. 18.13). Some of these differences may simply be the result of accumulated errors in developing N-budgets. However, Chesapeake Bay is a sediment-rich and eutrophic system with abundant suspended PN and sediment concentrations to facilitate burial. Of the estuaries examined by Nixon et al. (1996), only the Chesapeake systems had substantial N-burial losses, probably because of these features and moderately long water residence times. In addition, many Chesapeake Bay tributaries (e.g., Patuxent and Choptank) that export a small fraction of their N load have large areas of low-salinity tidal marshes which also sequester substantial amounts of both N and P (Greene, 2005b; Merrill, 1999). Thus, it's possible that some estuarine communities, such as those associated with tidal marshes and seagrass beds, may modify relative N export characteristics (Valiela et al., 2001). To expand our understanding of N dynamics in estuaries, there is a clear need for more direct calculations of N exchange with seaward waters and better estimates of other loss terms including burial and denitrification.

3.4. Ecosystem metabolism: Interactions with nitrogen cycling

Under steady-state conditions, the balance between inorganic N inputs and losses for an estuary is directly related to the net production (or consumption) of organic matter in the associated ecosystem, where net organic production and net fluxes of inorganic N are inversely related. Net ecosystem production (NEP) is supported by net uptake of inorganic N, and conversely, net consumption of organic matter results in net release of inorganic N. NEP, which is defined as the balance between gross primary production (P_G) and community respiration (R_C) of organic carbon, provides a measure of the excess production or consumption resulting in changes in internal storage or net export or import across the system boundaries (Fisher and Likens, 1973; Odum, 1956). Ecosystems with positive NEP are considered autotrophic, while those with negative NEP are considered heterotrophic. Although P_G tends to equal R_C (NEP $\approx$ 0) at large scales, imbalances between P_G and R_C at smaller scales support functional coupling between adjacent habitats through exchanges of organic carbon and inorganic nutrients (e.g., Hopkinson and Vallino, 1995; Kemp et al., 1997; Smith and Hollibaugh, 1997).

NEP can be calculated using different methods including long-term continuous records of dissolved oxygen or inorganic carbon (relative to saturation concentrations) at diel or seasonal scales (e.g., Howarth et al., 1992), and summation of individual production and respiration measurements broadly representing temporal and spatial scales and major habitats (e.g., Kemp et al., 1997). NEP can also be computed using estimates of the net balance between imports and exports of DIP (e.g., Smith, 1991), where rates are converted from phosphorus to carbon units assuming a fixed stoichiometry for primary production and nutrient recycling (e.g., C:N:P = 106:16:1). Conversely, NEP can be converted to nitrogen units using these stoichiometric assumptions; however, the net balance of DIN will also be affected by two important processes involving nitrogen gas production and consumption—denitrification and nitrogen fixation, respectively (e.g., Nixon and Pilson, 1984). In fact, rates of NEP and related biogeochemical fluxes have been

estimated for many estuaries and coastal bays worldwide combining simple water- and salt-balance models that compute advective and diffusive solute transport with data for DIN and DIP time-space distributions to infer non–conservative net fluxes as residuals in dynamic nutrient budget calculations (http://wwwold.nioz.nl/loicz/). Comparative analyses of these rates suggest broad regional relationships with hydrology and watershed development (Smith et al., 2003).

Estimates of NEP can also be made using time-varying water/salt balance calculations for an estuarine water volume segmented into two-layers and multiple regions (or boxes) along the salinity gradient (Hagy et al., 2000). Such a "box-model" was used to compute net non-conservative fluxes for nutrients and dissolved oxygen (O_2, corrected for air-sea exchange) in the Patuxent River estuary, USA (Kemp et al., 1999; Testa and Kemp, 2005). The inherently tight coupling between organic production (NEP) and N cycling can be seen in the highly correlated patterns of annual mean rates of net O_2 and DIN flux along the estuarine gradient for surface and bottom layers in hydrologically contrasting years (Fig. 18.14). In surface layers, interannual and longitudinal variations in DIN uptake generally correspond to patterns in net O_2 production, while in bottom layers, trends of net O_2 consumption appear as a mirror image of net DIN production rates. Bottom layer DIN fluxes are inversely correlated to O_2 fluxes ($r^2 = 0.81$); however, trends are complicated because negative fluxes in low salinity estuarine regions are from high water column nitrate concentrations diffusing into sediments to support denitrification, while positive net fluxes in the more saline regions are primarily due to ammonium efflux from decomposing organic matter in sediments.

Whereas annual rates of primary production tend to be regulated by inputs of total nitrogen for many coastal ecosystems (e.g., Boynton et al., 1982; Nixon et al., 1986), it appears that NEP (NEP $= P_G - R_C$) is controlled more by the balance between inputs of DIN and total organic nitrogen (TON) or carbon (TOC), where DIN inputs stimulate P_G, and TON (or TOC) inputs support R_C (Kemp et al., 1997). Comparative analysis of NEP calculations and loading rates for five estuaries and for mesocosms (MERL; Oviatt et al., 1986) at different nutrient treatments reveals a consistent relationship between NEP and the DIN:TOC loading ratio (Fig. 18.15). While strong relationships were evident for NEP from controlled experimental systems and for long term average rates in specific estuaries, substantial year-to-year variations in NEP are often evident for specific estuarine ecosystems. Such variations in metabolic rates may be related to fluctuations in climatic conditions that regulate, for example, inputs of organic matter from adjacent coastal upwelling regions (e.g., Smith and Hollibaugh, 1997), or river flow and associated nutrient loading and water residence time (Fig. 18.15, note differences in net fluxes for high and low flow years, and interannual variability in NEP in figure inset). Values for P_G and R_C computed from continuous diel O_2 measurements for a range of shallow North American coastal ecosystems revealed that NEP was generally negative (i.e., net heterotrophic), was responsive to climatic variations, and was related to N inputs (Caffrey, 2004). The heterotrophic nature of NEP in many coastal ecosystems combined with the correspondence between biogeochemical fluxes of C and N have been interpreted to suggest that N cycling, in general, and denitrification, in particular, may actually be limited by C availability (Smith and Hollibaugh, 1989). This is in contrast to the conventional view that carbon production and consumption are limited by N availability. In summary,
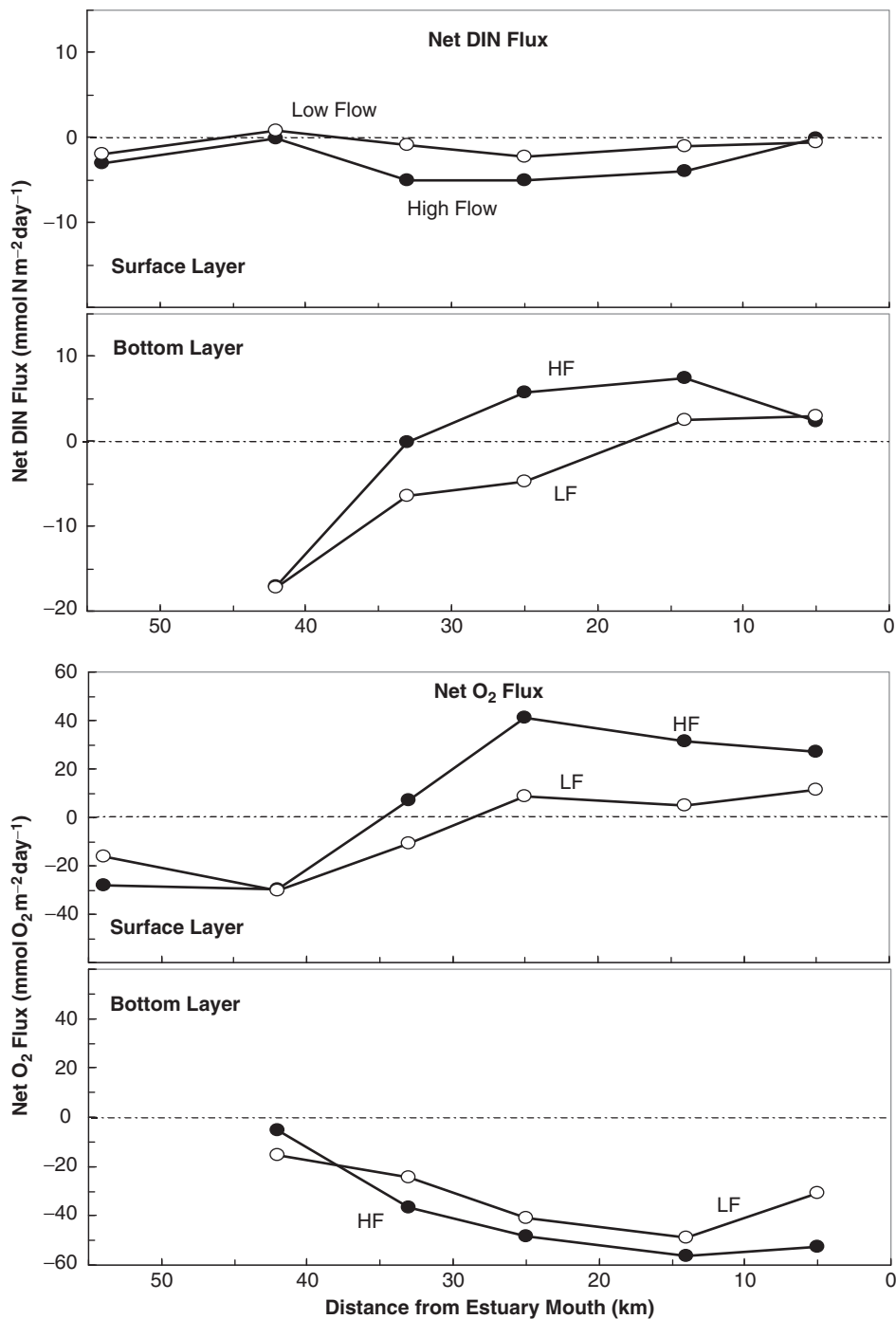
Figure 18.14 Net non-conservative biogeochemical fluxes of dissolved oxygen (O_2) and dissolved inorganic nitrogen (DIN) calculated using a box–model (Hagy *et al.*, 2000) for six regions and two vertical layers defined along the Patuxent River estuary for relatively wet (2000) and dry (2002) hydrologic years. Fluxes were estimated as residuals in mass-balance calculations for monitored O_2 and DIN concentrations (Kemp *et al.*, 1999).
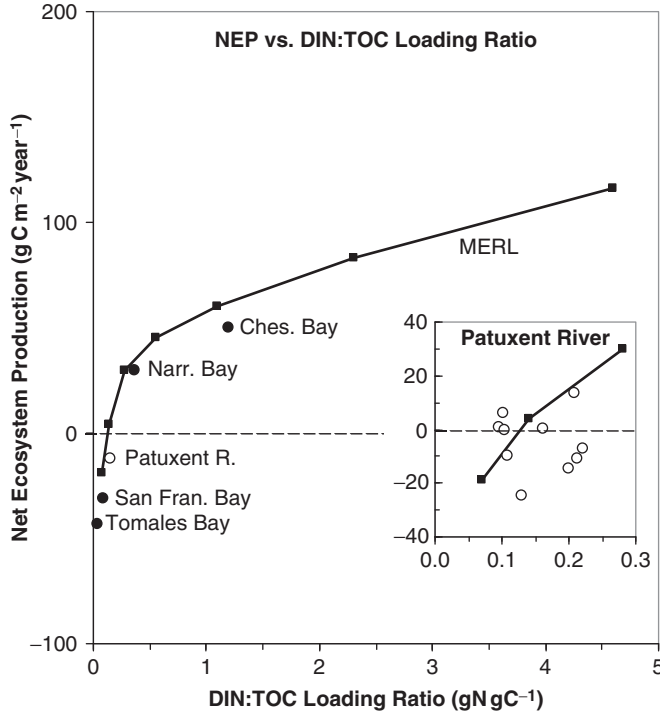
Figure 18.15 Comparative analysis of net ecosystem production for estuaries in relation to loading ratio for DIN:TOC. Data sources are: for MERL mesocosms (Oviatt *et al.*, 1986), for Narragansett Bay (Nixon *et al.*, 1995), for Tomales Bay (Smith and Hollibaugh, 1997), for San Francisco Bay (Jassby *et al.*, 1993), for Chesapeake Bay (Kemp *et al.*, 1997), and for Patuxent River estuary in 1985–1994 (Kemp *et al.*, 1999). Figure was adapted from Kemp *et al.* (1997). The DIN:TOC load ratio was based on external inputs of DIN ($NO_3 + NO_2 + NH_4$) and Total Organic Carbon (TOC) to each site.

we conclude total primary production and net ecosystem production are tightly coupled to inputs and cycling of N in most coastal environments, and that these relationships vary with hydrologic and climatic conditions.

4. Sediment-water Solute Fluxes

One of the distinguishing features of estuarine ecosystems is their relatively shallow water depths compared to many lakes and the coastal ocean. One consequence of a short water column is that the upper photic waters that support primary production are closely connected to zones of nutrient remineralization in estuarine sediments. This results in strong benthic-pelagic (B/P) coupling, which is one of several reasons suggested for the high primary and secondary productivity of estuarine systems (Kemp and Boynton, 1992). While B/P coupling implies bi-directional influences, we focus here on benthic N cycling and the efflux of DIN across the sediment-water interface. During the past twenty years a substantial number of sediment–water flux measurements have been made in diverse estuarine and coastal

marine systems, providing opportunity for a synthetic analysis. In the following section we consider ammonium effluxes because they have been widely measured and because NH_4 is usually the primary N compound released by sediments. However, we also included oxygen and DIP fluxes because they are also widely measured and because O_2, DIP and NH_4 fluxes are stoichiometrically linked in complex ways. We recognize that other N forms, such as NO_3, can occasionally play a significant role in sediment nutrient dynamics. In recent years, a growing number of sediment–water fluxes have been measured in shallow, clear-water systems where sediments are in the photic zone (e.g., Eyre and Ferguson, 2002; McGlathery et al., 2001; Reay et al., 1995; Rizzo et al., 1992; Rysgaard-Petersen et al., 1994; Tyler and McGlathery, 2003). N cycling under those conditions is apparently even more complex, with benthic photosynthesis supporting N-assimilation, shifting redox conditions and associated biogeochemical processes. A detailed discussion of these data can be found elsewhere in this book (Chapter 23 by McGlathery, this volume). Finally, we did not review DON fluxes associated with estuarine sediments because these have not been routinely measured and because Bronk and Steinberg (Chapter 8, this volume) and Joye and Anderson (Chapter 19, this volume) address this issue in detail.

4.1. Overview of flux magnitude

Sediment–water fluxes of ammonium (NH_4), dissolved oxygen (O_2), and dissolved inorganic phosphorus (PO_4) were organized based on data from 52 studies (6, 19, 26, and 1 from the 1970s, 1980s, 1990s and 2000s, respectively) conducted in 48 estuarine and coastal marine areas. A total of 701 flux measurements were included in the summary developed by Bailey (2005). Measurements included those made in situ or with ship–board or laboratory sediment core incubations. In all cases, measurements of analyte concentrations (O_2, NH_4, and PO_4) were made during an incubation period in the dark at ambient water temperature, and fluxes were computed using concentration temporal rates of change. Studies using modeling techniques or fluxes estimated from sediment pore water concentrations were not included.

Mean values of flux magnitudes and ratios exceeded median values (Table 18.4); there were a small number of large values for each of these fluxes (Fig. 18.16A).

Table 18.4 Summary of Net Sediment-Water Oxygen and Nutrient Exchanges and Flux Ratios from 48 Estuarine and Coastal Ecosystems Developed by Bailey (2005)

Type of flux or flux ratio	Minimum	Maximum	Mean	Median	n
NH_4	−83	2700	125	42	641
PO_4	−231	900	22	4.2	506
O_2	0	−18229	−1302	−781	554
N: P	0	193	13	7.8	369
O: N	0	1450	63	24	413

All measurements were made in environments where the sediments were aphotic and all sediment incubations were made in the dark. Flux units are: umol O_2, N or P m^{-2} h^{-1}; N: P and O: N ratios atomic basis. Negative values indicate fluxes into sediments.
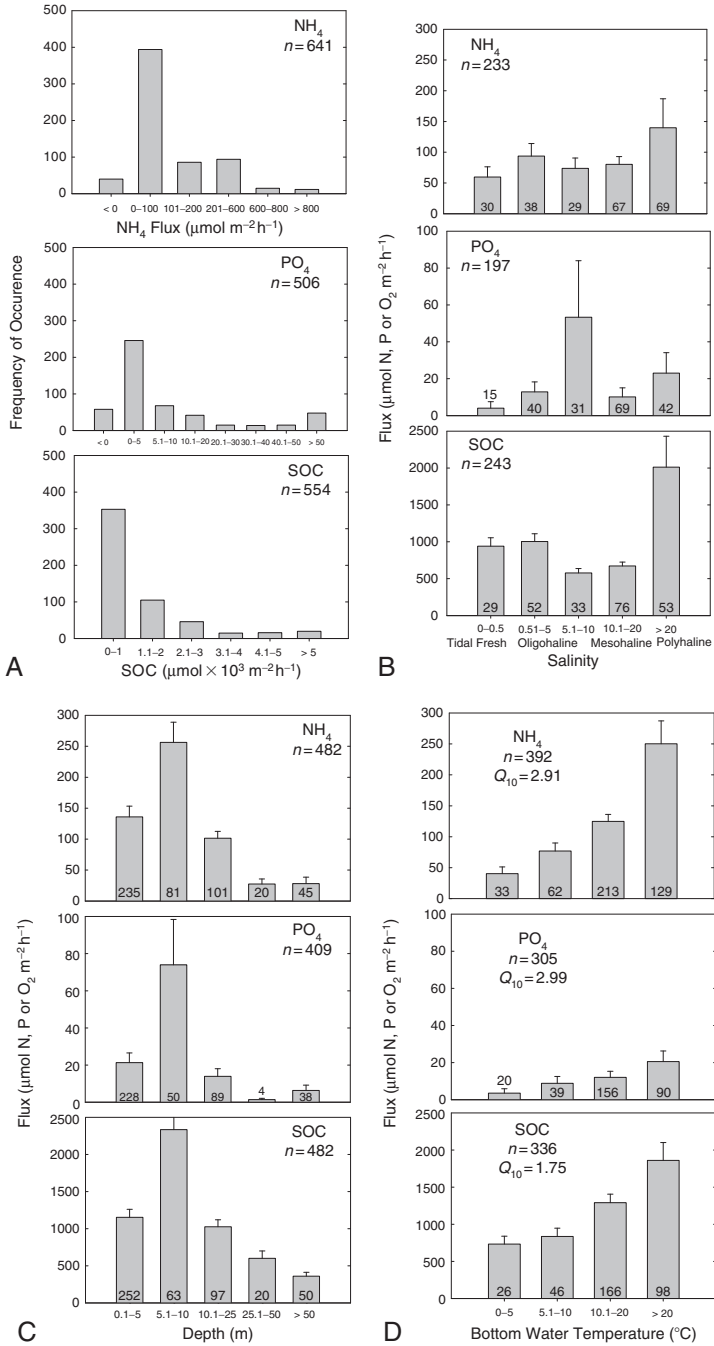
Figure 18.16 A summary of sediment-water NH$_4$, PO$_4$ and sediment oxygen consumption (SOC) rates, including: (A) frequency histogram of rates; (B) rates as a function of salinity regime; (C) rates as a function of system depth; (D) rates as a function of temperature. Data were organized by Bailey (2005).

Sediment oxygen consumption (SOC) rates ranged from 0 to 18,229 μmol m^{-2} h^{-}, ammonium fluxes from -83 to 2700 μmol m^{-2} h^{-1} and phosphate fluxes from -231 to 900 μmol m^{-2} h^{-1}. Flux ratios are also of interest because they can provide an indication of active sediment biogeochemical processes. Median values of N: P and O: N flux ratios were lower and higher, respectively, than would be expected based on Redfield phytoplankton composition ratios (N: P $\sim$ 16; O: N $\sim$ 13.3). Both ratios suggest less N being recycled relative to recycled P and to oxygen consumption rates. While several explanations are possible, much of this pattern is probably attributable to loss of N-solutes via nitrification-denitrification in estuarine sediments (e.g., Jenkins and Kemp, 1984; Rysgaard-Petersen et al., 1994; Seitzinger, 1988).

4.2. Spatial patterns of flux

All fluxes tended to be higher in saltier than in fresher waters (Fig. 18.16B). Although one might anticipate that NH_4 and SOC fluxes would be inversely related to salinity with highest values closest to terrestrial organic matter and nutrient sources, this was not the case. Apparently, more phytoplanktonic debris, which is more labile with lower C:N ratios, tends to be the dominant source of organic matter fueling benthic processes in higher salinity zones. On the other hand, DIP efflux from sediments tended to peak in the low mesohaline (salinity = 5–10) region. This is probably a manifestation of both loosely sorbed P being released into solution as a consequence of increased salinity (Froelich, 1988) and of the dissolution of iron–phosphate complexes after chemical reduction of iron and precipitation of iron sulfides in anaerobic sediments (Jensen et al., 1995; Krom and Berner, 1980).

In general, SOC tends to decline with water depth (e.g., Hargrave, 1973; Kemp and Boynton, 1992; Kemp et al., 1992), as plankton respiration causes a smaller percentage of sinking organic matter to reach the bottom due to longer transit times in deeper water columns. Efflux of NH_4 from sediments has previously been related to water column depth (Harrison, 1980) across large gradients (10–2000 m). We found a generally similar response for NH_4, PO_4, and SOC fluxes (Fig. 18.16C) for a relatively small depth range (0.2–50 m). Fluxes were 5 to 10 times higher in water of 5–10 m depth than in waters greater than 50 m depth, consistent with earlier reports for SOC. However, mean fluxes in the dark were lower at very shallow depths (0.2–5 m). The shallow water sediments are typically within the zone where autotrophic processes tend to dominate sediment biogeochemistry, with high rates of N and P assimilation by benthic algae and seagrasses and oxidized sediments which promote adsorbtion and precipitation of PO_4 and coupled nitrification-denitrification of N. Thus, NH_4 and PO_4 effluxes tend to be low in these shallow sediments. In addition, sediments and organic matter may be exposed to resuspension via wave action and the less dense labile organic particulates transported to deeper waters where they are remineralized. The flux-depth pattern observed with our large data set is not consistent with several other analyses including those of Nixon (1981) and Seitzinger and Giblin (1996) where depth ranges of 3–70 m and <200 m, respectively, were examined and did not exhibit strong relationships with depth.

4.3. Regulation of sediment–water fluxes

There are many factors that may play important roles in regulating sediment biogeochemical processes and associated solute exchanges. There is a substantial literature concerning this topic, extending back several decades. The list of potential influencing factors includes temperature, activities of infaunal communities, redox conditions near the sediment-water interface, solute concentrations in overlying water, and rates of organic matter supply. Effects of infauna can be both direct (i.e., excretion) and indirect (i.e., burrowing, pumping, and stimulation of microbial communities). For example, Banta *et al.* (1995), Flint and Kamykowski (1984), Hammond *et al.* (1985), Kanneworff and Christensen (1986), and Webb and Eyre (2004) reported a variety of macrofaunal influences on sediment-water exchange rates and other processes. Others have reported on the influence of redox and water quality conditions on sediment processes (e.g., Sundby *et al.*, 1992). The challenge of quantitative modeling of sediment-water processes and associated interactions was captured in a recent book by DiToro (2001).

Previous studies have concluded that, ultimately, organic matter supply rate to sediments was the overarching factor regulating sediment biogeochemistry and solute flux across the sediment-water interface. For example, comparative analysis among diverse estuarine systems indicates that benthic respiration rates are highly correlated with organic matter production rates (Nixon, 1981). The relationship appeared to be linear across a very large range of primary production rates ($\sim$75–1400 g C m^{-2} year^{-1}), including data from Chesapeake Bay (Kemp and Boynton, 1992). In northern European waters, rates of sediment respiration and ammonium and phosphorus fluxes tend to respond rapidly to deposition of spring and autumn algal blooms (Graf *et al.*, 1982; Jensen *et al.*, 1990). Although temperature appears to affect response time, similar relationships have been reported for sediment anaerobic respiration in North American systems (Marvin-DiPasquale *et al.*, 2003; Sampou and Oviatt, 1991).

Interacting effects of organic matter supply and temperature are illustrated with two examples from Chesapeake Bay aphotic sediments. In the first case (Cowan and Boynton, 1996), sediment chlorophyll *a* concentration (as an index of recent organic deposition) was related to sediment-water NH$_4$ fluxes measured during three years at three stations along the estuarine salinity gradient (Fig. 18.17). These stations varied substantially in terms of mean depth, O$_2$ conditions, sediment type, and macrofaunal characteristics; the strength of the observed relationship, therefore, emphasizes the overall importance of organic matter supply. A second point of interest is the lag time used in this analysis. Specifically, sediment chlorophyll *a* concentrations were averaged from just prior to spring bloom deposition through the summer period, while NH$_4$ fluxes were averaged from mid-spring through summer. Biogeochemical processes in this system, which has a large annual temperature range (0–33°C), are apparently not adapted to cold water (Sampou and Kemp, 1994). It is argued that sediment respiration and nutrient remineralization respond to spring bloom deposition and labile organic matter accumulation primarily after temperature increases beyond 10°C (Cowan and Boynton, 1996). Thus, there is a period of organic matter
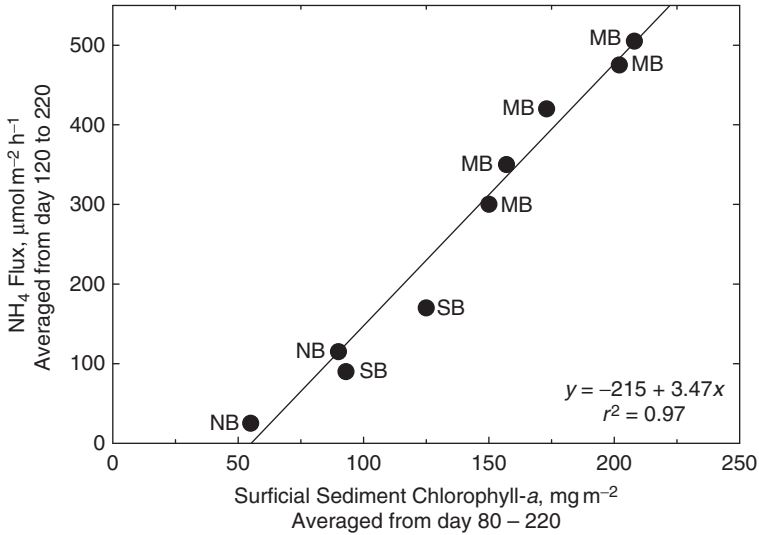
Figure 18.17 A scatter plot of sediment NH_4 fluxes versus surface sediment chlorophyll *a* concentration collected for several years at three sites along the salinity gradient of Chesapeake Bay. Note that sediment chlorophyll *a* data were averaged from late winter through summer while sediment fluxes were averaged from late spring through summer. Figure was redrawn from Cowan and Boynton (1996). NB–Northern Bay (oligohaline); MB–Mid Bay (mesohaline); SB–South Bay (polyhaline).

loading to sediments followed by a period of NH_4 release. Other investigators have found a more immediate response to temperature (e.g., Banta *et al.*, 1995). However, the importance of organic matter supply rate is clearly evident in these results. The second example is from the Patuxent River, where multiple NH_4 flux measurements were made during three summer periods, all within the mesohaline region of the estuary (Stankelis *et al.*, 1999). Water column and sediment characteristics were also measured at flux site locations and a reasonably simple regression model was developed in which sediment chlorophyll *a*, as an index of labile organic matter supply, again played an important part (Fig. 18.18). An additional twist in this analysis was the apparent role played by sediment redox conditions. The model indicated that as sediment redox conditions became more positive, less NH_4 was released. This is consistent with the idea that sediment N is more likely to be nitrified when sediments are oxidized (Kemp *et al.*, 1990; Rysgaard-Petersen *et al.*, 1994).

We also examined flux data with respect to water temperature at the time of measurement using the sediment-water flux data set developed by Bailey (2005; Fig. 18.16D). In all cases there were sharp increases in rates with increased temperature. Estimated Q_{10} (0–30°C) values for NH_4, PO_4 and SOC fluxes were 2.9, 3.0, and 1.8, respectively. It is also useful to note there is considerable bias in the temperature range in which these measurements are made. Less than 10% of the reported rates were from temperatures <5°C, and only a slightly higher percentage were from temperatures of 5–10°C. About 50% of all measurements
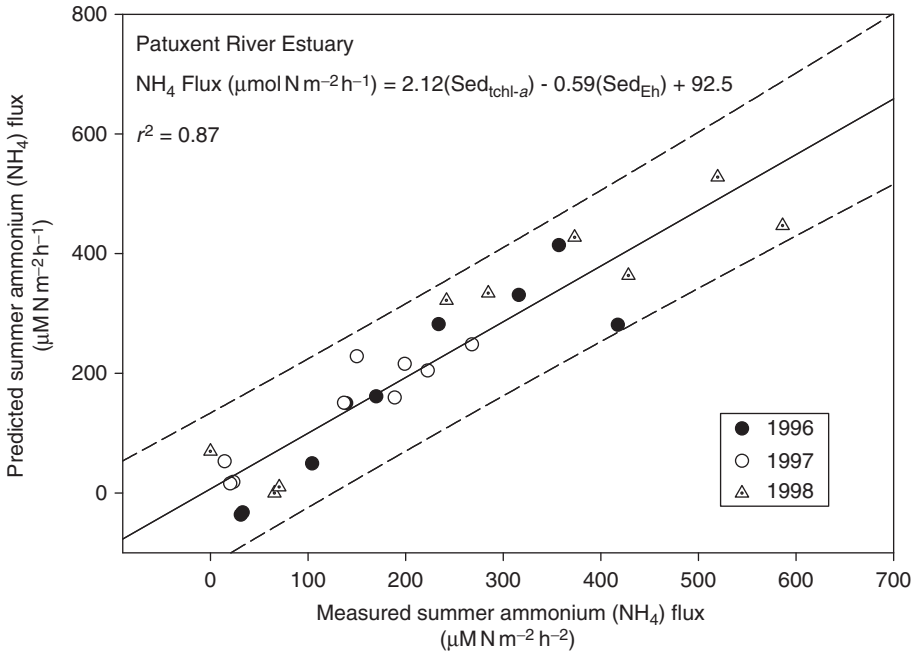
Figure 18.18 A scatter plot of predicted versus measured NH$_4$ fluxes for the mesohaline region of the Patuxent River estuary. Data and figure are from Stankelis *et al.* (1999).

were made at temperatures between 10°C and 20°C, a fact that suggests a *Goldilocks and the Three Bears* approach to ecology wherein measurements were made when it was neither too hot nor too cold, but just right (Brett, 1987). To examine the influence of temperature on sediment fluxes in more detail, we organized a rare 9 year time-series of sediment NH$_4$ fluxes collected from two Chesapeake Bay tributaries- the Potomac and Patuxent River estuaries (Boynton and Rohland, 1998). For comparison, monthly NH$_4$ fluxes were expressed as percent of maximum flux observed during each year, averaged by month for the 9 year record, and then plotted as a function of average temperature at the time of measurement (Fig. 18.19). Results from the heavily nutrient enriched Potomac and moderately enriched Patuxent indicated increasing fluxes with increasing temperature through mid-summer but then sharply reduced fluxes at comparable or slightly higher temperatures through late summer and fall. A similar pattern was reported from a site in Chesapeake Bay (Cowan and Boynton, 1996). The substantial differences in flux magnitude before and after July indicate that other factors, such as supply of labile organic matter, limit remineralization in late summer and autumn. However, there appear to be a variety of seasonal patterns of sediment fluxes among estuaries. For example, Banta *et al.* (1995) and Nixon *et al.* (1976) found strong relationships between temperature and sediment ammonium fluxes in Buzzards Bay and Narragansett Bay. The seasonal pattern of sediment denitrification may also strongly influence the pattern of sediment ammonium fluxes (Hopkinson *et al.*, 1999).
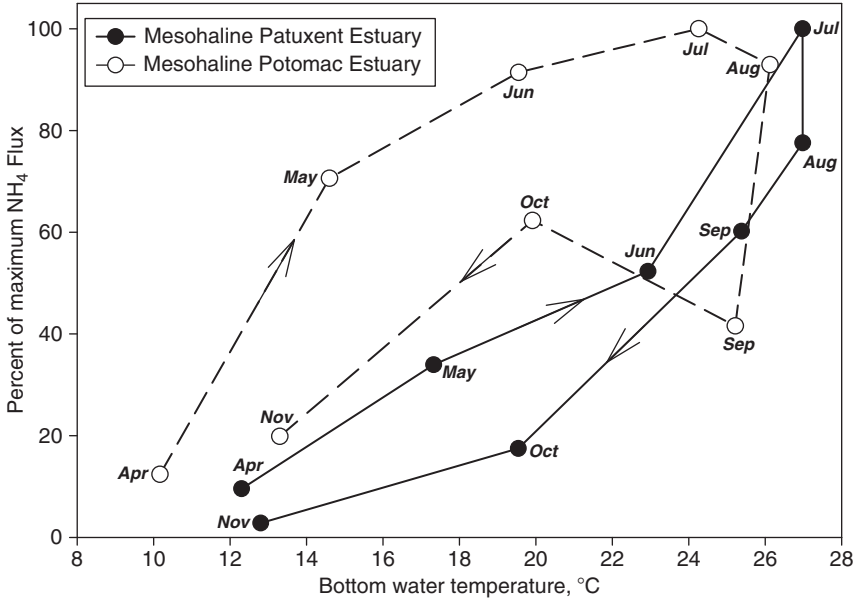
Figure 18.19 A scatter plot of sediment NH_4 fluxes as a function of temperature from two tributary rivers of Chesapeake Bay. NH_4 fluxes were based on 9 years of monthly samples. Fluxes were expressed as a percent of the maximum flux observed in each year and then averaged, again by month, for all years. Temperature was the average temperature during each month of the 9 year record at each site. The Potomac site was very nutrient enriched and the Patuxent site was moderately enriched. Data were from Boynton and Rohland (1998).

5. Nitrogen and Primary Production

In many estuarine systems primary production by phytoplankton and other autotrophs is affected by N inputs and concentrations (e.g., Cloern, 2001; Howarth, 1988; Kelly, 2001; Nixon, 1992; Smith, 2003). For this review, we have compiled estimates of annual primary production and simple models used to relate production to nutrient conditions. We also discuss the evolution of thinking concerning nutrient limitation of pelagic primary production in estuaries. Finally, we review the conceptual models concerning benthic and pelagic primary production and consider the influence of N on these different autotrophic components.

5.1. Phytoplankton primary production

Annual rates of phytoplankton primary production from 120 different estuarine and coastal marine systems were organized in a frequency plot (Fig. 18.20). The most frequently encountered rates were those between 100 and 300 g C m^{-2} year^{-1}, corresponding to mesotrophic status in the classification suggested by Nixon (1995). Despite very real concern about increasing eutrophication of coastal systems, only 32%
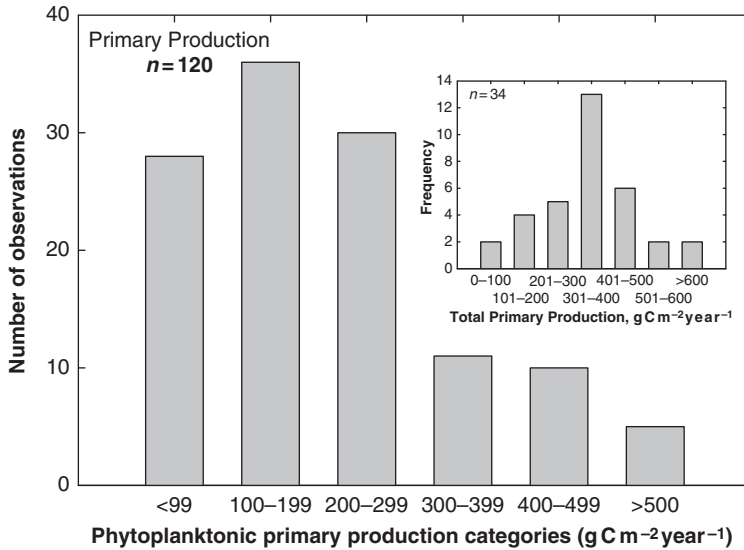
Figure 18.20 Frequency distribution of annual phytoplankton primary production rates for a selection of estuarine and coastal marine systems. Data were from Borum and Sand-Jensen (1996), Boynton *et al.* (1982), Chen *et al.* (2000), Cloern (2001), Conley *et al.* (2000), deJonge *et al.* (1994), Harding *et al.* (2002), Kelly (2001), Nixon (1997), and Pennock and Sharp (1986). Inset diagram shows frequency distribution for total primary production (pelagic plus benthic) for a limited number of estuaries. Inset data were from Borum and Sand-Jensen (1996).

of these systems were in the eutrophic or hypertrophic categories. It is unclear, however, how representative this sample is of estuarine systems worldwide. It is almost certain that vast amounts of data remain to be mined from unpublished sources and that incorporation of this material might substantially change the observed pattern. In addition, most estimates of primary production are estimates of pelagic primary production; few included benthic primary production, which is especially important in shallow systems. We used the summary of total system (pelagic plus benthic) primary production compiled by Borum and Sand-Jensen (1996) and examined categories of production relative to trophic state (Fig. 18.20 inset). In this smaller sample, rates were generally higher, as expected, with 68% of the sites having annual productivity rates greater than 300 g C m^{-2} year^{-1}. Most of these sites were shallow, even by estuarine standards, and exhibited very high production rates, clearly indicating the importance of benthic primary producers. Borum and Sand-Jensen (1996) also pointed out that it was rare for both benthic and planktonic rates to be high in the same system, and indicated that conditions which enhance one group of primary producers tend to inhibit the other.

5.2. Statistical models of nitrogen and production

We also compiled reports of statistical models developed to relate primary production or algal biomass to N (Table 18.5). All but one of these were generated during the last two decades, and the models take a variety of forms, for example, using N

Table 18.5 A summary, by date of publication, of statistical models relating phytoplankton primary productivity or biomass to nitrogen (concentration or loading rates) or other variables

Location	Independent variable, X (units)	Dependent variable, Y (units)	Predictive equation	r^2/n	Reference
Multiple estuaries	TN loading (g N m^{-2} year^{-1})	Phytoplk Prod (g C m^{-2} year^{-1})	$Y = 25 + 13X$	0.60/14	Boynton $et\ al.$, 1982
San Francisco Bay other estuaries	Composite parameter $X = f(B, Z_p, I_0)$	(mg C m^{-2} year^{-1})	$Y = 15 + 0.73X$	0.82/211	Cole and Cloern, 1987
Narragansett Bay and Univ RI MERL	Composite parameter $X = f(B, Z_p, I_0)$	(g C m^{-2} year^{-1})	$Y = 220 + 0.70X$	0.82/1010	Keller, 1988
Multiple estuaries and Univ RI MERL	DIN-loading (mol N m^{-2} year^{-1})	(g C m^{-2} year^{-1})	$\log Y = 0.44\log(X) + 2.33$	0.93/19	Nixon $et\ al.$, 1996
Multiple estuaries	TN loading (g N m^{-2} year^{-1})	(g C m^{-2} year^{-1})	$Y = 244 + 175\log(X)$	0.36/51	Borum and Sand–Jensen, 1996
Boston Harbor	Composite parameter $X = f(B, Z_p, I_0)$	(mg C m^{-2} year^{-1})	$Y = 285 + 0.79X$	0.66/12	Kelly and Doering, 1997
Waquoit Bay system	Annual average DIN conc (μM)	(g C m^{-2} year^{-1})	$Y = 60.9 + 13.96X$	0.61/12	Valiela $et\ al.$, 2001
Chesapeake Bay	TN(x_1), TP(x_2) load (kg mol^{-1})	(g m^{-2} year^{-1})	$Y = 335 + 18.9X_1 - 164X_2$	0.67/11	Harding $et\ al.$, 2002

Multiple estuaries	DIN (m M m^{-3}); tidal range (m)	Phytoplk Biomass (µg I^{-1})	na; positive slope	na/163	Monbet, 1992
Multiple systems/ MERL	DIN input (mmol m^{-3} year^{-1})	(µg I^{-1})	na; positive slope	na/34	Nixon, 1992
Chesapeake Bay mesohaline region	River flow (m^3 day^{-1}) (proxy for N-load)	(kg estuary^{-1})	na; positive slope	0.70/34	Harding *et al.*, 1992
Maryland lagoons	TN load (g N m^{-2} year^{-1})	(µg I^{-1})	$Y = 16.9 + 0.70X$	0.96/9	Boynton *et al.*, 1996
Danish coastal waters	TN concentration (µg I^{-1})	(µg I^{-1})	na; positive slope	0.64/168	Borum, 1996
Canadian estuaries	TN concentration (µg I^{-1})	(µg I^{-1})	$\log Y = -4.06 + 1.78 \log X$	0.72/15	Meeuwig, 1999
Chesapeake Bay and Tributaries	Scaled TN load (mg N m^{-2} year^{-1}) (R_{time}, years)$^{-1}$	(mg m^{-2})	$Y = 18.9 + 0.078X$	0.82/17	Boynton and Kemp, 2000
Danish estuaries	TN concentration (µg N I^{-1})	(µg I^{-1})	$\ln(Y) = 0.96 \ln(X) - 4.19$	0.30/1347	Nielsen *et al.*, 2002

concentration or loading rate as an independent variable. Most models were computed on annual time scales, and for several it was unclear how temporal and spatial variability was taken into account. Sample size used to develop these models varied widely, and some were based on multi-system comparisons while others were based on multi-year data collected for one system. Given the myriad factors known to influence production and algal biomass accumulation, it is impressive that N alone was able to account for a large portion of the variability of phytoplankton production or algal biomass.

There are, of course, some serious limitations with these analyses. These are basically correlations and thus do not demonstrate causation; results may be related to some other co-variate. More importantly, other possible explanations for production or biomass variability were not always thoroughly examined (Peters, 1991). For example, in some of these papers it appeared that N was assumed, *a priori*, to be the key explanatory variable, while other factors, such as P or light availability, were not examined with equal rigor. One could make the case that sufficient data are now available to initiate a more comprehensive, comparative synthesis of estuarine primary production (both pelagic and benthic) that considers a wider selection of independent variables and uses dimensional scaling to the extent needed to ensure adequate comparability among different estuarine locations. In short, estuarine ecologists need to take a lesson from the limnologists who began doing just that some 30 years ago (e.g., Vollenweider, 1976) and developed tools useful for both scientific understanding and for lake management.

5.3. Nutrient limitation

The question of nutrient limitation is fundamentally important for understanding controls on estuarine production and practically important for understanding how to mitigate eutrophication. In recent decades, nutrient limitation concepts have been better defined, explanations for differences in nutrient limitation among systems have been clarified, and experimental approaches have been organized in a heiarchial suite, from short-term small-scale bioassays, to intermediate-scale mesocosm studies, to whole-system biogeochemical analyses. The history of development of nutrient limitation studies has been reviewed (Howarth, 1988) and more recent studies have discussed the use of data from these studies for nutrient management in such areas as Chesapeake Bay and the Baltic Sea (Elmgren and Larsson, 2001; Malone *et al.*, 1993). The basic story is summarized below.

In the 1970s limnologists produced convincing evidence that for many lakes P was the important limiting nutrient (e.g., Schindler, 1977). With the growing sense that estuaries and coastal waters were showing signs of serious nutrient-based eutrophication, the lake model was assumed to apply. However, contemporaneous studies in coastal areas (e.g., Ryther and Dunstan, 1971) suggested that N was more important than P in estuarine and coastal systems, and studies conducted during the 1980s generally supported that view (e.g., Caraco *et al.*, 1987; D'Elia *et al.*, 1986; Graneli, 1987). P limitation in lakes had been demonstrated at various scales of complexity/realism (i.e., bioassays, mesocosms and whole-lake experiments), but the same degree of testing had not been completed in estuaries and hence the N conclusion

needed to be viewed with caution (Hecky and Kilham, 1988). For many estuaries, however, long-term field studies (e.g., Jordan *et al.*, 1991), multi-year, system-wide bioassay studies (e.g., Fisher *et al.*, 1992, 1999), whole-year mesocosm experiments (D'Elia *et al.*, 1986; Harrison *et al.*, 1990; Oviatt *et al.*, 1995) and some whole–system experiments (Conley *et al.*, 2000; Elmgren and Larsson, 2001; Paerl *et al.*, 2004) have been completed, and the emerging story is more complete and complex.

In general, pelagic estuarine productivity tends to be limited by N; however, light, P, Si and other factors also play into the story. Nitrogen limitation appears to be more often the case in higher salinity than in tidal fresh waters and more intense in summer/fall than in winter/spring. These temporal/spatial patterns tend to parallel trends in dissolved inorganic nutrient ratios (DIN:DIP) in the water column which, in turn, are often reflective of the nutrient composition of inputs. Many authors indicate P limitation associated with high N: P ratios (>90, Caraco *et al.*, 1987; >90, D'Elia *et al.*, 1986; >80, Harrison *et al.*, 1990; >60, Kemp *et al.*, 2005; >30, Pennock and Sharp, 1994), a condition that often occurs during winter/spring in estuaries with significant freshwater inflows not dominated by sewage. Dodds (2003) reviewed use of DIN: DIP ratios as an indicator of nutrient limitation and argued for the use of TN:TP ratios rather than the inorganic ratios more commonly used in estuarine analyses. When N supply is extremely high (e.g., south China coast), P may be more generally limiting, but again, it seems probable that outside the plume of low salinity, N–rich water, limitation switches back to N (Harrison *et al.*, 1990). There is also some empirical evidence that P limitation is a more general feature of warm temperate embayments, such as those along the Gulf of Mexico (e.g., Murrell *et al.*, 2002).

In overview, the emerging picture is one of a mosaic of general N limitation, but with substantial time-space regions where productivity is limited by P and other factors. This description is certainly consistent with a view of estuaries as gradient-rich, dynamic ecosystems that lie between the land and the sea and are influenced by both. Given the diversity of responses reported for estuaries, a uniform nutrient management directive does not seem possible. In large temperate coastal systems that include a full salinity gradient from tidal fresh to polyhaline, it may be prudent to consider a "duel-nutrient reduction strategy" wherein both N and P loads are reduced. This approach ensures that eutrophic conditions will be reduced both in tidal freshwater areas where P tends to be limiting, and in meso- and polyhaline areas where N is more important (Conley, 1999; Elmgren and Larsson, 2001; Fisher *et al.*, 1999; Paerl *et al.*, 2004).

5.4. Benthic autotrophs and nitrogen

Although the majority of the discussion regarding N-productivity interactions has focused on phytoplankton, N is also an important control on benthic primary production in estuarine ecosystems. During the past two decades, there has been much research examining how nutrient additions have contributed to global seagrass decline (e.g., Duarte, 1995; Kemp *et al.*, 1983). The cause-effect linkage of seagrass decline and nutrient additions typically involves increased phytoplankton standing stocks and associated reduced water transparency (e.g., Nielsen *et al.*, 2002).

In addition, in some areas, enhanced epiphytic growth on seagrass leaves further reduces light availability, eventually leading to loss of this community (e.g., Taylor *et al.*, 1995; Twilley *et al.*, 1985).

In other shallow ecosystems, macroalgae play a prominent role in the response to N addition: as nutrient inputs are increased, macroalgae first replaces seagrasses and then with further nutrient increases, macroalgae are themselves replaced by dense phytoplankton stocks (Valiela *et al.*, 1992). This sequence is modified by the influence of water residence time and the nutrient buffering influence of adjacent fringing ecosystems (e.g., Valiela *et al.*, 2001). Bowen and Valiela (2001) traced historical changes in N loads to a cluster of coastal embayments and reported a doubling of loads between 1938 and 1990, progressive increases in phytoplankton and macroalgae stocks and a decline in seagrass coverage. It is somewhat dispiriting to learn that seagrass declines, at least in these coastal systems, were associated with N loads as low as 3 g N m^{-2} year^{-1}, rates far lower than for many estuaries (Fig. 18.9). A series of papers concerning nitrogen effects on autotroph assembledges in Danish estuaries were especially comprehensive considering water residence times, nutrient demands, growth rates, C:N:P ratios and light requirements. (Borum, 1996; Borum and Sand–Jensen, 1996; Pedersen and Borum, 1996; Sand-Jensen and Borum, 1991). These studies found that nutrient enrichment changed plant community composition such that slow growing macro-algae were replaced by fast growing algal species, both of which rely primarily on water column rather than sediment nutrient sources.

In overview, several things seem clear. Very high primary production rates can be maintained in benthic dominated systems (Fig. 18.20, inset) with sufficient light reaching the bottom. Benthic autotrophs (microalgae, macroalgae and seagrass) are capable of obtaining N needed for growth either from sediment porewaters or from overlying water column pools depending on concentration gradients. There appears to be a generally predictable sequence relating predominant autotrophic groups to nutrient enrichment level. This sequence is likely related to competition for nutrients and light, where seagrasses dominate at one end of the gradient (low nutrients) because of their ability to tap deep sediment porewater nutrients, and phytoplankton dominate the other end (high nutrients) because of their ability, at high densities, to shade out benthic autotrophs.

6. Nitrogen and Secondary Production

Inputs and transformations of N and other nutrients directly influence production of all populations of consumer organisms, be they small or large. On one hand, nutrient additions generally result in increased primary production, which propagates forward to support increased growth of consumer populations, including fish. The amplitude of nutrient enrichment effects, however, tends to be damped as it is passed from one consumer population to the next, with responses being relatively large for primary producers, modest for herbivores, and small for top-carnivores (e.g., Micheli, 1999). On the other hand, spatial or temporal shifts in the abundance of fish and other consumers can result in marked effects on nutrient transport and recycling

processes (e.g., Kitchell *et al.*, 1979). Primary production is passed on to diverse consumer organisms through feeding interactions and food-webs (e.g., Clarke, 1946; Lindeman, 1942). These webs can often be described with equivalent straight food-chains, where plants are consumed by herbivores, which are consumed by first-level carnivores, and so on (Ulanowicz and Kemp, 1979). Food-webs and food-chains are considered to be controlled by "bottom–up" or "top-down processes," depending on the relative responses to changes in nutrient inputs at the bottom or changes in carnivorous predation at the top, respectively (e.g., Carpenter *et al.*, 1985). Here, we address the double-edged question of how secondary production and nutrient cycling processes are related. We focus on N because it tends to be the limiting nutrient for primary production on annual time scales in most estuaries.

6.1. Effects of nitrogen enrichment on secondary production

It has been suggested by Caddy (1993, 2000) that nutrient enrichment affects secondary producers through a range of mechanisms that follow a sequence of three stages. At relatively low nutrient loading rates, the first stage involves enhanced production of demersal and pelagic species as a result of increased food availability. In the second stage, at moderate nutrient loading rates, benthic invertebrates and demersal fish tend to decline but planktonic consumers and pelagic fish species continue to increase with more nutrient additions. In the hypothesized third stage of this model, there is a general decline in total production of invertebrates and fish as water quality and habitat conditions become severely degraded. There is substantial direct evidence for the first two stages of this proposed sequence, while evidence for the third stage is more limited and indirect.

Comparative analysis of fisheries landings and primary production among diverse coastal systems (Nixon and Buckley, 2002) reveals a significant positive relationship (Fig. 18.21A). This may illustrate Caddy's first stage, where increasing inputs of nutrients support higher rates of primary production that in turn support higher rates of fish production and harvest. At least for herbivorous fish such as Atlantic menhaden, this relationship is supported by controlled experiments (Keller *et al.*, 1990) and bioenergetic models (Luo *et al.*, 2001) which indicate that production of estuarine fish tends to increase with nutrient enrichment. Although this relationship is impressive, there are key underlying assumptions which need to be considered. Perhaps the most obvious of these is the assumption that fisheries harvest provides a dependable index of fish production. The relationship (Fig. 18.21A) also suggests that exploited fish populations are generally food-limited and relatively unaffected by fishing mortality, conclusions that are difficult to reconcile with fundamental principles of fisheries science (e.g., Chesney *et al.*, 2000). Alternative explanations include the possibility that human population density (and associated nutrient loads) and fishing pressure are highly correlated when comparing different estuaries or regions. Consequently, densely populated coastal regions, which tend to have eutrophic estuarine waters and high rates of primary production, also support heavily exploited fisheries, often dominated by species feeding at lower trophic levels (e.g., Pauly *et al.*, 1998). Nevertheless, the strength of this relationship and the fact that similar patterns have been reported previously for lakes are undeniable (e.g., Nixon, 1988).
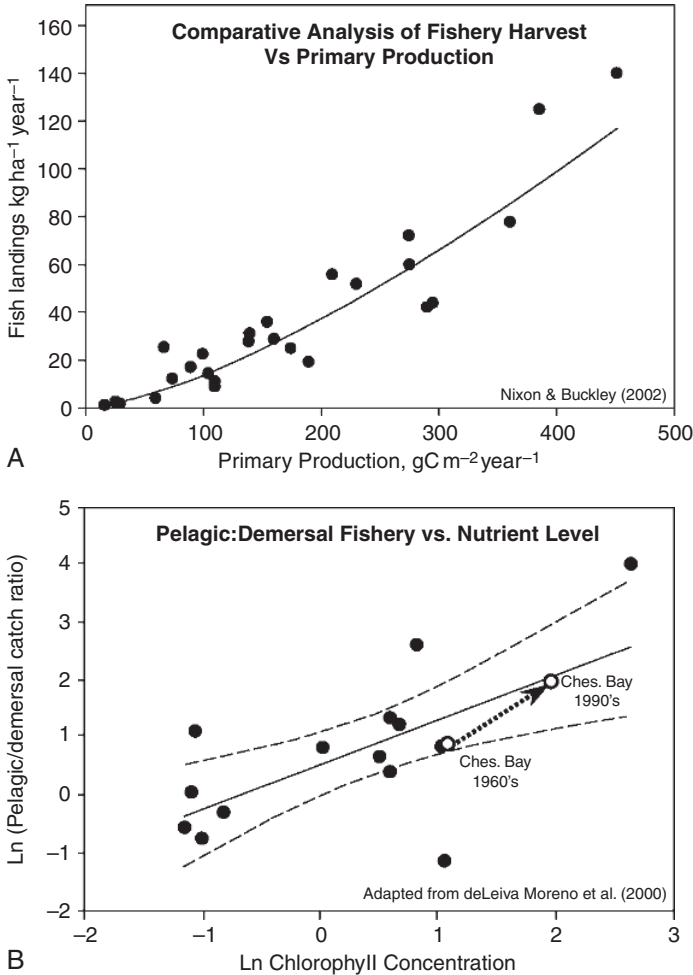
Figure 18.21 Comparative analyses of fisheries characteristics in relation to trophic status of estuarine and coastal ecosystems around the world: (A) fisheries harvest versus primary production (re-drawn from Nixon and Buckley, 2002); (B) ratio of pelagic-to-demersal fish caught in fishery plotted versus phytoplankton chlorophyll concentration (natural logarithm of both x and y variables). Figure 18.21 (B) was re-drawn from de Leiva Moreno *et al.* (2000) with Chesapeake Bay data added for two time periods.

Consistent with Caddy's second stage, recent data indicate that continued nutrient enrichment causes estuarine benthic habitats to become degraded, thereby contributing to marked shifts in food-web structure (e.g., Kemp *et al.*, 2005). For example, increased nutrient loading leads to decreased water clarity, which tends to cause loss of seagrass habitat in shallow areas (e.g., Duarte, 1995; Kemp *et al.*, 1983) and related reductions in fish and invertebrate production (e.g., Lubbers *et al.*, 1990). In some coastal lagoons, the loss of seagrass may be followed by outbreaks of dense benthic macroalgae stands with episodic hypoxic events that further degrade these

habitats for fish and benthos (e.g., Deegan, 2002). Enhanced phytoplankton production from N enrichment can also degrade deeper estuarine habitats by inducing prolonged seasonal hypoxia and anoxia in stratified water columns (e.g., Hagy *et al.*, 2004). As a result of this deep-water hypoxia, the behavior of bottom-dwelling animal populations is altered (e.g., Brandt, 1993; Phil *et al.*, 1991) and their abundance, diversity and production tend to decline (Howell and Simpson, 1994; Kemp *et al.*, 2005). A major consequence of benthic habitat degradation is a general switch from food-webs dominated by demersal pathways under low N-loading to ones dominated by pelagic pathways in more eutrophic estuaries (e.g., de Leiva Moreno *et al.*, 2000). It appears that this trend is often reflected in fisheries harvests with the ratio of pelagic-to-demersal (P/D) catch being directly related to indices of nutrient loading such as phytoplankton abundance (Fig. 18.21B). Time-series fisheries data in Chesapeake Bay from 1960 to 2000 reveal a trend of generally increasing P/D ratio that parallels increases in nutrient loading (Fig. 18.21B; Kemp *et al.*, 2005).

There is also evidence that N and P enrichment can significantly alter coastal ecosystems through changes in trophic structure that are not related to degradation of benthic habitat. With coastal eutrophication N and P loading tends to increase more rapidly than Si inputs, thereby resulting in decreasing Si:DIN ratios, limiting diatom growth and allowing smaller algal cells to dominate (e.g., Turner, 2001). The preponderance of smaller food particles reduces the efficiency by which primary production is transferred to herbivorous zooplankton and tends to increase the relative fraction of phytoplankton production that sinks to the benthos or enters the pelagic microbial loop (Kitchell and Carpenter, 1993). In addition to declining Si:DIN ratios, another mechanism causing a larger fraction of phytoplankton production to shift from zooplankton grazing to microbial degradation with nutrient enrichment involves predation-limitation on the growth of herbivore populations (e.g., Kemp *et al.*, 2001). Modeling studies suggest that this shift from metazoan grazers to microbial decomposers may be a general feature of all pelagic food-webs where exploited fisheries cause strong top-down control (Brooks *et al.*, 2005). Indeed, many investigators provide evidence to suggest that eutrophication tends to cause this shift from herbivorous grazing food-chains to microbial pathways (e.g., Baird *et al.*, 2004). Although detailed mechanistic understanding of these trophic responses to nutrient enrichment are generally lacking, recent evidence suggests that some systems may be currently experiencing Caddy's third stage (e.g., Yamamoto, 2003).

6.2. Effects of estuarine animals on nitrogen-budgets and cycles

In addition to being affected by N-loading to coastal waters, many animal populations in estuaries and other environments markedly influence the balance and cycling of N and other nutrients (Kitchell *et al.*, 1979). For example, respiration and excretion by Atlantic menhaden traveling in large schools can significantly deplete oxygen and increase ammonium concentrations in estuarine waters (Oviatt *et al.*, 1972). In pelagic estuarine mesocosm experiments, fish have been shown to enhance phytoplankton growth both through top-down effects on zooplankton grazing and through bottom-up effects of nutrient excretion (e.g., Micheli, 1999). In most cases, however, such large direct effects of fish on estuarine N

dynamics are confined to dense schools of herbivores or planktivores. In some estuaries with productive fisheries, harvest can account for a modest loss ($\approx$2–8%) of N inputs to the system, and an additional N sink of similar, or even larger magnitude, may be associated with emigration of fish using the estuary as a seasonal feeding ground (e.g., Boynton *et al.*, 1995; Deegan, 1993).

Benthic invertebrate populations can also influence N cycling in estuarine environments. As discussed earlier in this chapter, benthic invertebrates living as deposit-feeding infauna can influence nutrient cycling both through ventillation and bioturbation of sediments and through direct excretion and related metabolic processes. Reefs and other dense populations of benthic filter-feeders can, however, exert substantial influence on estuarine N cycling and transformation processes via a range of mechanisms. It is well established that filtration by clams, mussels and oysters can substantially reduce phytoplankton populations in overlying waters (e.g., Cloern, 1982). In many estuaries, native benthic filter-feeding populations have been depleted to levels that currently have little impact on plankton dynamics (e.g., Newell, 1988). In other estuaries, however, non-native species of benthic bivalves have grown to sufficient densities to readily control phytoplankton abundance in overlying waters (Cohen *et al.*, 1984). In a few coastal systems, healthy bivalve populations can still control plankton communities under certain hydrologic conditions (e.g., Møhlenberg, 1995). Similar densities of benthic bivalves, such as hard clams, have been shown to cause significant increases in rates of sediment N cycling through both direct excretion and increased turn-over of organic matter (e.g., Doering *et al.*, 1987). Under extreme conditions of intense benthic filtration associated with commercial culture, rates of N and P recycling can be greatly enhanced (e.g., Souchu *et al.*, 2001) stimulating growth of benthic macro-algae (e.g., Rafaelli *et al.*, 1998). In contrast, the eastern oyster, which maintains high clearance rates that are relatively independent of food concentration, produces large amounts of particulate N and P as psuedofeces (Newell *et al.*, 2004). Much of this pseudofecal material is deposited initially in shallow areas, where it supports enhanced production of deposit-feeding animals and increased nutrient burial (Newell *et al.*, 2004). In addition, recent experiments indicate that such oyster bio-deposits may stimulate N_2 loss via coupled nitrification-denitrification (Newell *et al.*, 2002). Although many questions remain, we are beginning to understand how heterotrophic organisms from bacteria to fish are linked together through diverse pathways and relationships in estuarine N cycles.

7. Summary and Future Directions

We indicated at the beginning of this paper that the last two decades have seen significant advances in understanding many aspects of N in estuaries. Scientific progress is, perhaps, difficult to comprehend on times-scales less than decades because the slow, incremental adaptation to new or refined ideas tends to mute our sense of progress. Our review of the estuarine N literature from the twenty years since the publication of Carpenter and Capone (1983) has revealed important new

developments in this field. A vastly expanded pool of measurements, ranging from simple N concentrations to far more sophisticated rate measurements, supports this conclusion. Improved measurement techniques and wider access to new instrumentation have also contributed to this progress. Twenty years ago, there were simply too few observations reported to develop robust generalizations about spatial and temporal patterns and controls on N cycling processes. In addition, a matured understanding of nutrient-limitation of primary production in estuarine systems during this time has contributed to development of science-based nutrient and eutrophication abatement programs in many estuarine systems.

Synthesis of these diverse observations into useful estuarine models of N transport, transformation, assimilation, and storage has, however, made less progress during this time period (e.g., Cloern, 2001; Nixon *et al.*, 1996). Using the broad, ecosystem scale perspective adopted in this paper, we emphasize the continued need for more rate measurements, data syntheses and predictive models of estuarine N cycling. Most estuarine research and monitoring programs have focused on measuring N concentrations, with much less emphasis on rate measurements that are needed to assess system dynamics. For example, N loss terms due to burial, denitrification and export are rarely measured at annual time-scales for whole systems, while other key rate processes such as primary production rates of benthic autotrophs have been virtually ignored until very recently (Chapter 23 by McGlathery, this volume). An important goal is to find ways to incorporate rate measurements into long-term, system–wide monitoring programs rather than relegate such measurements to research programs focused at smaller and shorter scales. For many coastal systems worldwide, vast and expanding water quality data sets call for integrated synthesis focused on N cycling and related issues. Nutrient budgets represent one type of synthesis that requires a variety of processes to be linked for computing the sources and fates of N in estuaries. The challenge is to develop multi-year N–budgets for diverse estuarine systems using consistent protocols (e.g., Gordon *et al.*, 1996). Future comparative analyses of these budgets will contribute to a deeper understanding of physical, geological and climatological controls on estuarine N dynamics.

Finally, we suggest that innovative conceptual, analytical and forecasting models need to be developed and tested against field and experimental observations. Recent conceptual schemes proposed to describe and explain ecological interactions associated with estuarine eutrophication (Cloern, 2001; Kemp *et al.*, 2005) need to be quantified toward the goal of developing reliable numerical models for testing our scientific understanding and for providing useful forecasts for estuarine nutrient management. It is particularly important that conceptual models relating N-loading to fish production and harvest (e.g., Caddy, 1993; Nixon and Buckley, 2002) be quantified, tested and improved to support ecosystem based management of water quality and fisheries. It is clear that current estuarine science is rich with empirical observations but relatively poor on integrated understanding. Nowhere is the need to apply such integrated, predictive scientific knowledge more severe than in the world's estuarine regions, where disturbance associated with densely populated human societies threatens the quality of these productive ecosystems.

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Nitrogen Cycling in Coastal Sediments

Samantha B. Joye *and* Iris C. Anderson

Contents

1. Introduction

Nitrogen (N) availability is frequently cited as the factor limiting primary production in marine systems over annual time scales (Capone and Kiene, 1988; Ryther and Dunstan, 1971; Vitousek and Howarth, 1991) though phosphorus (P), silica (Si) limitation or co-limitation by N, P, and/or Si sometimes occur on seasonal time scales (Conley *et al.*, 1993; Malone *et al.*, 1996; Officer and Ryther, 1980). In offshore waters, the presence of bioavailable iron can also regulate primary productivity, particularly that by diatoms (Coale *et al.*, 1996; Hutchins and Bruland, 1998; Martin *et al.*, 1994). Sediment N pools reflect a balance between inputs (external or internal) and outputs (sedimentation, denitrification (DNF), long-term burial, or export), and this balance is affected by a variety of environmental and physiological factors (Joye, 2002). Within the sediment, N is cycled primarily by microbially-mediated redox reactions between more highly oxidized forms (nitrate, NO_3^-, or nitrite, NO_2^-) and more reduced forms (ammonium, NH_4^+, amino acids, or organic nitrogen compounds). Gaseous products of these redox reactions may include dinitrogen, N_2, nitric oxide, NO, and nitrous oxide, N_2O. One characteristic of the sediment N cycle is the coupling between aerobic and anaerobic processes. Another characteristic is that for a given species, e.g., NO_3^-, several competing pathways and potential fates may exist. In this chapter, we review the factors regulating sediment N cycling, both in the bulk sediment environment and on a process level.

2. The Sediment Nitrogen Cycle: Overview

Sediment processes are integral components of the N cycle, particularly in shallow waters that often have higher sedimentation rates than deeper waters. In shallow coastal ecosystems, sediments contribute to ecosystem metabolism and provide habitat for a variety of microorganisms, invertebrates, and plants. Because sediments are porous media comprised of water, inorganic particulates, and living and nonliving organic materials, they are characterized by interactions between physical, biological, and chemical processes (Van Cappellen and Gaillard, 1996), including advective and diffusive transport, resuspension, bioturbation, and bioirrigation. Because of the relative magnitudes of these physical–biological interactions, sediments often exhibit steep gradients of electron donors and acceptors and process rates. The organic nitrogen in sediments may derive from water column or benthic primary production, nitrogen fixation (NFIX), or allochthonous sources (e.g., river or terrestrial-derived particulate organic matter). Allochthonous N inputs may also include particulate inorganic components (e.g., ammonium sorbed onto particles). The rate at which organic nitrogen is mineralized to inorganic forms depends on its source and characteristics such as its C/N ratio (Burdige, 2006). Nitrogen content of

sediments in estuarine and coastal systems varies by over an order of magnitude, primarily depending on the sediment type. For example, in shallow sites located in Chesapeake Bay, sediment nitrogen varied from 0.0075% to 0.577% (Anderson, in preparation) and at sites on the continental shelf of Eastern Massachusetts ranged from 0.03% to 0.52% (Hopkinson *et al.*, 2001).

The presence of a solid matrix in sediments slows rates of diffusive and advective transport of chemicals, creating steep redox gradients that microbes may exploit for energy generation. These redox gradients also facilitate coupling between aerobic and anaerobic processes, such as nitrification (NTR)–DNF, that drive sediment–water column fluxes of NH_4^+, NO_x^-, N_2, and N_2O. The sulfur cycle, which plays an important role in mediating organic matter decomposition and whose intermediates regulate N-cycling processes, also requires close coupling between aerobic and anaerobic processes. Transport through sediments is related to porosity, which decreases exponentially with depth due to compaction (dewatering) of sediments (Rabouille and Gaillard, 1991). Transport is mediated by diffusion (Glud *et al.*, 2003), advection (horizontal or vertical; Huettel *et al.*, 2003; Ziebis *et al.*, 1996), and biological processes such as nonlocal mixing via bioturbation and bioirrigation (Aller, 1982, 1994; Aller and Aller, 1998; Meile *et al.*, 2001) or plant root release of materials (Frederiksen and Glud, 2006). The physical and chemical processes influencing sediment nitrogen processing are discussed further in Section 3.

The sediment N cycle consists of oxidative and reductive processes that are frequently coupled in space and/or time (Herbert, 1999) and are strongly influenced by chemical, physical, and biological factors (Figs. 19.1 and 19.2). We present
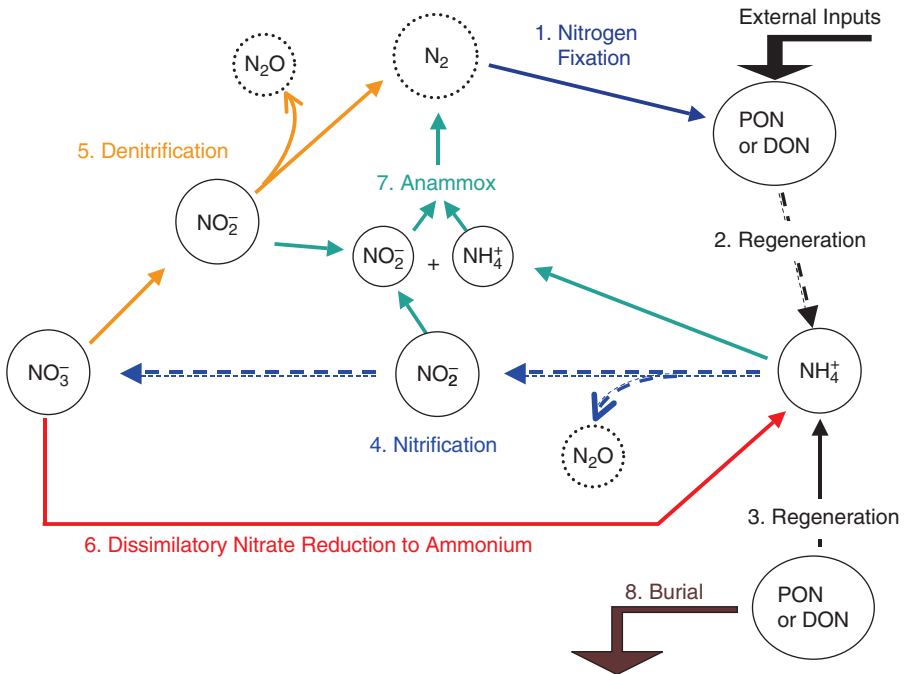
Figure 19.1 Processes in the sediment nitrogen cycle. Hashed lines reflect aerobic processes while solid lines reflect anaerobic processes. Species in hashed circles are gaseous.
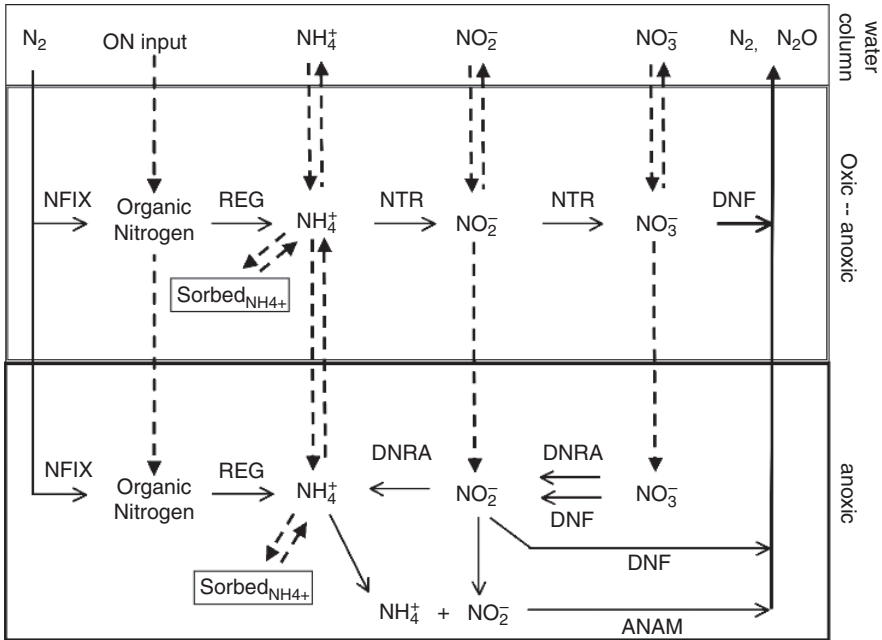
Figure 19.2 Spatial segregation of N cycle processes in sediments. Solid lines represent biological processes (NFIX = dinitrogen fixation; REG = regeneration; NTR = nitrification; DNF = denitrification; ANAM = anammox) and dashed lines represent exchange between boxes by diffusive, advective, or surface exchange (in the case of exchangeable ammonium) processes.

available ranges of rates of key processes; however, we note that a major problem with comparing rates available in the literature is the inconsistency of units for which rates are presented. We have limited our summary of activities to those that were either presented in, or easily converted to, units of mmol N m^{-2} d $^{-1}$. In addition, the ability to accurately determine most N-cycling process rates has suffered from methodological limitations; as a result the technology used to measure processes such as DNF has changed rapidly over the last 10 years, making it difficult to compare rates. The methodologies used to assess N-cycling process rates are discussed in Section 5. Throughout this chapter, we refer to rates >1.5 mmol m^{-2} d^{-1} as "high" and rates <0.25 mmol m^{-2} d^{-1} as "low."

2.1. Regulation of nitrogen fixation

NFIX is the biologically mediated conversion of molecular nitrogen (N_2) to cellular N (e.g., amino acids). NFIX rates in sediments are often lower than rates of the other N cycle processes because NFIX is inhibited by the high concentrations of bioavailable dissolved inorganic nitrogen (DIN) that are often present in sediments (Fig. 19.1, arrow 1; Capone, 1988; Seitzinger and Gardner, 1987). However, NFIX can occur at high rates, exceeding 2.5 mmol m^{-2} d^{-1}, in some sediments (Table 19.1). NFIX is mediated by a variety of organisms, including primary

Table 19.1 Rates of nitrogen fixation (NFIX) in Sediments

Location	NFIX rate (mmol m^{-2} d^{-1})	Method	Reference
Mekong Delta, Vietnam	0.5–2.85	ARA	Alongi et al., 2000
Sawi Bay, Thialand	0–0.6	ARA	Alongi et al., 2001
Missionary Bay, AU	0.14–0.3	ARA	Boto & Robertson 1990
Bahamas (seagrass bed)	0.36–0.98	ARA	Capone and Taylor 1980
Barbados (seagrass bed)	2.69	ARA	Capone and Taylor 1980
Caete Estuary, Brazil	2.3	ARA	Dittmar & Lara 2001
Magatorda Bay, TX	0.06	MIMS/IPT	Gardner et al., 2006
Nueces Estuary, TX	0.46	MIMS/IPT	Gardner et al., 2006
Ishigaki Island (NW Pacific)– sed+seagrass	0.1–0.3	ARA	Miyajima et al., 2001
Joyuda Lagoon, Puerto Rico	0.53	ARA	Morell and Corredor 1993
Oyster Bay, Jamaica	0–2.4	ARA	Nedwell et al., 1994
Phuket, Thailand (mangrove)	0.28–0.39[a]	ARA	Kristensen et al., 1998
Bassin d'Arcachon, France	<0.01–0.5	ARA	Welsh et al., 1995

[a] ~μmol/m^2/day, all others mmol/m^2/day.
ARA, acetylene reduction activity; MIMS, membrane inlet mass spectrometry.

producers (e.g., cyanobacteria), chemolithotrophs (e.g., photosynthetic bacteria and methanogens), and heterotrophs (e.g., sulfate reducers). NFIX requires a substantial input of energy; thus N_2 fixing microbes are often reductant limited. Rates of light–driven NFIX often exceed rates of chemolithotrophic (Van Gemerden, 1993) or heterotrophic NFIX (Bebout et al., 1987; Steppe and Paerl, 2002; Welsh et al., 1995), but elevated rates of heterotrophic NFIX have been observed in some wetland and seagrass habitats (Gotto and Taylor, 1976; Sundareshwar et al., 2003; Welsh et al., 1995).

In sediments with active communities of micro- (Paerl et al., 1993) or macro- (An et al., 2001; McGlathery et al., 1998) primary producers or with substantial rates of labile organic matter input (e.g., dissolved organic matter (DOM) leakage from roots; Howarth et al., 1988), rates of NFIX can be substantial. In general, rates of NFIX in subtidal sediments are lower than rates in intertidal sediments (Joye and Paerl, 1993) because light energy is more limiting in subtidal sediments, making heterotrophic NFIX the dominant process. Many of the factors that influence water column NFIX are less of an issue for benthic N_2 fixers. For example, metals, e.g., bioactive Fe, can be abundant in sediments but may limit water column NFIX

activity (Mills *et al.*, 2004; Paerl *et al.*, 1994). Oxygen concentration is a regulator of NFIX in both water column and benthic habitats. Highest NFIX rates tend to occur in tropical habitats but only limited data are available for temperate sediments and "hotspots" of NFIX in these habitats may remain to be discovered. Regulation of benthic N_2 fixation is discussed further in Chapter 4 by Ed Carpenter and Doug Capone; Chapter 21 by Judy O'Neil and Doug Capone, this volume.

2.2. Ammonium regeneration by mineralization

Organic nitrogen is introduced to sediments via either external (allochthonous) or internal (autochthonous, NFIX) mechanisms. The breakdown of sediment organic nitrogen occurs in several phases. Particulate organic nitrogen (PON) can be mineralized directly to ammonium (NH_4^+) or it may be metabolized to dissolved organic nitrogen (DON), which is further mineralized to NH_4^+. Mineralization of PON or DON to NH_4^+ occurs under both oxic and anoxic conditions (Fig. 19.1, arrows 2 and 3; Fig. 19.2). Ammonium regeneration is inherently linked to organic carbon oxidation; the fate of mineralized NH_4^+ depends in part on the C:N ratio of the reacting organic matter (Blackburn, 1979). Some PON escapes mineralization and is buried and subsequently sequestered in sediments for the long term (>100 or more of years; Fig. 19.1, arrow 8). Ammonium regenerated in sediments may either (1) be sorbed onto particles, (2) flux from the sediment to the overlying water, (3) be consumed by biological processes within the sediment or at the sediment–water interface (see Fig. 19.3, Section 4), (4) be transformed to nitrite (NO_2^-) and then nitrate (NO_3^-) by nitrifying microorganisms (Fig. 19.1, arrow 4; Fig. 19.2), or (5) be transformed to molecular nitrogen (N_2) via the anammox (ANAM) process (Fig. 19.1, arrow 7; Fig. 19.2). Ammonium can reversibly adsorb onto sediment particles (Rosenfeld, 1979) and adsorption can be described using a linear adsorption coefficient (Mackin and Aller, 1984). Ammonium adsorption onto particles may vary according to redox conditions (Morse and Morin, 2005) and possibly as a function of the NH_4^+ concentration if the solid phase is almost saturated with NH_4^+. Finally, some organic nitrogen may be transformed to form geopolymers via abiotic chemical condensation reactions (Burdige, 2006; Tisset and Welte, 1978). Geopolymers are resistant to microbial degradation and, thus, their formation represents a long-term sink for organic nitrogen in sediments; such compounds would be represented by Fig. 19.1, arrow 8.

2.3. Coupling of nitrification, denitrification, dissimilatory nitrate reduction to ammonium, and anammox

Because NTR links the reduced and oxidized sides of the N cycle, it can be considered a central process that provides substrate to microbes that employ nitrate or nitrite as oxidant (see Chapter 5 by Ward, this volume; Fig. 19.1, arrow 4). Like NH_4^+, the products of NTR, NO_2^-, and NO_3^-, may experience one of several possible fates, including (1) flux from the sediment, (2) assimilation within the sediment or at the sediment–water interface, or (3) reduction by one of three possible dissimilatory pathways: DNF, dissimilatory nitrate reduction to ammonium (DNRA), or ANAM (Fig. 19.1, arrows 5, 6, and 7; Fig. 19.2). Uptake of NO_x^- by
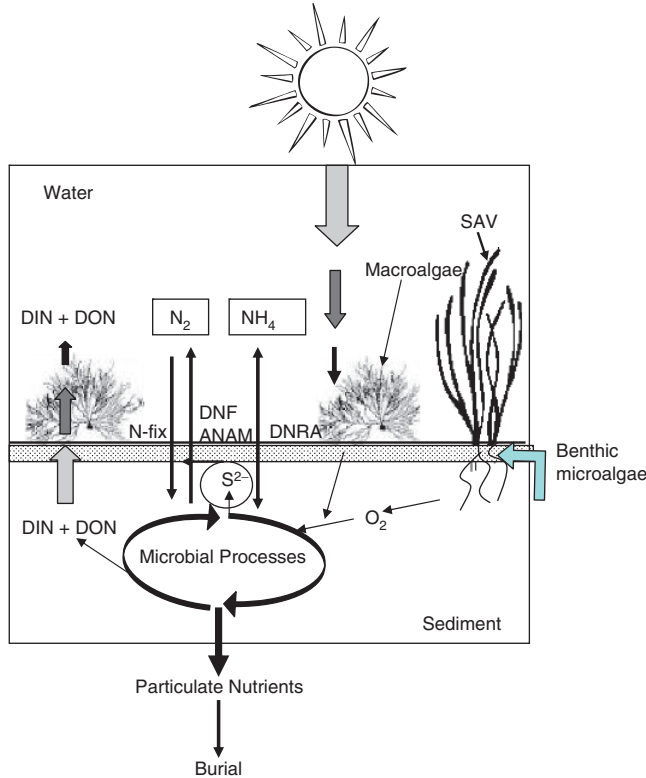
Figure 19.3 Impact of benthic primary producers on N cycle processes.

dissimilatory anaerobic processes requires diffusion of NO_x^- into anoxic zones or temporal separation of oxic production and anoxic consumption (e.g., daytime NO_x^- production followed by nighttime dissimilation).

Coupling of NTR and DNF or ANAM results in a net loss of fixed N, as gaseous forms, from the sediment (Fig. 19.2). DNF is the microbially-mediated conversion of NO_3^- to NO_2^- and then gaseous products, first nitric oxide, then nitrous oxide, N_2O, and finally dinitrogen, N_2, that are not generally considered to be bioavailable (see Chapter 6 by Devol, this volume). DNF rates have been reported for an extremely large number of sediments across the globe using a variety of methods (see Table 19.2). DNF rates vary as a function of NO_3^- availability, temperature, DOC concentration, and salinity; activity is much higher in shallow coastal estuaries (up to 30 mmol m^{-2} d^{-1}) compared to continental shelf or slope sites (~2 mmol m^{-2} d^{-1}; Table 19.2). Sediment DNF rates are often coupled to NTR rates, so DNF rates may provide insight regarding NTR rates in systems with low water column NO_3^- concentrations.

Until recently, DNF was considered the dominant dissimilatory NO_3^- sink in sediments (Hulth *et al.*, 2005); however, recent work has illustrated the importance of DNRA (An and Gardner, 2002; Binnerup *et al.*, 1992) and ANAM (Dalsgaard and

Table 19.2 Denitrification Rates in Sediments

Estuary/coastal	Denitrification rate (mmol m^{-2} d^{-1})	Method	Reference
Baffin Bay, TX	0.4–1.63	MIMS/IPM	An and Gardner 2002
Laguna Madre, TX	0.2–0.98	MIMS/IPM	An and Gardner 2002
Galveston Bay, TX	0.1–2.2	TCD	An and Joye 2001
Laguna Madre, TX	0.48	MIMS/IPM	An et al., 2001
Humber Estuary, UK	1.2–10	AIT	Barnes and Owens 1998
Norsminde Fjord, DK	0–8.1	15N2/AIT	Binnerup et al., 1992
Bremer River, AU	3.78	MIMS	Cook et al., 2004
Huon Estuary, AU	0.01–0.12	MIMS	Cook et al., 2004a (Meps 280)
Skagerrak/Kattegat, DK	0.1	IPM	Engstrom et al., 2005
Venice Lagoon, IT	0.34–6.85	TCD	Eriksson et al., 2003
Shallow lagoons near Sydney, AU	0.19–1.66	MIMS	Eyre and Ferguson 2002
Brunswick Estuary, AU	0.07–1.4	MIMS	Eyre and Ferguson 2005
Neuse River Estuary, NC	0–6.62	MIMS	Fear et al., 2005
Brunswick Estuary, AU	0.38–5.95	MIMS	Ferguson et al., 2004
Black Sea (Danube Delta)	3	Stoichiometry	Friedrich et al., 2002
Matagorda Bay, TX	0.54 ± 0.1	MIM/IPT	Gardner et al., 2006
Nueces Estuary, TX	0.96 ± 0.2	MIM/IPT	Gardner et al., 2006
Concepcion Bay, CL	0.57	IPM	Graco et al., 2001
Kertinge Nor, DK	3.1–7.7	Stoichiometry	Hansen and Kristensen 1997
Raritan Bay, NJ	2	MIMS probe	Hartnett and Seitzinger 2003
Parker River Estuary, MA	0–3.5	Stoichiometry	Hopkinson et al., 1999
Patuxent River Estuary, MD	1.85–2.14	Stoichiometry	Jenkins and Kemp 1984
Tomales Bay, CA (intertidal)	0.7–7.1	AIT	Joye and Paerl 1994
Tomales Bay, CA (subtidal)	0.2–10	AIT	Joye et al., 1996
Choptank River, MD	0.96–2.95	MIMS	Kana et al., 1998
Choptank River, MD	3.2–5.3	MIMS + NO^{3-}	Kana et al., 1998
Delaware Inlet, NZ	0.07	AIT	Kaspar 1983

Table 19.2 (*continued*)

Estuary/coastal	Denitrification rate (mmol m^{-2} d^{-1})	Method	Reference
Gt. Ouse Estuary, North Sea	0.1–3.6	Diagenetic model	Kelly-Gerreyn et al., 2001
Phuket Thailand, mangrove	0.01–0.04	IPM	Kristensen et al., 1998
Banzu flat, Tokyo Bay, JP	2.39	AIT	Kuwae et al., 1998
Waquoit Bay, MA	0–17	TCD	LaMontagne and Valiela 1995
Tamar Estuary, UK	3.62–10.8	AIT	Law et al., 1991
Santa Monica Bay, CA	0.9–2.5	Stoichiometry	Lehmann et al., 2004
Richmond River, AU	1–4.8	Stoichiometry	McKee et al., 2000
Scheldt Estuary, ND	0.1–8.44	TCD	Middelburg et al., 1995
Ishigaki Island (NW Pacific)-sediment	0.04–0.16	AIT	Miyajima et al., 2001
Ishigaki Island (NW Pacific)-sed +seagrass	0.06–0.28	AIT	Miyajima et al., 2001
Chesapeake Bay, MD	5.4–12	MIMS	Newell et al., 2002
Norsminde Fjord, DK	0.2–38	IPM/ Modeling	Nielsen et al., 1992
Tokyo Bay, JP	0.38–0.12	15N2 production	Nishio et al., 1982
Tama Estuary, JP	3.4–7.4	15N2 production	Nishio et al., 1983
Odawa Bay, JP	1.11	15N2 production	Nishio et al., 1983
Massachusetts Bay, MA	0.72–3.81	TCD	Nowicki et al., 1997
Narragansett Bay, RI	3.96	TCD	Oviatt et al., 1995
Neuse River Estuary, NC	0–2.49	AIT	Piehler et al., 2002
Sapelo Island, GA	0.1–0.2	MIMS/IPM	Porubsky et al. 2008a
Dover Bluff, GA	0–0.4	MIMS/IPM	Porubsky et al. 2008a
Okatee Estuary, SC	0–0.2	MIMS/IPM	Porubsky et al. 2008a
Randers Fjord, DK	5.2–8.0	MIMS/IPM	Risgaard–Peterson et al., 2004
Norsminde Fjord, DK	4.6–5.7	MIMS/IPM	Risgaard–Peterson et al., 2004

Table 19.2 Denitrification Rates in Sediments (*continued*)

Estuary/coastal	Denitrification rate (mmol m^{-2} d^{-1})	Method	Reference
Terminos Lagoon, MX	0.59	AIT	Rivera-Monroy and Twilley 1996
Tokyo Bay, JP	0.26	AIT	Sayama 2001
Narragansett Bay, RI	0.9–2.6	TCD	Seitzinger et al., 1984
Ochlockonee Bay, FL	0–5.0	TCD	Seitzinger 1987
Tomales Bay, CA	1.6–18.1	Stoichiometry	Smith et al., 1991
Randers Fjord, DK	0.99	AIT	Sørensen 1978
Gulf of Bothnia, Baltic Sea	0–0.94	AIT	Stockenberg and Johnstone 1997
Gullmar Fjord (Skagerrak), SW	0.4	IPM	Sundback et al., 2004
Ringfield Marsh, VA	1.8–17.6	15N/mass balance	Tobias et al., 2001
Tama Estuary, JP	5.1–30.2	IPM	Usui et al., 2001
St. Lawrence Estuary, CA	0.04–0.79	NO3 flux	Wang et al., 2003
Readings Bay, Brunswick, AU	1.2–7.2	MIMS	Webb and Eyre 2004
Guadalupe Estuary, TX	0.1–0.83	TCD	Yoon and Benner 1992
Nueces Estuary, TX	0.09–1.7	TCD	Yoon and Benner 1992
Galveston Bay, TX	0–0.96	TCD	Zimmerman and Benner 1994
Shelf/slope/deep sea			
North Sea (cont. shelf)	0.55	AIT	Brion et al., 2004
Louisiana Continental Shelf	0.96–2.59	AIT	Childs et al., 2002
Global continental shelf sediments	0.354	Stoichiometry	Christensen et al., 1987
Louisiana Gulf Coast	0.2–0.7	AIT	Delaune et al., 2005
Continental Shelf (Eastern N. Pacific)	0.4–2.6	TCD	Devol and Christensen 1993
Chukchi, W Beaufort, & Bering Seas	0.5–2.88	Benthic "lander"	Devol et al 1997
Cont. shelf, central Chile	0.64–2.9	IPM	Farias et al., 2004
French JGOFS (2300 m)	0.06–0.14	Stoichiometry	Gehlen et al., 1997
Greenland E and W coasts	0.03–0.27	IPM	Rysgaard et al., 2004
Mass. Bay/Gulf of Maine	1.9	Stoichiometry	Hopkinson et al., 2001

Table 19.2 *(continued)*

Estuary/coastal	Denitrification rate (mmol m^{-2} d^{-1})	Method	Reference
Stellwagen Basin (Gulf of Maine)	1.3	Stoichiometry	Hopkinson *et al.*, 2001
Norway fjords/ Svalbard archipelago	0.2–0.6	IPM	Kostka *et al.*, 1999
Mid-Atlantic Bight	1.74	MIMS	Laursen and Seitzinger 2002
Santa Monica Bay (shelf)	0.9–2.5	Stoichiometry	Lehmann *et al.*, 2004
North Sea (cont. shelf)	0.13–0.17	AIT	Lohse *et al.*, 1996
North Sea (cont. shelf)	0.23–0.32	IPM	Lohse *et al.*, 1996
>45°N Latitude	0.90	various	Seitzinger and Giblin 1996
20°N–45°N	2.60	various	Seitzinger and Giblin 1996
0–20°N	1.42	various	Seitzinger and Giblin 1996
Equatorial Atlantic	0.0007	AIT	Sørensen *et al.*, 1984
SE Bering Sea Shelf	0.7–1.5	Isotope tracer	Tanaka *et al.*, 2004
Baltic Proper (northern)	0.15–0.65	IPM	Tuominen et al 1998
Gulf of Finland (Baltic Sea)	0.03–0.11	IPM	Tuominen *et al.*, 1998

MIMS/IPM, membrane inlet mass spectrometry/isotope pairing method; TCD, gas chromatography–thermal conductivity detector; AIT, acetylene inhibition technique; IPM, isotope pairing method with isotope ratio mass spectrometry; MIMS, membrane inlet mass spectrometry; Stoichiometery, benthic flux DIC:DIN stoichiometry; MIMS + NO3, membrane inlet mass with NO3 amendment; 15N2 production, 15NO3 or 15NH4 conversion to 15N2.

Thamdrup, 2002; Thamdrup and Dalsgaard, 2002a) in the sediment nitrogen cycle. DNRA converts NO_3^- to NH_4^+, thereby retaining N in a readily bioavailable form (Binnerup *et al.*, 1992; Jørgensen, 1989; Porubsky *et al.*, 2008a). ANAM microorganisms use NO_2^- as the oxidant for anaerobic NH_4^+ oxidation, and the metabolic end product is N_2 (Strous *et al.*, 1999). In fact, NO_2^- reduction coupled to NH_4^+ oxidation leading to N_2 generation was first documented in a *Nitrosomonas*-type nitrifying bacterium (Bock *et al.*, 1995; Poth, 1986). While DNF and ANAM lead to N_2 production and, thus, fixed N loss from the sediment, DNRA maintains fixed N in a biologically available form that can fuel subsequent microbial production. Compared to DNF, far fewer estimates of ANAM and DNRA rates are available in the literature (Table 19.3). For ANAM few studies report rates in units of mmol m^{-2} d^{-1}. For DNRA, rates are often comparable to DNF rates, though the maximum reported rates of DNRA are lower than those of DNF; this could result from the paucity of studies on published reporting DNRA rates.

A final component of the sediment N cycle that warrants mention is manganese-mediated conversion of NH_4^+ to NO_x^- or N_2. The reduction of Mn-oxides can be

Table 19.3 Rates of anammox (ANAM) and dissimilatory nitrate reduction to ammonium (DNRA) in Sediments

Location	Anammox rate (mmol m^{-2} d^{-1})	Method	Reference
Skagerrak (Baltic/ North Sea)	0−0.86[a]	IPM	Dalsgaard and Thamdrup 2002
Long Island Sound, NY	0.096[b]	IPM	Engstrom et al., 2005
Skagerrak/Kattegat, DK	0.96[b]	IPM	Engstrom et al., 2005
Randers Fjord, DK	0.3−0.6	MIMS/IPM	Risgaard-Peterson et al., 2004
Norsminde Fjord, DK	0	MIMS/IPM	Risgaard-Peterson et al., 2004

Location	DNRA rate (mmol m^{-2} d^{-1})	Method	Reference
Baffin Bay, TX	0.48−1.4	15NO3/HPLC	An and Gardner 2002
Laguna Madre, TX	0.9−3.6	15NO3/HPLC	An and Gardner 2002
Norsminde Fjord, DK	<0	15NO3/irMS	Binnerup et al., 1992
Magatorda Bay, TX	0.08	MIM/IPT	Gardner et al., 2006
Nueces Estuary, TX	0.25	MIM/IPT	Gardner et al., 2006
Baltic Sea	0.8−1.1	15NO3/mass balance	Karlson et al., 2005
Lower Great Ouse, UK	0−24	15NO3/mass balance	Kelly-Gerreyn et al., 2001
Tokyo Bay, JP	1.27	N mass balance	Nishio et al., 1982
Tama Estuary, JP	19.9	N mass balance	Nishio et al., 1983
Odawa Bay, JP	1.4	N mass balance	Nishio et al., 1983
Tokyo Bay, JP	0.99	mass balance	Sayama 2001
Ringfield Marsh, VA	0.9−6.1	15N/mass balance	Tobias et al., 2001

[a] μmol cm^{-3} d^{-3}
[b] μmol L^{-1} h^{-1}

MIMS/IPM, membrane inlet mass spectrometry/isotope pairing method; IPM, isotope pairing method with isotope ratio mass spectrometry; $^{15}NO_3$/HPLC, $^{15}NO_3$ tracer and quantification of $^{14}NH_4$ via HPLC; N mass balance, benthic flux mass balance.

coupled to the oxidation of NH_4^+ producing NO_x^-, and the oxidation of Mn^{2+} can be coupled to NO_3^- reduction and N_2 production. These processes are thermodynamically favorable at circumneutral pH (Luther et al., 1997). Interactions between manganese reduction and ammonium oxidation have been inferred from pore water data collected at a variety of sedimentary locations (e.g., Hulth et al., 1999, 2005; Luther et al., 1997, 1998; Mortimer et al., 2002; and references therein). However, attempts to quantify rates of this process directly using ^{15}N tracers have been unsuccessful thus far (Thamdrup and Dalsgaard, 2000), making the role of this process

in the N cycle under naturally occurring substrate concentrations and environmental conditions questionable (Hulth *et al.*, 2005).

3. Chemical and Physical Factors Influencing Sediment Nitrogen Transformations

3.1. Interactions between the nitrogen and sulfur cycles

A variety of chemical species and physical factors influence the fate of N in sediments, including salinity, pH, oxygen concentration, substrate concentrations, temperature, and residence time. The impacts of some of the common factors known to influence N cycle processes are shown in Table 19.4. The concentrations of many chemical species are regulated by biological activity. For example, hydrogen sulfide (H_2S) concentration is influenced by rates of microbial sulfate reduction and also by the sediment iron (Fe) oxide content because Fe oxides can titrate H_2S, thereby reducing the amount of H_2S in solution (Canfield, 1989). Sulfide inhibits both steps of NTR (Joye and Hollibaugh, 1995) and short-circuits DNF (Sørensen *et al.*, 1980), resulting in elevated production rates of N_2O relative to N_2 and reduced rates of overall DNF activity (Joye, 2002; Senga *et al.*, 2006). However, H_2S may stimulate DNRA (An and Gardner, 2002) and NFIX (Bebout *et al.*, 1993; Tam *et al.*, 1982). The effect of H_2S on ANAM is unknown.

In systems with high water column NO_3^- concentrations, high concentrations of H_2S in sediments may promote coupling of NO_3^- reduction and H_2S oxidation by vacuolate sulfur oxidizing bacteria (Otte *et al.*, 1999; Sayama, 2001; Zopfi *et al.*, 2001). These microorganisms appear to couple H_2S oxidation to DNRA, thus promoting N retention at the expense of N loss via DNF or ANAM. H_2S may also stimulate DNRA by additional fermentative microorganisms (An and Gardner, 2002). Thus, the overall effect of H_2S on the sediment N cycle is to promote sediment N retention or gain and reduce N loss through coupling of NTR and DNF (or ANAM). The presence of

Table 19.4 Impact of Various Physical Parameters and Chemical Species on Rates of Nitrogen Cycle Processes

Process	Factor						
	Oxygen	T(°C)	Labile DOC	H_2S	NH_4^+	NO_2^-	NO_3^-
Nitrogen fixation	−	+	+	+	−	−	−
Nitrification	+	+	−	−	+	−/?	N
Denitrification	−	−	+	−	N	+	+
Anammox	−	−	?	?	+	+	+
Dissimilatory NO_3^- rdn	−	+	+	+	N	+	+

(+) denotes stimulation while (−) denotes inhibition of activity. For temperature, the effect of temperature (>16°C) is noted. The impact of low temperature can be considered the opposite. N, no direct effect known; ?, unknown.

H_2S thus acts as a positive feedback on the N cycle, promoting N recycling and retaining N in the system so that it can fuel additional biological production, the regeneration of which further stimulates sulfate reduction and H_2S production.

3.2. Regulation of nitrogen cycle processes by oxygen

Oxygen is a potent regulator of most processes in the N cycle (Fig. 19.2; Table 19.4). The only process in the N cycle that is not strongly regulated by O_2 concentration is organic N mineralization (NMIN) ($\sim NH_4^+$ regeneration). Ammonium mineralization occurs consistently throughout the sediment column under both oxic and anoxic conditions, although rates may be slightly higher under oxic conditions. Decreases in rates of NMIN over depth are probably more strongly influenced by reductions in organic matter reactivity than by the transition from oxic to anoxic conditions (Blackburn, 1979). While surficial (recent) sediment organic nitrogen is dominated by amino acids and proteins, these labile components are lost during early diagenesis and more recalcitrant organic N forms (e.g., heterocyclic N) dominate at depth (Burdige, 2006; Dereene et al., 1998; Gelinas et al., 2001).

Of the other processes in the N cycle, NFIX, ANAM, and DNRA are obligate anaerobic processes while NTR is an obligate aerobic process (though anaerobic ammonium oxidation by Nitrosomonas has been reported; Poth, 1986). DNF is a facultative process meaning that most denitrifiers prefer to respire O_2 instead of NO_3^- when O_2 concentrations exceed $\sim 20\ \mu M$ (Tiedje, 1982). Though many N_2 fixing microbes survive exposure to oxic conditions, the process of NFIX is tightly regulated by O_2 because O_2 exposure deactivates key enzymes (i.e., nitrogenase; Postgate, 1998). Most of the microbes responsible for ANAM and DNRA are obligate anaerobes and while they are able to tolerate oxic conditions, their activity is limited largely to anoxic conditions (Dalsgaard et al., 2005; Kelly-Gerreyn et al., 2001; Risgaard-Petersen et al., 2004a,b, 2005). An exception to this is the vacuolate sulfur bacteria (e.g., Beggiatoa or Thioploca) that mediate DNRA. These facultative anaerobes are chemoautotrophs (Nelson and Jannasch, 1983) that concentrate NO_3^- in a central vacuole (McHatton et al., 1996) and use it as the electron acceptor for anaerobic H_2S oxidation (Otte et al., 1999; Sayama, 2001). The role of vacuolate sulfur bacteria in the sediment N cycle is increased when both NO_3^- and H_2S are present (Sayama et al., 2005). Species such as Thioploca can link the N and S cycles when surface NO_3^-–rich and deep H_2S-rich zones are separate by vertical migration of filaments within sheaths (Zopfi et al., 2001).

Variations in the concentration and distribution of oxygen (O_2) in sediments can result from benthic primary production, release of O_2 from plant roots, advective mixing of overlying water into the sediment (bioturbation), or diffusive exchange of O_2 between the overlying water and sediments. The activity of benthic primary producers drives microscale (μm to cm) variation in O_2 concentrations and distributions in sediments (Revsbech et al., 1989; Wiltshire et al., 1996) while macrobiological activity, including bioturbation, bioirrigation, or plant-mediated root dynamics, can alter O_2 distributions at spatial scales from centimeter to tens of centimeter. The presence of benthic primary producers at the sediment–water interface also drives dramatic day–night variations in both the O_2 concentration and the vertical extent of the oxygenated zone (Revsbech et al., 1983). Rooted

macrophytes or the presence of tube-dwelling animals further intensifies horizontal and vertical O_2 distributions (Aller, 1982, 1994; Aller and Aller, 1998; Frederiksen and Glud, 2006; Kemp and Murray, 1986). Variations in rates of benthic production, root O_2 release and/or animal activity generate spatial and temporal complexity in sediment O_2 distributions and thus in the cycling of N in sediments. These topics are discussed further in the "biotic controls" section (Section 4).

The concentrations of different oxidized N species, such as NO_2^- and NO_3^-, hereafter referred to as NO_x^-, are largely regulated by biological processes. However, elevated water column concentrations of NO_x^- can result from septic or agricultural and groundwater inputs as well as from *in situ* biological processes (water column NTR) occurring in rivers (Berounsky and Nixon, 1990; Pakulski *et al.*, 1995). In some cases, high water column NO_x^- concentrations support NO_x^- diffusion into sediments, which fuels dissimilatory processes. However, in most cases sediment NTR of NH_4^+ generated by mineralization of PON or DON provides the majority of NO_x^- consumed by dissimilatory processes or by benthic primary production, suggesting that NTR limits most other nitrogen requiring processes, such as, ANAM, DNRA, DNF, and GPP (gross primary production) (An and Joye, 2001; Anderson *et al.*, 2003; Dollhopf *et al.*, 2005).

Fluctuating oxic–anoxic conditions may facilitate coupling between NMIN, NTR, and the dissimilatory processes of DNF, DNRA, or ANAM (An and Joye, 2001; Risgaard-Petersen *et al.*, 2005; Rysgaard *et al.*, 1994). Strictly oxic or anoxic conditions may lead to efflux of NO_3^- or NH_4^+, respectively, from the sediment (Kemp *et al.*, 1990; Fig. 19.2; Table 19.4). Shallow, well-lit sediments exhibit high rates of benthic production and strong diurnal variability in O_2 dynamics. Such habitats may favor metabolically versatile microorganisms that can quickly adapt to changing environmental conditions. For example, the metabolic flexibility of denitrifying microbes may provide them a competitive advantage over microbes conducting ANAM or DNRA. In the absence of NO_x^-, denitrifiers may either conduct aerobic respiration or utilize alternate electron acceptors (e.g., Fe oxyhydroxides). Denitrifiers can also survive extended periods of low or zero NO_x^- concentration (Risgaard-Petersen *et al.*, 2005). While ANAM microorganisms are not immediately killed by oxygen exposure and appear to recover ANAM activity fairly quickly after oxygen exposure (Strous *et al.*, 1997), the ability of denitrifiers to respire O_2 may offer them a competitive advantage.

Hypoxia, which refers to low oxygen conditions ($\sim$3 mg L^{-1} or 90 μM dissolved O_2; Diaz and Rosenberg, 1995), occurs when rates of oxygen consumption exceed rates of oxygen supply to a water body. Hypoxia is one of the most common symptoms of eutrophication (Boesch, 2002). Hypoxic waters provide poor habitat for pelagic and benthic fauna and persistent or intermittent water column hypoxia can exert profound effects on the sediment N cycle. For example, hypoxia can impact the N cycle by reducing the density and activity of benthic infauna responsible for bioturbation and bioirrigation; by increasing the organic deposition rate to sediments as a result of asphyxiation and subsequent demise of water column fauna; by stimulating benthic anaerobic respiration and associated nutrient recycling such as sulfate reduction to H_2S, with associated inhibitory effects on N-cycling processes and on benthic primary producers which assimilate N; or, by thinning the sediment oxic zone, which reduces or eliminates the activity of O_2-dependent N cycle processes, like NTR (Diaz and Rosenberg, 1995; Kemp *et al.*, 1990, 1992; Ritter and Montagna, 1999).

Water column hypoxia results from a variety of interrelated factors including water column stratification, elevated rates of nutrient or organic matter loading and increased fluxes of H_2S (which reacts rapidly with and hence consumes O_2) from the sediments to the overlying water (Ritter and Montagna, 1999). Hypoxia decreases rates of coupled NTR–DNF (Kemp and Boynton, 1990; Kemp et al., 1990) while enhancing rates of DNRA. As NH_4^+ accumulates and fluxes to the water column, primary production and organic matter sedimentation to the benthos will increase, resulting in a positive feedback whereby benthic respiration and O_2 consumption increase further exacerbating hypoxia.

3.3. Advective and diffusive transport through sediments

Physical factors such as transport clearly influence N cycling in sediments at a variety of spatial and temporal scales. Diffusive and advective processes generate flow through sediments. Sediment texture plays a major role in regulating transport through the sediments, and transport influences the rates and pathways of N processing. In low permeability muddy sediments molecular diffusion and infaunal irrigation control pore fluid transport through the sediments (Aller, 1982). In high permeability sandy sediments waves (Riedl et al., 1972) and/or bottom topography and currents (Huettel and Gust, 1992; Huettel et al., 2003) enhance fluid exchange between the sediments and overlying water column (Huettel and Webster, 2001).

Generally speaking, when sediment permeabilities exceed 10^{-12} m^2, advection of fluids through sediments can increase exchange by up to two orders of magnitude relative to that expected from molecular diffusion alone (Huettel et al., 2003). Variable density gradients in sediment pore waters relative to the overlying water (e.g., lower pore water salinity relative to the salinity in the overlying water) may also enhance fluid flow. Such density gradients can result in pore water convection and enhanced mixing (Webster et al., 1996).

Advective fluid flow has been shown to influence the rates and pathways of N processing in sediments. Bottom topography and currents result in local changes in NH_4^+ and NO_2^-/NO_3^- concentrations and likely stimulate rates of NTR and possibly DNF (Huettel et al., 1998). Advection can enhance the sediment oxygen uptake by ~25% in medium-grained and coarse-grained sands while NH_4^+ fluxes decrease and NO_3^- fluxes increase as grain size increases (Janssen et al., 2005). Increased permeability and advection-enhanced fluid flow through sediments appear to favor conversion of NH_4^+ to NO_3^- may stimulate sequestration of bioavailable N into microbial biomass in sediments (Huettel et al., 1998; Janssen et al., 2005) and may increase exchange of reduced metabolites (e.g., NH_4^+, Fe_2^+) from the sediments to the overlying water (Huettel et al., 1998; Precht et al., 2004). This occurs because elevated rates of fluid flow through sediments not only increase the oxidant supply but may also increase the supply of organic material to the sediments because of trapping (e.g., filtering out) of suspended particulate organic matter from the overlying water, thus increasing sediment respiration rates. Increased rates of organic matter mineralization can increase NH_4^+ fluxes but may reduce NTR rates due to O_2 limitation (Caffrey et al., 1993).

3.4. Porewater exchange—Resuspension

Physical processes that mix sediment pore fluids with the overlying water column can also affect the N cycle because the concentrations of N species in sediments are much higher than concentrations in the overlying water column. For example, NH_4^+ can reach millimolar concentrations in pore fluids while surface water concentrations are typically micromolar or less. These concentration differences lead to substantial rates of diffusive- and advective-driven effluxes of nutrients from sediments to the water column (Rowe *et al.*, 1975), which may support high rates of water column production. Resuspension of sediment (Fanning *et al.*, 1982), which results from wind or wave disturbance of bottom sediments (Chang *et al.*, 2001), barge traffic, trawling or dredging (Morin and Morse, 1999; NRC, 2002; Pilskaln *et al.*, 1998), also transfers nutrients into the overlying water. While some previous studies concluded that resuspension is of minor consequence for overlying water nutrient budgets (Blackburn, 1997; Sloth *et al.*, 1996), others have shown that resuspension can significantly increase DIN exchange between sediments and overlying waters (Fanning *et al.*, 1982; Morin and Morse, 1999). For example, release of NH_4^+ from sediments related to a single dredging of the main channel in Laguna Madre (TX, USA) introduced as much NH_4^+ into the water column as the diffusive NH_4^+ efflux from the sediments to the water column over the summer. Other studies have postulated that trawling activities play an important role in transferring mineralized nutrients from the sediments to the overlying water (Pilskaln *et al.*, 1998). The impact of dredging and trawling activities on the transfer of nitrogen from the sediments to the water column and on sediment N processing deserves further study (NRC, 2002).

3.5. Relationship between water residence time and nitrogen fate in coastal systems

Understanding the relationship between water residence time and nitrogen dynamics is complicated by the difficulty in quantifying residence time and by the fact that many of the data used to develop residence time–N cycle relationships were obtained in dark (rather than light) incubations. Thus, more work is required in this area but the available data will be summarized here. The residence time of a water body, which denotes the average length of time a parcel of water remains in the system, influences the fate of N and other nutrients within a system and also impacts nutrient export. Nielsen *et al.* (1995) and Nixon *et al.* (1996) first suggested that on a system-wide basis DNF in estuaries was proportional to the residence time within the system as a whole: A longer water residence time provided more opportunity for DNF to consume N, thus reducing N export from a system. A logarithmic relationship between N cycle processes and residence time in estuaries was later observed (Dettmann, 2001): Increases in residence time led to increased rates of DNF, which corresponded to reduced N export from the system. DNF was assumed to account for a constant fraction of N removal (69–75%) and total N removal increased substantially as residence time increased (Dettmann, 2001). Though DNF accounts for a large fraction of the total N removed, less than 40%

of the N load to a system is removed via DNF if the residence time is about 3 months; this fraction levels out to around 80% in systems with residence times greater than 100 months (Dettmann, 2001).

However shallow systems may behave differently than deeper estuarine systems. For example, residence time in shallow coastal bays has been shown to be highly variable across systems. Thus, the influence of N-cycling processes in both the pelagic and benthic zones on whole system export may depend upon position within the system (Fugate et al., 2006). Most coastal estuaries have residence times less than 100 days, making DNF an inefficient mechanism for reducing total N loading under most circumstances. For example, only 8% of the N load to Boston Harbor was denitrified and Nowicki et al. (1997) attributed this to the short residence time (2–10 days) of the system. Similarly, in Norsminde Fjord (Denmark), a rapidly flushed system with a 2–13 day residence time, DNF removed only 2–3% of the N load (Nielsen et al., 1995). Sporadic increases in freshwater delivery to coastal systems via storm-derived runoff can result in "contact limitation" of DNF, in that sediment denitrifiers do not have the opportunity to remove N before it is exported from the system (Joye and Paerl, 1993). As such, N export is generally much higher during high flow events (Nixon et al., 1996).

In some shallow coastal ecosystems with short residence times, benthic primary production can regulate coupled NTR–DNF. When N loads are high, benthic primary production can significantly stimulate coupled NTR–DNF resulting in substantially higher rates of N loss via DNF than would be predicted from the system residence time. For example, Joye and An (1999) studied DNF in Galveston Bay, TX, a shallow estuary with a seasonally varying residence time. The residence time of Galveston Bay is short (30 days) during the wet winter and spring periods and long (100 days) during the dry summer period. DNF rates determined in two separate studies were lowest in winter and spring and highest during summer as predicted by residence times (Joye and An, 1999, in preparation; Zimmerman and Benner, 1994). However, DNF rates determined in dark chamber incubations yielded system-wide rates of DNF that amounted to about 7% of the N load to the system (Zimmerman and Benner, 1994). This value agrees well with the DNF rate that would be predicted from the residence time–DNF relationship reported by Nixon et al. (1996) and Dettmann (2001). However, DNF rates determined in light chamber incubations yielded system-wide DNF rates that amounted to about 50% of the N load to the system, a value that falls significantly off the regression line of the published residence time–DNF relationship (Joye and An, 1999). Joye and An (1999) attributed the observed stimulation of DNF in the light to oxygenation of the sediments by benthic microalgae, thereby increasing rates of NTR and subsequent DNF. Thus, high rates of benthic primary production can offset short residence times and enhance DNF-based N removal in systems that one might assume to be quite inefficient at removing N based solely on measurements of residence time. On the other hand, in the shallow coastal bays of Virginia's Delmarva Peninsula, with residence times varying from 10 to 23 days for most of the system and with limiting water column DIN, McGlathery et al. (2004) attributed the observed low rates of DNF to competition between denitrifiers and benthic microalgae.

Whole estuary ^{15}N-cycling studies in which $^{15}NO_3^-$ is dripped into a tributary over multiple weeks allow determination of N uptake, processing, transformation, and export under natural conditions. This approach provides a powerful way to evaluate N cycling at the ecosystem scale. For example, the NISOTREX I experiment, conducted in the Parker River estuary of northern New England, demonstrated that when flushing rates were low and water column primary production was high, the planktonic centric diatom, *Actinocyclus normanii*, effectively removed all of the water column NO_3^- with little export of DIN downstream. Planktonic diatoms and their phytodetritus played the most important role in fueling production of numerous estuarine consumers including planktonic copepods, benthic amphipods, grass shrimp, mud crabs, and both planktivorous and benthivorous fishes (Holmes *et al.*, 2000; Hughes *et al.*, 2000). Since nitrogen demand of phytoplankton in the Parker River greatly exceeded riverine supply, production was supported mainly by benthic regeneration of N taken up by planktonic diatoms and subsequently sedimented to the benthos. On the other hand, during NISOTREX II, conducted in the Rowley River estuary, also in northern New England, but with a residence time shorter than the doubling time of phytoplankton, benthic microalgae rather than phytoplankton were responsible for most of the system's primary production and N demand (Tobias *et al.*, 2003b), and regenerated N was mainly exported from the system.

3.6. The influence of salinity on nitrogen cycle processes

The salinity of coastal estuaries varies on daily, seasonal, and annual time scales as a function of freshwater inputs and tidal fluctuations. Salinity influences many processes in estuarine ecosystems, including sediment N-cycling processes ranging from ammonium sorption and regeneration to NTR and DNF. The impact of salinity on sediment NH_4^+ pools, DNF, and NTR has received considerable study but the impacts of salinity on other sediment N cycle processes (NFIX, ANAM) are not well known. Furthermore, the physical or physiological consequences of varying salt content versus impacts resulting from other constituents associated with seawater, e.g., sulfate and associated increases in sulfate reduction rates, are unclear.

The pool of exchangeable NH_4^+ in sediments decreases with increasing salinity; this pattern has been documented in a variety of aquatic sediments (Seitzinger, 1988; Seitzinger *et al.*, 1991; Simon and Kennedy, 1987). In low salinity sediments, a primary consequence of the higher exchangeable NH_4^+ concentrations is elevated NTR, and coupled DNF rates. For example, Boynton and Kemp (1985) showed that benthic NH_4^+ fluxes increased with increasing salinity while benthic NO_3^- fluxes decreased. Gardner *et al.* (1991) and Seitzinger *et al.* (1991) expanded on this work, showing that changes in sediment NH_4^+ fluxes and N cycle processes were driven largely by variations in ionic strength (i.e., salinity), which altered the exchangeable NH_4^+ pool size.

Salinity effects may be both indirect, by controlling substrate pool sizes, or direct, by regulating physiological processes. Kana *et al.* (1998) showed that sediment DNF rates were independent of salinity at low salinities (<13 ppt) but were dependent on the NO_3^- concentration in the overlying water. Rysgaard *et al.* (1999) were the first to illustrate a salinity-linked physiological inhibition of both NTR and DNF in

estuarine sediments. The capacity of sediments to bind NH_4^+ decreased with increasing salinity, as noted previously. Results of assays for NTR and DNF in substrate amended incubations over a salinity range between 0 and 30‰ demonstrated that both NTR and DNF decreased with increasing salinity, suggesting that increasing ionic strength inhibited both processes (Rysgaard et al., 1999). In subsequent studies, evidence for a physiological inhibition of NTR or DNF with increasing salinity has been less clear. Fear et al. (2005) found no relationship between increased salinity and DNF in the Neuse River (NC, USA). And, Magalhaes et al. (2005) found that DNF rates in the Douro Estuary (Portugal) were strongly correlated with NO_3^- concentrations and that NO_3^- concentrations co-varied with salinity. They suggested that the pattern of decreasing DNF rates with increasing salinity reflected the limited NO_3^- supplies at higher salinities. NTR showed no consistent pattern with respect to salinity or NH_4^+ concentrations (Magalhaes et al., 2005). Thus, while salinity appears to physiologically inhibit the processes of NTR and DNF to some extent, substrate availability or the presence of inhibitory compounds (e.g., H_2S concentrations increase with salinity and H_2S inhibits NTR and DNF rates; Joye, 2002; Joye and Hollibaugh, 1995) may be a more important factor in determining absolute rates of these processes and other N cycle transformations.

4. Biotic Factors Influencing Sediment Nitrogen Transformations

Numerous biotic factors influence N processing within sediments, including the composition of the microbial, macrofaunal, and macrophyte communities, behaviors of the various infaunal communities, and trophic transfer of carbon and nitrogen between communities. Interactions between benthic communities may be physical, chemical, or biological in nature and, in turn, are influenced by the physical nature of the environment, sediment composition, and autotrophic dominance (Fig. 19.3).

4.1. Benthic–pelagic coupling: Impact of benthic primary production

Prior to the 1980s most studies of water column primary production ignored the role of the benthos in regenerating, transforming, or retaining nutrients. It was assumed that phytoplankton production was supported by a combination of allochthonous inputs of nutrients and by recycling of particulate organic matter in the water column (Johannes, 1969; Ryther and Dunstan, 1971). There was little to no consideration of benthic–pelagic coupling (Nixon, 1981). Measurements of benthic fluxes were generally performed in the dark; thus, the influence of benthic autotrophs on benthic fluxes was generally not realized (McGlathery et al., 2004), although Henriksen et al. (1980) noted the importance of benthic microalgae in taking up water column nutrients. More recent studies have demonstrated that in many coastal systems benthic remineralization of particulate organic matter, derived from a variety of sources, is responsible for supporting a large portion of both pelagic

and benthic primary production (Anderson *et al.*, 2003; Nixon, 1981). Whereas in the dark or in deep water benthic and pelagic processes are closely coupled, in the light benthic autotrophs, including seagrasses, macroalgae, and benthic microalgae, may decouple the benthic and pelagic zones by retaining, and transforming nutrients in sediments (McGlathery *et al.*, 2004).

The impact of benthic primary production on sediment–water fluxes of NH_4^+ is clearly demonstrated when cores collected from shallow water sites are incubated in the light and dark. Cores collected from a wide variety of shallow sites located along the axis of Chesapeake Bay in Maryland and Virginia demonstrated that regardless of salinity or degree of anthropogenic disturbance, NH_4^+ release from sediment to the water column was either less in the light or there was uptake (instead of release) by sediments (Anderson *et al.*, in preparation; Fig. 19.4). Similar results are evident when sediment–water NH_4^+ fluxes from shallow littoral zone sites (<2 m mean low water (MLW)) are compared to fluxes from deeper water sites (>2 m MLW). Fluxes from shallow water sediments are less than those from deeper water sediments (Fig. 19.5), suggesting autotrophic uptake at shallower depths. In addition to light temperature, by regulating the rates of benthic microbial processes, is also expected to influence the magnitude and direction of NH_4^+ fluxes. In studies conducted at numerous latitudes, average annual NH_4^+ fluxes were low or directed into sediment at low and high latitudes and were directed out of sediments in mid-latitudes (Fig. 19.6).

4.2. The influence of benthic macrophytes on nitrogen cycle processes

Benthic macrophytes, including rooted seagrasses and marsh grasses, and ephemeral and longer-lived macroalgae have been estimated to account for 40% of the primary production in shallow coastal ecosystems (Duarte, 1995). They play an important role in influencing microbial N transformations in sediments (Tobias *et al.*, 2001) and are described extensively in Chapter 23 by McGlathery (this volume) and in Chapter 22 by Hopkinson and Giblin (this volume). Detrital material derived from rooted macrophytes or their epiphytes is a source of both particulate and DOM fueling sediment metabolic processes, N transformations, benthic fluxes, microbial N immobilization, burial, and transfer of N to higher trophic levels. However, macrophyte-derived detrital material may not be quantitatively as important a source of N or C as the particulate organic matter sedimenting from the water column or resuspended from the benthos and physically trapped by either seagrasses or marsh grasses (Boschker *et al.*, 1999; Canuel, 1997; Currin *et al.*, 1995; Dahllöf and Karle, 2005; Duarte and Cebrian, 1996; Gacia *et al.*, 2002). Burial of both C and N has been observed in sediments of seagrass meadows such as *Posidonia oceanica*, due to low mineralization rates of particulate organic matter, derived from deposited seston and seagrass detritus (Gacia *et al.*, 2002). On the other hand, Evrard *et al.* (2005) observed rapid root uptake of N mineralized from ^{15}N-labeled phytodetritus (diatoms) injected into the sediment surface of an Indonesian sea grass bed dominated by *Thalassia hemprichii*, *Cymodocea rotundifolia*, *Halodule unineris*, *H. pinifolia*, *Halophila ovalis*, and *Syringodium isoetifolium*. *Zostera marina* has also been shown capable of taking up both urea and dissolved free amino acids (DFAAs) through their leaves (Hansen *et al.*, 2000). The rate of mineralization and burial of particulate organic
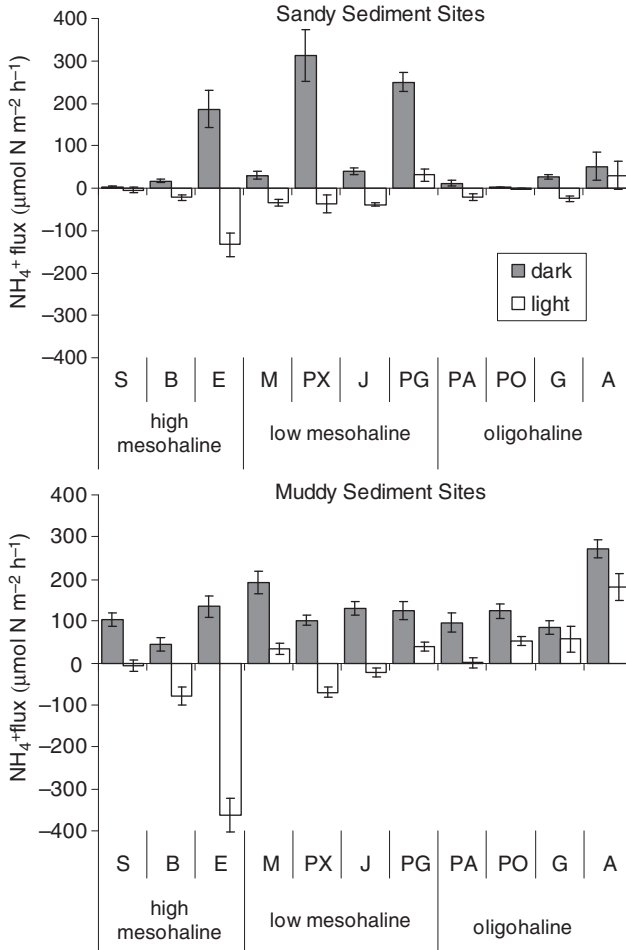
Figure 19.4 Light versus dark. Mean NH_4^+ sediment–water fluxes (mmol N m^{-2} h^{-1}; ±SE bars) in sandy and muddy littoral zone sites of the Chesapeake Bay (Anderson, Stanhope, and Schaffner, in preparation). S = Severn River, Virginia; B = Back River at Langley Air Force Base, Virginia; E = Elizabeth River, Virginia; M = Monie Bay, Maryland; PX = Patuxent River at Patuxent Naval Air Station, Maryland; J = James River at Fort Eustis, Virginia; PG = Pagan River, Virginia; PA = Pamunkey River, Virginia; PO = Potomac River at Quantico Marine Corp Base, Virginia; G = Gunpowder River at Aberdeen Proving Grounds, Maryland; A = Anacostia River, Virginia.

matter has been shown to be strongly influenced by sediment resuspension, which may mix porewater DOM into the water column and oxygenate the sediments (Arzayus and Canuel, 2004) or mix small particulates such as algae or bacteria into porous sandy sediments (Ehrenhauss *et al.*, 2004). In addition to serving as a source of detrital particulate organic matter or as a sink for mineralized dissolved nitrogen, macrophytes also release dissolved organic carbon, which may support processes such as NFIX (McGlathery *et al.*, 1998; Whiting *et al.*, 1986), DNF, (Eyre and Ferguson, 2002), and DNRA (An and Gardner, 2002). Indeed the partitioning of nitrate reduction between DNF and DNRA appears to be heavily influenced by the quality and quantity of available dissolved organic carbon (Christensen *et al.*, 2000).
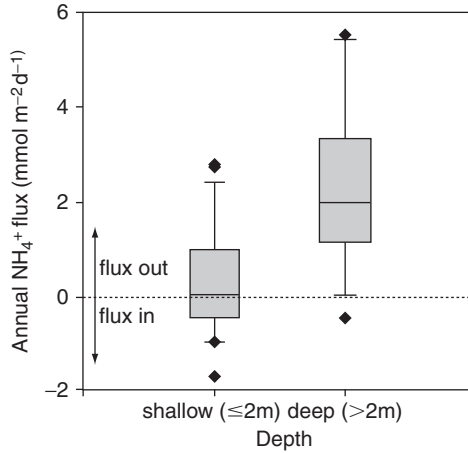
Figure 19.5 Annual mean NH_4^+ sediment–water fluxes (mmol m^{-2} d^{-1}) for shallow ($\leq$2 m) and deep ($>$2 m) sites. Shallow sites ($n = 18$) were included only if fluxes were measured in both light and dark using either *in situ* chambers or incubated sediment cores (Ferguson *et al.*, 2004; Fisher *et al.*, 1982; Nowicki and Nixon, 1985; Reay *et al.*, 1995; Rizzo, 1990; Sundback and Miles, 2000). Flux measurements from deep sites ($n = 12$) were measured at *in situ* light levels or only in the dark (Christensen *et al.*, 2000; Cowan and Boynton, 1996; Dollar *et al.*, 1991; Fisher *et al.*, 1982; Hopkinson *et al.*, 2001; Ibarra–Obando *et al.*, 2005; Pomroy *et al.*, 1983; Rizzo and Christian, 1996). Boxes and error bars represent 10th, 25th, 50th, 75th, and 90th percentiles and circles are outliers.
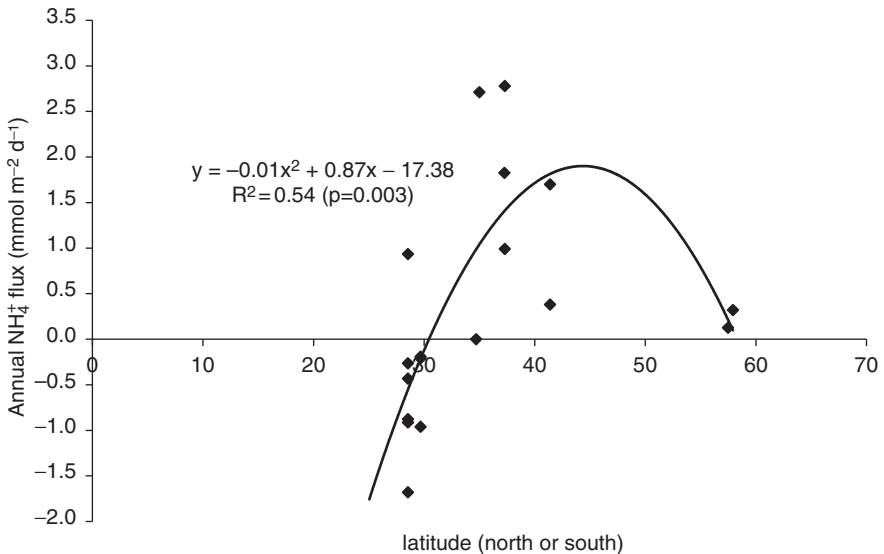
Figure 19.6 Latitudinal variation in annual mean NH_4^+ sediment–water fluxes (mmol m^{-2} d^{-1}) for shallow ($\leq$2 m) sites. Shallow sites ($n = 18$) were included only if fluxes were measured in both light and dark using either *in situ* chambers or incubated sediment cores (Ferguson *et al.*, 2004; Fisher *et al.*, 1982; Nowicki and Nixon, 1985; Reay *et al.*, 1995; Rizzo, 1990; Sundback and Miles, 2000).

4.3. The influence of macroalgae on sediment nitrogen cycle processes

In shallow, coastal littoral zone systems, macroalgae have been shown to alter benthic–pelagic coupling (Sundback and McGlathery, 2005; Valiela *et al.*, 1997). While alive, macroalgae intercept DIN, DFAA, and urea fluxing from sediments and release N as dissolved combined amino acids (DCAAs) (Tyler *et al.*, 2001, 2003). However, the ephemeral macroalgae (*Ulva lactuca* and *Gracilaria tikvahiae*) often found in eutrophied systems tend to undergo "boom and bust" cycles, dying off as summer temperatures peak in temperate zones. Following senescence macroalgae may decompose releasing much of the N stored in their biomass as dissolved inorganic and organic nitrogen (Tyler *et al.*, 2001). The fate of macroalgae depends on numerous factors, including its chemical composition, elemental stoichiometry, grazing, and physical parameters including temperature and energy of the surrounding environment. Since ephemeral macroalgae tend to have lower C:N and less structural material than rooted macrophytes, they are more rapidly and completely degraded by bacteria (Duarte, 1995).

In moderately eutrophied coastal ecosystems, bloom-forming "nuisance" macroalgae may become the dominant autotrophs. These nuisance macroalgae occur mainly as filamentous (e.g., the green algae: *Cladophora*, *Enteromorpha*, and *Chaetomorpha* species and the red algae: *Gracilaria*, *Spyridea*, and *Laurencia*) or sheet-like (e.g., *Ulva* species) forms and may accumulate as thick mats over the sediment surface (McGlathery *et al.*, 2004; Sundback and McGlathery, 2005; Valiela *et al.*, 1997) where they out compete the slower–growing seagrasses for N and reduce light availability for both seagrasses and benthic microalgae due to shading. Macroalgae can temporarily store a significant portion of the N load to a coastal embayment. For example, in Waquoit Bay, MA, macroalgal N was of the same magnitude (greater than 5 g N m^{-2} d^{-1}) as the annual N load from the watershed (Peckol, 1994; Valiela *et al.*, 1997). Much of the DIN assimilated by macroalgae is rereleased as DON, with release rates ranging from 17% to 99% of gross N uptake (Tyler, 2002).

Self-shading within the macroalgal mat, light attenuation by phytoplankton, and high temperatures eventually result in decay of macroalgal blooms with concomitant release of stored N. In Hog Island Bay, Virginia, Tyler *et al.* (2001) reported dissolved inorganic and organic N release rates following the collapse of a bloom composed primarily of *Gracilaria vermiculophylla* and *U. lactuca* that were sufficient to completely mineralize the macroalgal biomass (up to 650 gdw m^{-2}) within ~13 days. Release of plant-bound nutrients following these "dystrophic" events may stimulate phytoplankton and bacterial metabolism in the water column (Lunsford, 2002; McGlathery *et al.*, 2001; Valiela *et al.*, 1997).

During periods when decomposition rates are high, sediments underlying macroalgal mats are typically heterotrophic with high rates of N turnover (Trimmer *et al.*, 2000). Sediments may become anoxic, have high concentrations of dissolved inorganic and organic nitrogen, and H$_2$S in their pore water, and may be a source of NH$_4^+$ and DON to the overlying water column depending upon the abundance and productivity of rooted macrophytes and/or benthic microalgae on the sediment surface (Anderson *et al.*, 2003; Bartoli *et al.*, 1996). Regeneration of DIN by mineralization of particulate organic matter under these mats may provide a

high percentage of the N demand of macroalgae (Anderson *et al.*, 2003; Sundbäck *et al.*, 2003) or benthic microalgae (Anderson *et al.*, 2003). With decreased N demand by seagrasses and benthic microalgae due to shading, a positive feedback will occur in which N retention in sediments will decrease; fluxes of N to the water column will increase and may result in a shift of dominance to phytoplankton (Duarte, 1995; Sundback *et al.*, 1996; Sundbäck *et al.*, 2003). Sulfide accumulation in anoxic sediment with high rates of organic matter decomposition has been shown to interfere with coupled NTR–DNF and shift NO_3^- reduction toward higher rates of DNRA, further increasing fluxes of NH_4^+ out of sediments (Joye and Hollibaugh, 1995; Viaroli *et al.*, 1996). Eyre and Ferguson (2002) observed a negative relationship between DNF efficiency, calculated as N_2-N/(N_2-N + DIN), and rates of C decomposition. On the other hand, Krause-Jensen *et al.* (1999) demonstrated in a laboratory experiment that dense macroalgal mats did not significantly influence rates of DNF but instead moved the zone of DNF from the sediments up into the mat with peak rates at the oxic–anoxic interface.

4.4. The relationship between production/respiration and nitrogen cycle processes

Rooted macrophytes, macroalgae, and benthic microalgae all influence O_2 availability and, thereby, sediment biogeochemistry both in the light, during photosynthesis, and in the dark, due to the respiration of photosynthate. As discussed above, most N-cycling processes are regulated by the availability of O_2, as are other biogeochemical processes, e.g., sulfur, iron, and manganese transformations, which indirectly regulate N cycling. The sediment gross primary production to respiration ratio (P/R) has been shown to be a useful indicator of various N-cycling process rates and fluxes. For example, in Australian estuaries, NH_4^+ fluxes out of sediments were inversely related and DON fluxes positively related to P/R (based on either O_2 or CO_2; Eyre and Ferguson, 2002, 2005). The relationship between P/R and DNF was more complex, with DNF decreasing with increased CO_2-based P/R up to 0.4 and then increasing as net autotrophy increased; the presence of high macrofaunal biomass confounded these relationships (Eyre and Ferguson, 2005). Anderson *et al.* (in preparation) performed studies in shallow sites along the axis of Chesapeake Bay and observed a similar inverse relationship between P/R and NH_4^+ fluxes, which may have resulted in part from the increased macrofaunal abundance also observed with decreasing P/R. Other variables, such as salinity, nutrient standing stocks, and anthropogenic disturbance, also tend to co-vary with P/R in Chesapeake Bay and may confound relationships between P/R and N-cycling process rates.

4.5. Effects of shifting autotrophic dominance on nitrogen cycle processes

As eutrophication proceeds in response to nutrient enrichment and depending upon water residence time in coastal ecosystems there may be a shift in autotrophic dominance from rooted macrophytes to perennial macroalgae to ephemeral macroalgae and finally to phytoplankton (McGlathery *et al.*, 2004; Valiela *et al.*, 1997) and

references herein (see Chapter 23 by McGlathery, this volume, for more detail). The composition of the autotrophic community can influence nitrogen retention in sediments as well as microbial community structure. Degradation of macrophytes or macroalgae, such as *U. lactuca*, with low C/N and little cellulose and lignin in their biomass, occurs readily and may result in hypoxia–anoxia with efflux of NH_4^+ due to NMIN and influx of NO_3^- due to DNF. On the other hand, decomposition of *Z. marina* with higher C:N ratio and whose biomass contains greater quantities of both cellulose and lignin may result in net microbial immobilization of NH_4^+ to support microbial decomposition, as described by Dahllöf and Karle (2005).

4.6. Influence of benthic microalgae of sediment nitrogen cycle processes

Benthic microalgal (BMA) mats, containing primarily diatoms, but also including cyanobacteria, benthic dinoflagellates, and euglenoids, are important primary producers in shallow subtidal and intertidal systems. Because shallow sediments are frequently resuspended into the overlying water and phytoplankton are deposited into sediments, the distinction between pelagic and benthic species of microalgae is often blurred. The biomass of BMA normalized per square meter of surface often exceeds that of phytoplankton in the overlying water (MacIntyre *et al.*, 1996). In temperate and tropical systems, BMA are active throughout the year, and in polar regions they demonstrate changes in photophysiology and community composition in response to sea ice breakout (Glud *et al.*, 2002; McMinn *et al.*, 2004; Sundback and McGlathery, 2005). Although BMA primary production is generally confined to the top 1–3 mm of sediments, viable microalgal cells may be found at depths as great as tens of centimeters depending on sediment grain size, physical energy of the system, and abundance of bioturbating macrofauna (MacIntyre *et al.*, 1996). In intertidal sediments BMA migration as well as changes in species distribution and photophysiology occur during tidal cycles (down during flood tides and up again during ebb) as well as on diurnal cycles (Pinckney *et al.*, 1994; Underwood *et al.*, 2005). BMA biomass is usually in the range of 1–5 g C m^{-2} with a C/N ratio of $\sim$9 (Sundback *et al.*, 2000).

Benthic microalgae occupy a unique position both at the boundary between benthic and pelagic zones but also in many cases at the sediment oxic–anoxic interface. Thus, the environment to which BMA are exposed is dynamic both with respect to physical and biogeochemical factors. Benthic microalgae potentially impact the benthos in numerous ways, regulating sediment nitrogen cycling, sediment–water nutrient fluxes, sediment redox state, growth of macroalgae, supporting benthic food webs, and contributing to sediment stability. In shallow coastal estuaries and lagoons BMA compete for light and nutrients with phytoplankton, macroalgae, seagrasses, and bacteria. The outcome of this competition is dependent upon residence time and physical energy of the system, source of N (water column nutrient loading vs sediment DIN regeneration), and light availability (Fong *et al.*, 1993; Sundback and McGlathery, 2005; Sundback *et al.*, 2000; Valiela *et al.*, 1997). BMA production is not usually N limited in sediments because of high rates of sediment NMIN (Anderson *et al.*, 2003; MacIntyre *et al.*, 1996). However, recent studies in coastal Georgia have illustrated primary N and subsequently Si limitation of benthic diatom production (Porubsky *et al.*, 2008b), so in some cases, benthic microalgae may be nutrient limited. In mid-summer when macroalgal populations

decline because of self-shading or increased respiratory demand, BMA may become the dominant primary producers in subtidal systems (McGlathery *et al.*, 2001). In intertidal salt marsh dominated systems BMA production may represent 30–40% of macrophyte production even though the sediment surface is shaded to some extent by the macrophytes (Anderson *et al.*, 1997; Pinckney and Zingmark, 1993).

Numerous studies have shown that when sediments are net autotrophic BMA and the associated benthic bacterial community play a role in reducing the flux of remineralized nutrients from sediments to the overlying water column (Anderson *et al.*, 2003; Bartoli *et al.*, 2003; Cerco and Seitzinger, 1997; Eyre and Ferguson, 2002, 2005; Sundback *et al.*, 2000; Veuger *et al.*, 2007a). Thus, the benthic microbial community effectively decouples N remineralization within the sediments from primary production by both phytoplankton and macroalgae. If sediment nutrient sources are insufficient to meet growth demands, there also may be an influx of nutrients from the water column to the benthic community. Whether or not BMA effectively ''cap'' the sediment efflux of nitrogen over long time scales depends upon the fate of BMA biomass, which turns over on the scale of days or less (Cebrian, 2002; Tobias *et al.*, 2003a,b). Consequently, BMA only serve to ''cap'' the sediment nutrient flux if their own biomass is converted into forms of N and C that are either not released to the water column, or are released in a less biologically reactive form. Working in Rehobeth Bay, Delaware, Cerco and Seitzinger (1997) suggested that on annual time scales BMA may actually enhance and extend the duration of phytoplankton blooms by sequestering mineralized nutrients in the winter and spring and releasing them again in summer when BMA become light-limited due to increased phytoplankton shading. More work is needed to track the fate of macroalgal carbon and N through the benthic community over various time scales.

The influence of BMA on sediment biogeochemistry is both direct, due to competition for limiting nutrients with nitrifying and denitrifying bacteria, and indirect due to oxygenation of sediments by photosynthetic processes and also due to production and excretion of DOC, which serves as an energy source for bacterial processes (Veuger *et al.*, 2007a; Cook *et al.*, 2007). The effect of BMA on sediment–water fluxes of NH_4^+ is demonstrated in Figs. 19.4 and 19.5. In the light fluxes from sediments to the water column are either reduced relative to dark fluxes or fluxes are reversed and NH_4^+ is taken up by sediments. On the other hand, NO_3^- fluxes did not appear to vary in a predicable way in light versus dark incubations (Anderson *et al.*, in preparation).

Benthic microalgae cope with their high energy physical environment by releasing extracellular polymeric substances (EPS), which stabilize sediment structure and reduce resuspension. The release of extracellular DOC (Otero and Vincenzini, 2004; Underwood and Smith, 1998; Underwood *et al.*, 2005; Wolfstein *et al.*, 2002), which may account for more than 50% of the C fixed by BMA, also plays a role in BMA motility and influences N-cycling processes. Uptake of DIN by shallow euphotic sediments from both the water column and sediment pore water may be in response to the release of EPS by BMA. In order to use this carbon rich DOM, bacteria may depend on uptake of DIN to meet their N requirements (Cook *et al.*, 2007). The partitioning of NO_3^- reduction between DNF and DNRA may be regulated by the relative abundance of electron donors, such as labile DOC, to electron acceptors, such as NO_3^- (Tiedje, 1988). Although generally autotrophic, the BMA community has also been shown to take up DFAA under both enriched

and depleted inorganic N conditions (Admiraal *et al.*, 1984, 1987; Nilsson and Sundback, 1996, Veuger and Middelburg, 2007b).

Regulation of coupled NTR–DNF by benthic microalgae depends to a large extent on the availability of limiting N either in the water column or sediment. When N is limiting, DNF in benthic communities with actively photosynthesizing BMA is usually reduced both due to competition for substrate as well as by the O_2 produced (Eyre and Ferguson, 2005; Risgaard-Petersen, 2003; Sundback and Miles, 2000). BMA can similarly out-compete nitrifying bacteria for limiting NH_4^+ in the photic zone both because of their higher uptake rates and faster growth rates (Risgaard-Petersen *et al.*, 2004a,b). Because most of the BMA are motile and capable of vertical migration, they may also effectively compete for N with denitrifiers even below the photic zone (Risgaard-Petersen *et al.*, 2004a,b). On the other hand, oxygen production by BMA photosynthesis may enhance coupled NTR–DNF under conditions when N is not limiting and when surface sediment is anoxic, as is often the case in organic-rich sediment (An and Joye, 2001; Sundback *et al.*, 2000).

Ammonium fluxes out of sediments have often been calculated based on measured rates of net ecosystem metabolism (NEM based on dissolved O_2 uptake or DIC release) (Burdige and Zheng, 1998; Hopkinson *et al.*, 2001). In oxic sediments where NTR is likely to play an important role in transformation of NH_4^+, total DIN rather than the NH_4^+ flux should be more closely related to the stoichiometry of NEM, whereas in anoxic sediments where DNF is important the estimated DIN flux will not equal that predicted by NEM stoichiometry. Calculation of DNF has often been based upon this "missing" DIN.

However, in net autotrophic systems where immobilization of DIN by either BMA or microorganisms occurs, N-cycling rates or sediment–water fluxes calculated based on NEM stoichiometry will often result in overestimation of those rates (Anderson *et al.*, 2003). In general, BMA uptake represents a major fate of mineralized N compared to coupled NTR–DNF in net autotrophic sediments (Risgaard-Petersen, 2003). This was documented during a review of data derived from the EU-funded Nitrogen Cycling In Estuaries project performed in 18 European estuaries (Risgaard-Petersen, 2003); in a study of Hog Island Bay, Virginia, sediments (Anderson *et al.*, in preparation); in southern England sediments dominated by macroalgae (Trimmer *et al.*, 2000); in the Tagus Estuary, Portugal (Cabrita and Brotas, 2000), and at sites along the west coast of Sweden (Sundback and Miles, 2000). The DIN fluxes observed in these littoral zone systems differ markedly from those observed in sublittoral zone systems, which typically exhibit release of NH_4^+ or DIN, closer to values predicted by NEM (Burdige and Zheng, 1998; Giblin *et al.*, 1997; Hopkinson *et al.*, 1999) (Fig. 19.5).

4.7. The influence of benthic infauna on nitrogen cycle processes and fate

Meio- and macrofauna impact benthic nutrient cycling through grazing and by physically mixing sediment during the processes of feeding, burrow construction, and irrigation (Aller, 1982, 1994; Aller and Aller, 1998). The process of bioturbation, which depends on the feeding mode of the macrofaunal species, increases exposure of sediment to oxygen, vertically distributes both dissolved and particulate nutrients and thereby influences a variety of microbial activities, including organic

matter mineralization and respiration and nitrogen–cycling process rates, including NTR, DNF, and DNRA (Kristensen and Mikkelsen, 2003; Welsh, 2003). For example, subsurface deposit feeders contribute to mechanical breakdown and mixing of fresh particulate organic matter into deeper sediment strata with deposit of fecal castings and pore waters at the sediment surface. Surface deposit feeders, many of which occupy U-shaped burrows, increase diffusive exchanges between sediments and the water column and reoxygenate sediment pore water. Like deposit feeders, suspension feeders similarly influence organic matter processing by bioturbation and ventilation; however, they also increase organic matter inputs to the sediments, with rates estimated to range from 1 to 70 g C m^{-2} d^{-1} (Graf and Rosenberg, 1997). Introduction of labile organic matter and electron acceptors to deeper sediment strata stimulates decomposition of ''refractory'' organic matter by surface deposit feeders, perhaps due to introduction of electron acceptors or by increasing bacterial co–metabolism (Graf, 1992). Depending upon the relative importance of organic matter inputs versus mixing of electron acceptors to deeper sediment layers, benthic macrofauna influence the balance among dissolved inorganic and organic N species exchanged between sediments and the water column. For example, high organic matter inputs by suspension feeders and the resulting anoxia, may inhibit NTR, increasing fluxes of NH_4^+ out of the sediments (Caffrey *et al.*, 1993). When water column NO_3^- is available, high organic matter inputs may stimulate DNRA relative to DNF, again resulting in increased fluxes of NH_4^+ out of the sediments (Welsh, 2003).

Sediments underlying an oxic water column are dissected by a wide variety of tubes and burrows constructed by polychaetes, crustaceans, and bivalves. These macrofaunal structures are irrigated with oxic surface water and thereby influence the distribution of oxic–anoxic interfaces across 3-dimensional space and play an important role in regulating nitrogen and carbon mineralization rates. The volume of oxic burrow walls may be several-fold higher than that of oxic surface sediment (Kristensen, 2000). Linings of burrows and tubes are usually enriched in organic matter compared to surrounding sediments and have variable permeabilities, ranging from 10% to 40% of that in free solution. Kristensen (2000) modeled organic matter decomposition in sediments populated with the deposit feeding polychaete *Nereis diversicolor* and calculated an enhancement in mineralization of old and partially degraded organic matter of 47%. Others have measured increases in potential NTR rates related to duration of irrigation on burrow walls of an order or more in magnitude (Kristensen *et al.*, 1985; Mayer *et al.*, 1995). High rates of potential NTR have been associated directly with animal surfaces (Welsh, 2003), and increased rates of both coupled and uncoupled DNF have been observed in sediments bioturbated by the amphipod *Corophium volutator* (Pelegri *et al.*, 1994; Rysgaard *et al.*, 1995).

4.8. The role of benthic suspension feeders in reducing eutrophication potential

The increasing eutrophication observed in estuaries such as the Chesapeake Bay results not only from the bottom–up effect of increased nutrient loading, but also from the top–down effect of decreased abundance (100–fold decline over 150 years)

of the eastern oyster, *Crassostrea virginica* (Kemp *et al.*, 2005). Although oysters remove seston, thereby increasing light available to support benthic primary production, they also deposit feces and pseudofeces to the sediments, from which DIN may be regenerated. The effectiveness of *C. virginica* to impact water column primary production depends in part upon the fate of the DIN regenerated from their feces and pseudofeces. The fate of DIN regenerated from POM in the light and dark in aerobic and anaerobic sediments varies: in the dark DNF accounted for 17% (high POM loading) to 24% (low POM loading) of DIN removal; in the light benthic microalgae effectively intercepted and competed with denitrifiers for regenerated DIN and NFIX was an additional source of N to benthic microalgae (Newell *et al.*, 2002). The long-term fate of the N retained by the benthic microbial community, about which little is currently known, depends upon potential transformations to recalcitrant forms and burial in sediments and transfer to higher trophic levels.

Other benthic suspension feeders may have increased in abundance to occupy the niche formerly filled by *C. virginica*. For example, in certain areas of the lower Chesapeake Bay it has been reported that 35–100% of net plankton community production may be transferred from the pelagic to the benthic zone to support production of the polychaete, *Chaetopterus* cf. *variopedatus* and its tubes, given an ecological transfer efficiency of 10% (Thompson and Schaffner, 2001). Tube production by suspension feeders may be a mechanism by which nitrogen is transferred to and retained in sediments. Approximately 12% of the N flux to the benthos in central Long Island Sound, NY, reportedly is necessary to support tube production by the sea anemone *Ceriantheopsis americanus* (Kristensen *et al.*, 1991).

4.9. Transfer of benthic microalgal-derived carbon and nitrogen to higher trophic levels

Benthic microalgae serve as a base for the benthic infaunal food web where they provide particulate organic matter for sediment microfauna (<63 μm size class, mainly ciliate protozoans), meiofauna (63–500 μm size class, mainly nematodes, oligochaetes, and harpacticoid copepods), and macrofauna (>500 μm size class). Observations suggest that BMA, which are a rich source of proteins and lipids, are preferred by deposit feeders compared to macrophyte detritus (Miller *et al.*, 1996). Natural abundance multiple stable isotope studies similarly demonstrate the importance of BMA as a food resource to consumers in salt marsh sediments (Currin *et al.*, 1995). On the other hand Veuger *et al.* (2007a) noted that incorporation of ^{15}N-labeled microbial biomass into macrofauna in an intertidal sediment was negligible relative to the ^{15}N in total benthic biomass. In Korean coastal bay systems, Kang *et al.* (2003) observed that the natural abundance isotopic signatures of subtidal benthic deposit feeders reflected a diet almost exclusively of BMA, whereas the isotopic signature of suspension feeders suggested a diet made up approximately of an equal mixture of BMA and marine phytoplankton. BMA community production is regulated by the interactions between bottom–up (nutrients and light) and top–down controls (Hillebrand *et al.*, 2000; Worm *et al.*, 2000); for example, in intertidal sediments production is tightly linked to meiofaunal grazing. Grazing pressure and BMA community structure vary seasonally. In a study conducted at intertidal sites in

Terrebonne Bay estuary, Louisiana Pinckney *et al.*, (2003), observed high BMA biomass, dominated by diatoms, and low meiofaunal abundance and grazing (as a percentage of biomass) in January and low BMA biomass, dominated by cyanobacteria with high meiofaunal abundance and grazing in June. BMA production rates were not significantly different between seasons suggesting that grazing may increase BMA production by allowing increased light penetration and nutrient regeneration.

5. Methodologies Used for Measurements of Nitrogen Cycle Process Rates

It is widely recognized that measurements of most N-cycling process rates suffer from methodological limitations, as described below. Although techniques have been evolving rapidly during the last 10 years, difficulties remain. The ongoing development of molecular techniques capable of quantifying the abundance of specific genes involved in nitrogen transformations holds a great deal of promise (Dollhopf *et al.*, 2005; Wuchter *et al.*, 2006; Zehr *et al.*, 2001) yet more research is required to determine how gene copy number relates to process rates measured by more conventional techniques. What follows is a discussion of the more common biogeochemical techniques used to assess N-cycling process rates in sediments.

5.1. Nitrogen fixation

NFIX in surface sediments is most often measured using the acetylene (C_2H_2) reduction technique. Surface cores are incubated in sealed vials with a headspace containing 10–20% (v/v) C_2H_2 in both light and dark. Acetylene reduction activity (ARA) is determined by the rate of production of ethylene, which is measured on a gas chromatograph equipped with a flame ionization detector (Currin *et al.*, 1996). This technique can also be adapted for assessing ARA activity by heterotrophic N-fixers in the rhizosphere of marsh or seagrasses. McGlathery *et al.* (1998) removed pore water from intact seagrass-vegetated cores, equilibrated the pore water with C_2H_2, and reintroduced the pore water back into the core by vacuum. An advantage of the perfusion technique in intact vegetated cores is that it allows determination of the effects of photosynthate on NFIX. Whiting *et al.* (1986) estimated heterotrophic NFIX in saltmarsh sediments by perfusing C_2H_2 within *in situ* chambers. One caveat to consider when attempting to relate ARA to NFIX is that the conversion (moles of C_2H_2 reduced to ethylene vs N_2 fixed into NH_4^+ + DON) can vary across a wide range and often exceeds the theoretical 3:1 ratio used by many to estimate NFIX from ARA (Seitzinger and Garber, 1987). Thus, it is important to calibrate the ARA technique by measuring $^{15}N_2$ uptake into sediment extractable ammonium and labile organic N. More recently NFIX has been measured by using membrane inlet mass spectrometry (MIMS) in combination with the isotope pairing technique (An *et al.*, 2001), a modification of the method of Kana *et al.* (1994). This method allows simultaneous measurement of both DNF and NFIX by monitoring the relative fluxes of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ from sediments enriched with $^{15}NO_3^-$. A problem noted with the MIMS method is its sensitivity to bubble formation, limiting its usefulness in net autotrophic systems in the light (Eyre *et al.*, 2002).

5.2. Sediment nitrogen mineralization

Measuring NMIN in sediments is complicated by the multiple processes that can produce or remove NH_4^+ and the sensitivity of these processes to environmental conditions such as availability of oxygen and sulfide. Net mineralization is often estimated as the net flux of DIN out of sediments in the dark. This does not account for loss of N due to DNF. The net N flux can be compared to that predicted based on inorganic carbon release or dissolved oxygen uptake during sediment respiration (Giblin *et al.*, 1997; Hopkinson *et al.*, 2001). In some cases the difference between the predicted and measured N fluxes is attributed to DNF (see Section 5.4). In net autotrophic sediments, net NMIN is usually low relative to gross NMIN, especially in the light. Gross NMIN represents turnover of the sediment NH_4^+ pool and can be measured by the $^{15}NH_4^+$ isotope pool dilution technique as described first by Blackburn (1979) and modified by Jochem *et al.* (2004) and Anderson *et al.* (1997). Sediment cores injected with $^{15}NH_4^+$ are incubated under ambient conditions, extracted with 2 M KCl, and the NH_4^+ isolated by diffusion as described by Brooks *et al.* (1989). Both the concentration and atom % enrichment of the extracted NH_4^+ are measured, and the mineralization rates determined using a model similar to that derived by Blackburn (1979) and described by Wessel and Tietema (1992), which takes into account both changes in enrichment of the ^{15}N-labeled pool and the total concentration of that pool ($^{15}N + {}^{14}N$).

5.3. Nitrification

Because of the steep redox gradients in many sediments and the often rapid removal of the products of NTR by DNF and other dissimilatory processes, measurements of NTR remain problematic. NTR has been estimated in the presence and absence of specific inhibitors by the accumulation of NH_4^+ (Henriksen *et al.*, 1980; Caffrey *et al.*, 2003) or by dark $H^{14}CO_3^-$ uptake into SOM (Dore and Karl, 1996). The inhibitors used include nitrapyrin (N-serve), methyl fluoride, dimethyl ether, acetylene, and allylthiourea. Problems involved with these methods include nonspecificity of inhibitors and difficulty in determining the correct conversion factor for ^{14}C uptake to NH_4^+ oxidized, which has been observed to range from 8.3 to 42 mol NH_4^+ oxidized per mol C fixed. Dore and Karl (1996) used an independent chemical assay of nitrite oxidation to verify the conversion factor used. NTR has also been estimated by the accumulation of nitrite in the presence versus absence of chlorate, an inhibitor of nitrite oxidation (Belser and Mays, 1980). $^{15}NO_3^-$ isotope dilution techniques, similar to those used to measure NMIN (see Section 5.2) have been used to measure NTR (Anderson *et al.*, 1997; Risgaard-Petersen *et al.*, 1994); however, when DNRA rates are high, the added $^{15}NO_3^-$ may disappear faster than it is sufficiently diluted to accurately estimate NTR.

5.4. Denitrification

The product of DNF, dinitrogen (N_2), is a major component of the atmosphere, meaning that natural waters contain high concentrations of N_2 (≥ 300 μM) when in equilibrium with the atmosphere. This high background makes measuring the

production of N_2 via DNF (or ANAM) difficult because one must quantify a small change against a large background; this is particularly problematic when DNF rates are low (Cornwell *et al.*, 1999; Joye, 2002). There are several different ways to estimate DNF rates in coastal sediments. Here, direct methods will be used to refer to techniques that measure the production of N_2 under approximate *in situ* conditions. Indirect methods measure the production of an intermediate, usually nitrous oxide, or a metabolite (O_2) than can be used as a stoichiometric proxy for total N_2 production rates. Direct and indirect rates can reflect *in situ* or potential activity; potential activity reflects rates in the presence of additional NO_3^- or organic carbon substrate.

The most common direct approaches to measure DNF include gas chromatography (thermal conductivity detection, TCD; Seitzinger *et al.*, 1980; Nowicki, 1994) and MIMS (Kana *et al.*, 1994, 1998). Techniques based on GC-TCD often require degassing to reduce background N_2 concentrations (Seitzinger *et al.*, 1980) but use of novel separatory columns such as calcium chabazite can alleviate this problem and permit quantification of small amounts of N_2 against a high background (An and Joye, 2001). The MIMS approach is more advantageous than the GC-TCD approach because of its quickness, ease, and small sample requirement.

Indirect methods used to estimate DNF include the acetylene block method (Sørensen, 1978), metabolite stoichiometry (Dollar *et al.*, 1991), or stable isotope tracers (Nielsen, 1992). Acetylene (C_2H_2) blocks the terminal step of DNF, the conversion of N_2O to N_2, and the DNF rate is estimated by quantifying the production of N_2O on a gas chromatograph with an electron capture detector. Problems with the acetylene block technique include blockage of NTR (which means that rates of coupled NTR–DNF cannot be obtained), inefficacy at low NO_3^- concentration, and interference by H_2S. Sulfide appears to alleviate the acetylene block of nitrous oxide reductase and permit full reduction of N_2O to N_2.

Metabolite stoichiometry provides a relatively straight-forward approach for estimating DNF rates without quantifying concentrations of N_2 gas. Typically, whole sediment cores are incubated under *in situ* conditions and fluxes of oxygen, dissolved inorganic carbon, or dissolved inorganic phosphorus are used to predict fluxes of dissolved inorganic N based on elemental stoichiometry. The predicted N flux is compared to the observed inorganic nitrogen flux and the difference is a proxy for the net DNF rate (Joye *et al.*, 1996, Giblin *et al.*, 1997). The errors associated with this method are typically large because of the heterogeneity associated with benthic fluxes between replicate cores. Abiotic and biotic oxygen consumption can lead to overestimates of O_2–based DNF rates. Finally, fluxes of DON are not accounted for in benthic flux mass balances and can lead to errors in DNF estimates.

Stable nitrogen isotopic tracers provide another way to estimate DNF rates and are often used in concert with the direct approaches (described above) to better constrain rates of coupled NTR–DNF. Heavy (^{15}N-labeled) NH_4^+ or NO_3^- is added to samples in tracer quantities and the subsequent production of ^{15}N-labeled gases ([15]N:[14]N or [15]N:[15]N) is quantified (Nielsen, 1992). The main drawback of this approach is that pore water N pools may not reach isotopic equilibrium during the incubation, which complicates calculation of DNF rates.

5.5. Dissimilatory nitrate reduction to ammonium

Measuring rates of DNRA requires a ^{15}N tracer approach (Binnerup *et al.*, 1992; Koike and Hattori, 1978); otherwise it is impossible to distinguish NH_4^+ generated from organic matter mineralization from that derived from NO_3^-. Relative to DNF, DNRA rates are poorly constrained but DNRA activity can be comparable to DNF activity in coastal sediments (An and Gardner, 2002; Binnerup *et al.*, 1992; Jørgensen, 1989). The general approach for estimating DNRA is to add ^{15}N-NO_3^- and track the production of ^{15}N-NH_4^+ over time. Because ^{15}N-NH_4^+ can rapidly adsorb onto particles, it is important to quantify both dissolved and solid phase ^{15}N-NH_4^+ pools. Often, only the pore water or overlying water ^{15}N-NH_4^+ pool is quantified, which can lead to significant underestimates of DNRA rates. The isotopic signature of ^{15}N-NH_4^+ can be estimated by high performance liquid chromatography (Gardner *et al.*, 1995) or by isotope ratio mass spectrometry (Holmes *et al.*, 1998).

5.6. Anammox

As no specific inhibitor is currently available to block ANAM without blocking DNF (Jensen *et al.*, in press), quantifying rates of ANAM remains an analytical challenge. The generally accepted procedure is to use a combination of ^{15}N-based tracers to tease apart the relative importance of DNF and ANAM (Dalsgaard and Thamdrup, 2002; Risgaard-Petersen *et al.*, 2004a,b; Trimmer *et al.*, 2003). Typically ^{15}N-NO_2^-, ^{15}N-NH_4^+, and ^{15}N-NO_3^- are added to separate samples, which are subsequently incubated anaerobically. ANAM activity is evidenced by the production of mass $^{29}N_2$ (^{14}N:^{15}N) from any of the three substrates (^{15}N-NO_2^-, ^{15}N-NH_4^+, or ^{15}N-NO_3^- while DNF is evidenced by the production of $^{30}N_2$ (^{15}N:^{15}N) from either ^{15}N-NO_2^- or ^{15}N-NO_3^- (note that no DNF should result from ^{15}N-NH_4^+ (via coupled dentrification) if sediments are incubated anaerobically). A major complication related to quantifying ANAM rates is the contemporaneous occurrence of DNRA. DNRA converts ^{15}N-labeled N oxides to $^{15}NH_4^+$; if that $^{15}NH_4^+$ were consumed by ANAM, it would generate $^{30}N_2$ (^{15}N:^{15}N), which would be interpreted as resulting from DNF using standard methods (Porubsky *et al.*, 2008a). More methods development is required to quantify ANAM rates in the presence of DNF and DNRA.

6. FUTURE RESEARCH

Nitrogen cycling in sediments influences the global N cycle and hence global patterns of primary production in aquatic sediments. In this chapter, we have summarized the factors regulating sediment N cycling from chemical, physical, and biological perspectives, showing that in most cases, several processes control the concentrations and fate of N in sediments. While some aspects of the sediment N cycle have become more clear with each passing year, e.g., we know that sediments exposed to fluctuating oxic–anoxic conditions, such as those with active benthic primary producer communities, are characterized by dynamic diel variability in the rates and patterns of N processing; other issues require additional, more detailed study.

Given the increasing nutrient, particularly N, loads characteristic of most coastal watersheds, much research has focused on understanding the factors that regulate N removal from the system. However, the complexity of the sediment N cycle has resulted in many studies that focus on one process in isolation, such as DNF, rather than on linkages between competing processes. As noted earlier, the number of studies focusing on DNRA and ANAM pale in comparison to the number of studies focusing on DNF. Part of the reason for this bias toward DNF is that DNRA rates are more difficult to quantify than DNF and ANAM was only recently discovered. Now that it is clear that DNF, DNRA, and ANAM may co-occur in sediments, we need to understand the environmental triggers that select for one pathway at the expense of another. Thus, documenting and understanding the biotic, chemical, and physical factors that regulate the fate of NO_3^- by dissimilatory processes in sediments is deserving of more detailed study.

Furthermore, the role of factors, such as salinity, metabolic status, grazing, trophic transfer, and residence time on controlling the fate of N in coastal systems warrants additional study. While some studies have suggested that salinity influences rates of NTR and DNF, others have suggested that the availability of substrates, NH_4^+ or NO_3^-, is more important. Clearly, sediments with productive benthic autotrophic communities exhibit different patterns of N processing than do sediments that are net heterotrophic. The role that meiofauna and macrofauna play in determining the fate of nitrogen taken up by benthic microalgae and sediment microbes remains unclear. A better understanding of the effect of ephemeral changes in metabolic status on N processing is needed. Finally, most of the data used to develop residence time–DNF relationships utilized methods that ignored the impact of benthic primary producers on DNF. More work is needed to quantify DNF rates in the presence of active benthic primary producers to determine how primary production alters N removal via DNA (or ANAM) at the system level.

An ideal way to understand the detailed controls on N processing within ecosystems is to employ *in situ* ^{15}N tracer experiments, such as those conducted by Tobias *et al.* (2003a,b) in two Massachusetts estuaries and by Veuger *et al.* in intertidal sediments (2007a). Such studies provide insight into the biotic, chemical, and physical factors regulating N flow and also permit tracking of the tracer into the benthic and pelagic food webs. For example, pulse–chase experiments performed by Veuger *et al.* (2007a, b) in intertidal sediments followed by determinations of incorporation of ^{13}C and ^{15}N into bacterial specific biomarkers such as D-alanine have greatly clarified the role of the benthic microbial community in sequestering and transforming nitrogen and hold great promise for elucidating the fate of nitrogen in sediments (Veuger *et al.*, 2005). These integrative studies require collaboration of a number of investigators with diverse backgrounds and can be expensive but the scientific payoff justifies the effort.

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MACROALGAL-DOMINATED ECOSYSTEMS

Peggy Fong

Contents

1. INTRODUCTION AND OBJECTIVES

The objective of this chapter is to summarize and synthesize what is known about the role of nitrogen (N) in forming the structure and defining the function of macroalgal–dominated marine ecosystems. Specifically, marine macroalgae will first be broadly defined and a perspective given on its global importance relative to other primary producers in the ocean. Second, I will describe community characteristics of the four major habitat types that may be dominated by macroalgae: (1) soft–sediment areas such as bays, estuaries, and lagoons; (2) coral reefs; (3) rocky subtidal zones; and (4) the rocky intertidal. For each, what is known about the role of changing supplies of N—both natural and anthropogenic—and the increasing frequency of phase shifts will be summarized. Third, evidence for and current approaches to determining the importance of N limitation across these systems will be reviewed and areas for methodological improvement identified. Fourth, what is known about the role of macroalgae in the N cycle will be discussed, including the relative importance of

various sources of N across macroalgal-dominated habitats, and the role macroalgae play in retention, cycling, turnover, and export of N at the local scale. Fifth, exciting new approaches to using macroalgae as indicators of N sources and supply rates will be reviewed; this is especially timely as N supplies to coastal marine systems will continue to increase in the future. Finally, I hope to identify knowledge gaps and suggest directions for future research.

2. Diversity and Productivity of Marine Macroalgae

Marine macroalgae form productive and highly diverse ecosystems along continental shelves worldwide. Members of this functional rather than phylogenetic group are from two Kingdoms and span at least four major divisions (Rhodophyta, Phaeophyta, Chlorophyta, and Cyanophyta) with worldwide distribution. Structurally, macroalgae include such diverse forms as simple chains of prokaryotic cells, single-celled yet multinucleate thalli over a meter in length, and giant kelps over 45 m in length with complex internal structures analogous to vascular plants. The overall percentage of total oceanic net primary productivity attributed to macroalgae ranges from 4.8% to 5.9% (calculated from Duarte and Cebrian, 1996). Although relatively low compared to oceanic (81.1%) and coastal (8.5%) phytoplankton productivity, on a global basis macroalgae ranks third most productive. However, local macroalgal productivity is comparable to some of the most productive terrestrial ecosystems such as tropical forests and varies greatly across the macroalgal-dominated systems discussed below (Mann, 1982).

Mechanisms that control net production of marine macroalgae are the same as for other primary producers: Geographic limits for growth are set by temperature and light and for removal by grazing and physical disturbance. Within these geographical limits, biomass accumulation is controlled by many interacting biotic and abiotic factors including light quantity and quality, water motion, intra- and interspecific competition, herbivory, and physical disturbance. However, in most places where macroalgae proliferate and dominate coastal communities, this dominance is a function of nutrient, usually nitrogen (N), availability (for reviews see Downing *et al.*, 1999; Howarth and Marino, 2006; Valiela *et al.*, 1997; Vitousek *et al.*, 1997). Despite this importance, our knowledge about the role of N limitation in determining community structure and dynamics and the role of macroalgae in N cycling varies dramatically among macroalgal-dominated ecosystems.

3. Macroalgal-Dominated Communities: Nitrogen Supply Controls Community Characteristics

3.1. Bays, estuaries, and lagoons

Macroalgae are important members of the primary producer community in shallow soft-sediment systems worldwide where light penetrates to large areas of the benthos. In soft-sediment systems subject to low N-loading rates, macroalgae are
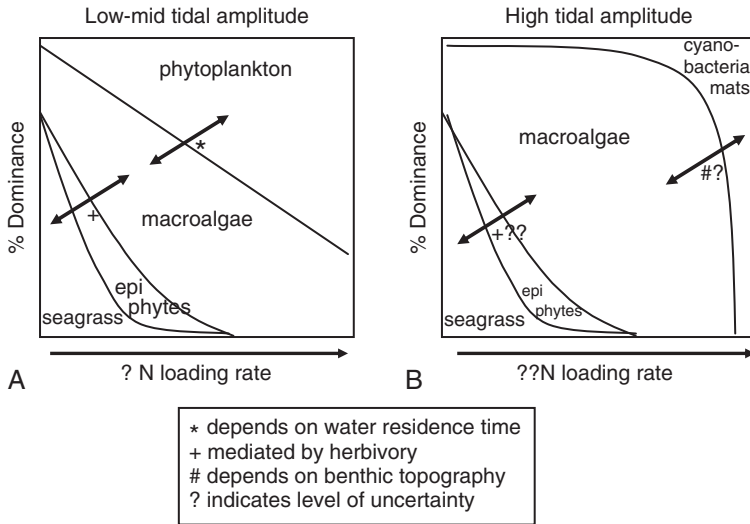
Figure 20.1 Conceptual model of the relationships among N-loading rate and the community composition of primary producers in bays, estuaries and lagoons. Adapted from Vaiiela *et al.* 1997.

an integral component of the seagrass community (Fig. 20.1), where they occur in relatively low abundance attached to the benthos, epiphytic on seagrass blades, or forming drifting mats. Within pristine tropical seagrass beds, calcareous green macroalgae are commonly attached to the benthos; calcification provides protection from most herbivores and ultimately contributes to the accumulation and stabilization of tropical sands (MacIntyre *et al.*, 2004). In both tropical and temperate seagrass beds, macro- and microalgae attach epiphytically to seagrass blades. Although even low abundances of epiphytes can have negative effects on seagrasses due to shading and interference with gas and nutrient exchange, in seagrass systems with low-nutrient loading and intact herbivore populations epiphyte biomass accumulation is modest. In low N systems, drift macroalgae are also present in low abundance, but are ecologically important as they may provide protection from predation and aid in dispersal of invertebrates and fishes (Holmquist, 1994; Salovius *et al.*, 2005). However, as biomass of drift macroalgae increases with N-loading rate (Fig. 20.1), effects on diversity and abundance of fauna associated with the benthos become negative (Norkko *et al.*, 2004).

There is empirical, experimental, and theoretical evidence that as N loading to shallow semi-enclosed coastal systems from developing watersheds increases, sea-grasses are replaced by blooms of opportunistic macroalgae (Fig. 20.1A; for reviews see Duarte, 1995; Valiela *et al.*, 1997). It is well documented that blooms of green or red macroalgae dominate many shallow estuaries and lagoons worldwide that are subject to N enrichment (e.g., Geertz-Hansen *et al.*, 1993; Hauxwell *et al.*, 1998; Hernández *et al.*, 1997; Kamer *et al.*, 2001; Marcomini *et al.*, 1995; Page *et al.*, 1995; Peckol *et al.*, 1994; Raffaelli *et al.*, 1989; Sfriso *et al.*, 1987, 1992; Valiela *et al.*, 1992, 1997). One mechanism driving this phase shift is increased growth and biomass accumulation of drift macroalgae with N enrichment (e.g., Hauxwell *et al.*, 2000;

McGlathery, 2001). These large floating or stationary mats smother seagrasses and lead to local declines in the diversity and abundance of benthic infauna (e.g., Cummins *et al.*, 2004). As the frequency, magnitude, and duration of nuisance blooms of macroalgae increase with rising N supplies, macroalgal blooms eventually replace seagrasses entirely. While this seagrass-to-macroalgal phase shift is well documented elsewhere, there is only anecdotal evidence of former seagrass dominance along the West Coast of the United States (Fig. 20.1B). Any losses that may have occurred predate scientific documentation in the southern California region and only a few estuaries now support remnant seagrass populations (Stewart, 1991); all have either continuous or episodic blooms of macroalgae (Kennison *et al.*, 2003). Thus, these systems deserve further study as potential models representing future stages in the estuarine degradation process.

There is mounting evidence that another phase shift may occur if N loading continues to rise in systems presently dominated by macroalgal blooms (Fig. 20.1). The driving mechanism is self-shading of macroalgal mats once they reach a critical density (Krause-Jensen *et al.*, 1999; Peckol *et al.*, 1994); decomposition of these mats leads to release of N to the water for use by other producers. In estuaries with low to moderate tidal amplitude, macroalgae are replaced by blooms of phytoplankon (Duarte, 1995; Valiela *et al.*, 1997), and this transition is thought to be dependent on water residence time (Valiela *et al.*, 2000). Results of the only *in situ* enrichment experiment on the West Coast of the United States suggest that this final ecosystem transition along a eutrophication gradient will be to benthic cyanobacterial mats (Armitage and Fong, 2004) rather than to phytoplankton. We hypothesize that this transition may be attributed to the higher tidal amplitudes and existence of broad areas of intertidal mudflats in these systems. These mats of cyanobacteria are unpalatable or may even be toxic to the dominant herbivores (Armitage and Fong, 2004).

Because coastal bays, estuaries, and lagoons are of immense economic and social importance, the effects of increased anthropogenic N loading are perhaps the best understood of all macroalgal-dominated ecosystems. These systems have been subjected to increased frequencies and magnitudes of harmful macroalgal blooms, resulting in phase shifts from seagrasses to macroalgae and changing food webs, energy flow, and N cycling. Ecosystem-process level impacts of blooms include decreases in biodiversity and disruption of biogeochemical cycling (Valiela *et al.*, 1997). Algal blooms reduce diversity in estuaries by depleting the water column and sediments of oxygen (Sfriso *et al.*, 1987; Valiela *et al.*, 1992; Young *et al.*, 1998). Low dissolved oxygen content in estuarine water results in fish (Coon, 1998) and invertebrate mortality and subsequent changes in both trophic and community structure (Bolam *et al.*, 2000; Raffaelli *et al.*, 1989, 1991). Effects on biogeochemical cycling include reduction in N retention, more rapid N regeneration, and uncoupling of benthic and water column N cycling in these systems (e.g., Valiela *et al.*, 1997, and see section 5).

3.2. Coral reefs

Coral reefs are productive, diverse, and economically important ecosystems that dominate hard-bottomed habitats in low-nutrient tropical and subtropical waters. On pristine coral reefs, it is widely accepted that fleshy macroalgae are rarely spatially
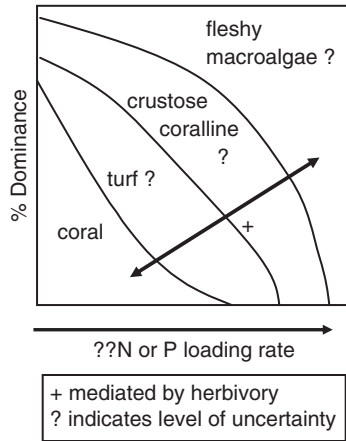
Figure 20.2 Relationships between N-loading rates and phase shifts among primary producers in coral reef communities.

dominant (Littler and Littler, 1984); rather, tropical reefs in low-nutrient waters are dominated by encrusting and turf-forming algae and dominance is maintained by high levels of herbivory (Fig. 20.2). These algae form the base of benthic food chains, contribute to biodiversity, and stabilize reef framework. Crustose coralline algae play an important role in reef accretion, cementation, and stabilization (Littler et al., 1995). Algal turfs, comprised of filamentous algae and cropped bases of larger forms, are ubiquitous throughout tropical reef ecosystems with high rates of primary productivity. Fleshy macroalgae have been documented to proliferate on reefs, especially those with chemical defenses, or in areas where there is overfishing of herbivorous fishes (Hughes et al., 2003; Jackson et al., 2001), in other spatial or temporal refuges from herbivory (Hay, 1984; Smith, 2005), and in areas subject to increased N loading.

Coral reefs are undergoing phase shifts to macroalgal domination on a global scale. The mechanisms causing these transitions are hotly debated, with the relative importance of nutrients and herbivory at the center (reviewed in McCook, 1999; Szmant, 2002). Some question whether algae in tropical systems are usually, or ever, limited by nutrients. One rationale advanced is that if tropical algal turfs are extremely productive even in areas characterized by low water column nutrients (Hatcher, 1988; McCook, 1999), then they cannot be nutrient limited. To support this, Williams and Carpenter (1998) provided evidence that supply of solutes to algal turfs may be more limited by boundary layers than concentration. A second rationale focuses on field studies where water nutrient concentrations do not correlate with algal growth or abundance in the field (e.g., McCook, 1999).

Microcosm and in situ field experimental studies of nutrient limitation of tropical algae have had conflicting results, adding fuel to this controversy. Across many systems and scales, effects of nutrient additions have varied from no effects (Delgado et al., 1996; Koop et al., 2001; Larkum and Koop, 1997) to orders of magnitude differences in effects on photosynthesis, growth, and biomass accumulation

(Lapointe, 1987, 1989; Lapointe et al., 1987; Schaffelke and Klumpp, 1997, 1998). Interpretation of experimental results is limited, in part, by the difficulty of relating results of laboratory or microcosm studies of the effects of nutrient addition to natural growth in high-energy, high-flow environments with variable nutrient supply typical of coral reefs (Fong et al., 2006), and the related methodological challenge of effectively conducting in situ experiments in these same environments (reviewed in McCook, 1999). Factors potentially confounding experimental microcosm approaches include, among others, the lack of recognition of the role of nutrient history and status of the experimental macroalgae (Fong et al., 2003a). Many experiments are conducted without setting them in the context of the natural spatial and temporal variability inherent in the environment. On the other hand, several processes that are difficult to control in the field may confound in situ enrichment experiments. On the local scale, preferential selection for enriched, fast-growing algae by herbivores may effectively mask the effects of enrichment in small-scale in situ experiments (Boyer et al., 2004; Fong et al., 2006). In addition, sources of nutrients in tropical systems may not be solely from the water column but may flux from sediments (e.g., Stimpson and Larned, 2000) and therefore not be included in traditional supply estimates. Because of these complex processes and many possible confounding factors, the importance of nutrient limitation for tropical algae is not well understood.

There is some indication that phase shifts may be attributed to strong interactions between the forces of nutrients and herbivory (Fig. 20.2). Increasing supplies of N and possibly P (see section 4) may stimulate productivity; however, at low to intermediate nutrient supplies this may not translate into increased algal biomass in the presence of intact herbivore populations (e.g., Hughes et al., 2003; Jackson et al., 2001; Pandolfi et al., 2003). Rather herbivores strongly mediate the position of the phase shift along the nutrient supply gradient, virtually acting as lawnmowers, limiting algal biomass accumulation and negating the impact of nutrients. This maintains the competitive dominance of slower growing corals. Once herbivores are removed, algae become competitively dominant and a phase shift from coral to algal dominance occurs. Others argue that reduced herbivory is not always needed to initiate a phase shift attributable to altered outcomes of competition. One alternative mechanism is for increased nutrient supplies to stimulate rapid growth and allow algae to escape control by herbivores, biomass to accumulate, a change in competitive dominance from coral to algae, and ultimately a phase shift from coral to algae (e.g., Lapointe, 1997). The only resolution to this controversy is to focus more research on the interactive effects of consumers and nutrients on relative abundance of coral and macroalgae in this important ecosystem.

3.3. Rock reefs

Rocky subtidal reefs support dense, diverse, and highly productive kelp forests that are constrained by light at high latitudes and by nutrients, warm temperature, and other macrophytes at low latitudes (for a review see Steneck et al., 2002). Within ~40–60° latitude, kelp forests dominate rocky substratum; dominance extends

toward the equator where gyres bring cold–water currents along coasts and where upwelling occurs. Kelp are foundation species or "eco-engineers," creating one of the largest biogenic structures found in any benthic marine community (Dayton, 1985). In southern California alone, kelp forests support over 200 species of algae, invertebrates, fishes, and marine mammals, many of which are commercially impor- tant (Graham, 2004). Kelp forest inhabitants are linked tightly to kelp forests via both trophic and habitat associations. Kelp forests are characterized by extremely high rates of primary productivity, comprise the base of a complex food web, form a three-dimensional structure that provides physical shelter for many inhabitants, have large algal standing stocks within forests, and export substantial biomass as drift algae that support other coastal ecosystems (Graham *et al.*, 2003; Steneck *et al.*, 2002). Kelp have high N requirements to support rapid growth. A long life span (max = 25 years (Steneck *et al.*, 2002)) coupled with tremendous biomass accumulation results in relatively slow turnover and recycling of N. Thus, kelp forests contain a very large and relatively stable reservoir of N compared to other algal dominated coastal systems.

N availability controls the large–scale biogeography of kelp (for a review, see Witman and Dayton, 2001), limiting distribution to cold regions and upwelling zones with high concentrations and supplies of inorganic N (Fig. 20.3). When temperatures remain high, as during an El Niño Southern Oscillation event when upwelling is suppressed in southern California, the combination of high respiration and N limitation causes the distribution, abundance, and size of kelp to decline (Dayton *et al.*, 1999). Large reserves of N in kelp tissues provide a mechanism of tolerance for high temperature, and may aid in surviving warm summers and cold winters in geographic margins (Gerard, 1997; Hernandez–Carmona *et al.*, 2001; Pueschel and Korb, 2001).

Outside of major climactic events and away from geographic boundaries, how- ever, N supply in kelp forests may be relatively less important in shaping community
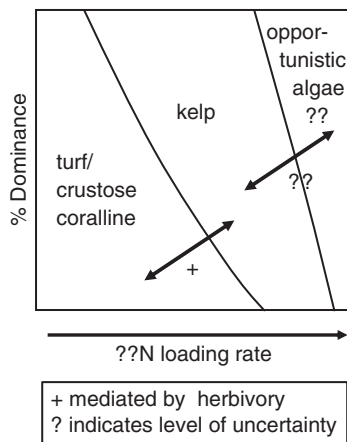
Figure 20.3 Relationships between N–loading rates and phase shifts in rocky subtidal habitats.

structure than other ecosystem processes. Witman and Dayton (2001) suggested that in N-rich areas, such as areas with predictable upwelling, N limitation may be relieved and the importance of biotic interactions may increase. Steneck *et al.* (2002) stated that within biogeographic boundaries, kelp distribution and abundance is most often limited by herbivory by abundant sea urchins (Fig. 20.3), and that the relative importance of urchin herbivory is increasing globally due to overfishing of apex predators. Even low abundances of herbivores may be important in marginal habitats (Russell and Connell, 2005) as these habitats demonstrate strong interaction between top–down and bottom–up factors. Overall, however, these studies suggest that within the geographic boundaries of kelp, N limitation is less important than herbivory in controlling kelp abundance. It is likely that internal stores of N within kelp forests are so large that they provide a buffer against short-term fluctuation in N supply.

The relative importance of N loading in kelp forests may change with anthropogenic disturbance. Although difficult to predict, global warming may have severe impacts on kelp forests by dramatically decreasing N supplies via suppressed upwelling and increased stratification (for a review see Steneck *et al.*, 2002). Decreases in N loading may result in phase shifts as in other macroalgal-dominated communities (Fig. 20.3); in this case, decreases in N may cause transitions to turf and crustose coralline algae, and, as in coral reef systems, may be strongly mediated by herbivores. At present, there is little evidence that increased anthropogenic sources of N have had any impact on kelp forests on open coasts (Steneck *et al.*, 2002). However, we do not know if there are threshold effects of varying N supplies as there are for herbivore abundance, so future increases in bottom–up control should not be ruled out (Fig. 20.3).

3.4. Rocky intertidal

Rocky intertidal zones worldwide are dominated by macroalgae and sessile invertebrates. Considering their relatively small area, virtually a one-dimensional ring bordering the world's coasts, the amount of research conducted in these habitats is extremely disproportionate (Witman and Dayton, 2001), and therefore our knowledge of many aspects of their structure and functioning is more complete than for other systems (Menge and Branch, 2001). The rocky intertidal zone is characterized by environmental extremes (temperature, salinity, desiccation, nutrient supply), yet there are also strong biotic interactions determining community structure. Macroalgae play important roles as the dominant *in situ* producers, forming the base of local food webs. Macroalgae in rocky intertidal habitats are highly diverse and abundant, especially in temperate regions. There is some evidence to suggest that rocky intertidal zones in tropical regions such as the Pacific coast of Panama are controlled by the same processes as temperate systems (Lubchenko *et al.*, 1984); however, rocky intertidal systems in tropical regions are far less studied.

Compared to the plethora of research on biotic and abiotic interactions in rocky intertidal habitats, comparatively few studies examine the role of nutrients in controlling macroalgal productivity (Menge and Branch, 2001). Some recent work

is focused on the importance of nutrient supply, especially N, at both the local and regional scales (for a review see Menge and Branch, 2001). These studies stress the importance of ''subsidies'' of organic and inorganic N from adjacent ecosystems such as coastal upwelling, detritus from kelp forests, or guano from sea bird colonies, rather than terrestrial sources or *in situ* regeneration and recycling (but see Bracken, 2004). Although rarely studied, it is likely that nutrient subsidies are also important in some tropical intertidal zones such as on the windward side of Oahu, Hawaii, where dense aggregations of *Sargassum* and *Turbinaria* occur (personal observation). This productivity may be supported by high water flow rates creating a high nutrient supply despite low water column concentrations. A few investigators have begun to quantify the effects of N subsidies on some well-known biotic interactions, finding that species interactions were much stronger with subsidies, with resultant differences in community structure (e.g., Menge *et al.*, 2003).

Recent experimental evidence may be pointing toward an emerging paradigm common to other macroalgal-dominated systems, one of a series of phase shifts along a N–supply gradient mediated by herbivores (Fig. 20.4). Enrichment experiments on rocky shores have provided mixed results; in Oregon alone results ranged from no effect at the population level (Pfister and Van Alstyne, 2003) to dramatic changes in community measures of algal diversity, evenness and complexity of thallus form (Nielsen, 2003). One possible explanation may be that these experiments started at different points along the N–loading gradient, and that different initial states resulted in differing outcomes (Fig. 20.4). Worm *et al.* (2002) and Worm and Lotze (2006) provided support for this explanation through a series of experiments and meta–analyses replicated across nutrient supply gradients. They determined that nutrients always increased macroalgal abundance and consumers moderated that response. However, areas with low ambient nutrients responded to enrichment by increased algal thallus
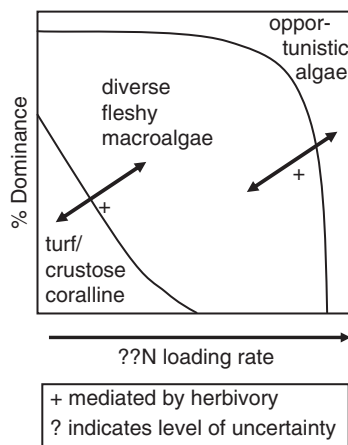
Figure 20.4 Relationships between N–loading rates and phase shifts in rocky intertidal habitats.

complexity and diversity (Fig. 20.4, phase shift 1), while more enriched areas already supporting more productive macroalgal assemblages responded with decreases in diversity due to a shift in dominance to opportunists (Fig. 20.4, phase shift 2). In addition, they found that, although grazing was important in controlling blooms of opportunistic macroalgae, it could not overcome the effects of increases in N loading across the whole supply gradient.

Overall, it is too soon to make generalizations on the relative importance of N-loading rate in controlling phase shifts among macroalgal communities in rocky intertidal zones compared to other biotic and abiotic processes that have been so well established. More work is needed to set the role of N in these systems into perspective.

4. Nitrogen Limitation of Marine Macroalgae: Evidence and Approaches

The importance of nutrients, especially N, in supporting coastal primary production has been firmly established (e.g., Downing et al., 1999; Howarth, 1988; Howarth and Marino, 2006; Nixon, 1995; Nixon et al., 1995; Vitousek et al., 1997). Overwhelming evidence has also accumulated that N is the most limiting nutrient to macroalgae in temperate regions (e.g., Lyngby et al., 1999; Taylor et al., 1995; Valiela et al., 1997). N limitation is also widespread in estuaries and lagoons with Mediterranean climates such as in Italy (Marcomini et al., 1995; Sfriso and Marcomini, 1997; Sfriso et al., 1987) and southern California (Kamer et al., 2004a,b). However, in Mediterranean climates there is some indication that the importance of N limitation may be seasonally variable and may relate to watershed development and algal type (Fong et al., 1993, 1994b; Lavery and McComb, 1991; Lavery et al., 1991).

For tropical marine macroalgae, quantifying the relative roles of N and phosphorus (P) limitation has been an important focus of research in recent years. In contrast to temperate systems (Fujita et al., 1989; Gallegos and Jordan, 1997; Rivers and Peckol, 1995; Sfriso and Marcomini, 1997; Taylor et al., 1995; Thybo-Christesen et al., 1993), some tropical studies found P to limit productivity and growth more frequently than N (Lapointe, 1987, 1989; Lapointe et al., 1992); however, even these studies found seasonal N limitation. Others found stimulation by both N and P (Lapointe, 1987; Schaffelke and Klumpp, 1998), or dual limitation (Fong et al., 2003a). There are several possible explanations why there is high spatial and temporal variability in the relative importance of N and P limitation in tropical systems. The strength of P limitation has been related to the amount of P-adsorbing carbonate in sediments (Delgado and Lapointe, 1994; Lapointe et al., 1992; McGlathery et al., 1994) and the habitat or substrate type the alga occupied (Lapointe, 1989; Littler et al., 1988). Others hypothesized that the relative importance of N and P limitation varied across a nutrient supply gradient, with N increasing in importance in more eutrophic systems due to saturation of the P adsorption capacity of sediments (Delgado and Lapointe, 1994; Downing et al.,

1999). Studies in Kaneohe Bay, Hawaii, an area with a history of nutrient enrichment, supported this hypothesis as N limited 9 of 10 species tested from a broad range of functional forms (Larned, 1998; Larned and Stimson, 1997). However, Fong et al. (2003a) found that several species of macroalgae in Puerto Rico differed in their response to N and P additions depending on their nutrient status; nutrient-replete algae did not respond strongly to either nutrient. Thus, more research is needed to further our understanding of the relative importance of N and P limitation in tropical marine ecosystems.

Two indirect approaches have been used extensively to determine if N and/or P limits productivity of marine macroalgae. In the first approach, N:P ratios of dissolved inorganic nutrients in the water column have been used as a measure of nutrient availability; water column N:P ratios are compared to nutrient requirements of algae to determine limitation (e.g., Duarte, 1992; Lapointe, 1989; Redfield et al., 1963; Wheeler and Björnsäter, 1992). However, different species or functional forms of algae may require nutrients in differing proportions; one study found that one alga was limited by N and another by P when grown together in seawater of the same N:P ratio (Fong et al., 1993). In addition, water column measures provide only a snapshot in time and may not adequately characterize availability in areas where nutrients are supplied in pulses as in the tropics (McCook, 1999) or in estuaries (Boyle et al., 2004; Fry et al., 2003). In the second approach, N:P ratios in algal tissue have been used to predict nutrient limitation (e.g., Lapointe et al., 1992; Wheeler and Björnsäter, 1992). However, this method also has limitations, because differing uptake and storage capacities of algae may confound the relationship. For example, if both N and P are abundant in the water, and an alga has a greater uptake ability and storage capacity for N than P, then the resultant high tissue N:P ratio would indicate P limitation when limitation by nutrients was not occurring. Sfriso (1995) suggested that either N or P could be considered limiting to a single algal species in Venice Lagoon, Italy, depending on what measure of limitation was used (tissue N content vs water column nutrients).

A third approach used to determine N or P limitation is factorial enrichment experiments adding N and P alone and in combination, and quantifying response variables such as photosynthesis, growth, and changes in tissue N and P content (e.g., Fong et al., 1993; Lapointe, 1987, 1989; Larned, 1998). When addition of a nutrient increased any of these response variables, it was considered to be limiting. This approach has been used across a range of scales, from laboratory or field microcosm dose–response experiments (e.g., Fong et al., 2003a) to large-scale and longer-term in situ experiments (Koop et al., 2001). The limitations include choice of realistic experimental enrichment levels, effectively scaling up the relatively short-term and small-scale responses from microcosm experiments, and effectively enriching in situ experiments in high-energy environments. The advantages, however, are that the experimental approach provides direct rather than indirect evidence of limitation. Although at present the disadvantages of this approach require careful interpretation and application of experimental results, the strength provided by direct evidence warrants further research and development.

5. The Role of Marine Macroalgae in Nitrogen Retention, Cycling, Turnover, and Loss

5.1. Sources of nitrogen to macroalgae

The primary source of ''new'' N to macroalgal-dominated marine communities has long been identified as terrestrial inputs via rivers from coastal watersheds (e.g., Downing *et al.*, 1999; Howarth and Marino, 2006; Nixon *et al.*, 1995; Vitousek *et al.*, 1997), although upwelling has also been recognized to be important along portions of the west coasts of major continents (Duxbury, 1979; Menge and Branch, 2001; Smith *et al.*, 1996; Fong *et al.*, in preparation). While these sources are still considered of primary importance, there is an increasing focus on other sources, including nitrogen fixation (for a review see Howarth and Marino, 2006), groundwater, and aerial deposition (e.g., McClelland and Valiela, 1998; Page, 1995; Whitall and Paerl, 2001). With the exception of upwelling and N-fixation, all of these sources of new N to macroalgae are rapidly increasing as a result of anthropogenic alterations of the global N cycle (for a review see Vitousek *et al.*, 1997). The role of anthropogenic versus natural sources of N in stimulating macroalgal blooms has been well established in estuarine systems (Fig. 20.5); evidence of the same processes occurring in rocky intertidal zones is accumulating (Worm and Lotze, 2006). In coral reefs it is highly debated, and in kelp forests it has yet to be demonstrated (Steneck *et al.*, 2002). Overall, systems more closely associated with terrestrial watersheds and with restricted water exchange such as estuaries are thought to be subject to and affected the most by increasing anthropogenic N supplies compared to open oceanic systems such as kelp forests (Fig. 20.5).

In situ sources of N to macroalgae include recycling from other biota, and, in soft-bottomed systems, regeneration from the sediments (Fig. 20.5). Across all systems, biotic sources include regeneration of N from primary producers during decomposition. In estuaries, macroalgal blooms may be sustained by nutrient release of N from senescent or self-shaded portions of the blooms (Peckol *et al.*, 1994) or by recycling of N from nearby vascular plant communities such as seagrass beds (for a review see McGlathery *et al.*, 2004). Flocculent material, most likely at least partially of biotic origin, settling on the surfaces of algal thalli has been identified as a source of N to macroalgae in coral reefs (Schaffelke, 1999). Animals in coral reefs and kelp forests that live in close association with macroalgae may have positive effects by releasing nitrogenous waste products (Bracken, 2004; Hurd *et al.*, 1994; Williams and Carpenter, 1988).

Across macroalgal-dominated systems, recycling of N from sediments and biota is most likely greatest in estuaries and coral reefs (Fig. 20.5). It is well known that macroalgae intercept fluxes of N regenerated from estuary and lagoon sediments in temperate and Mediterranean regions (e.g., Fong and Zedler, 2000; McGlathery *et al.*, 1997; Tyler *et al.*, 2001, 2003), in some seasons meeting up to 100% of macroalgae N demand (Sundbäck *et al.*, 2003). In tropical systems, recycling from sediments is most likely only a significant contribution in enriched systems (Stimpson and Larned, 2000). The relative importance of sediment regeneration
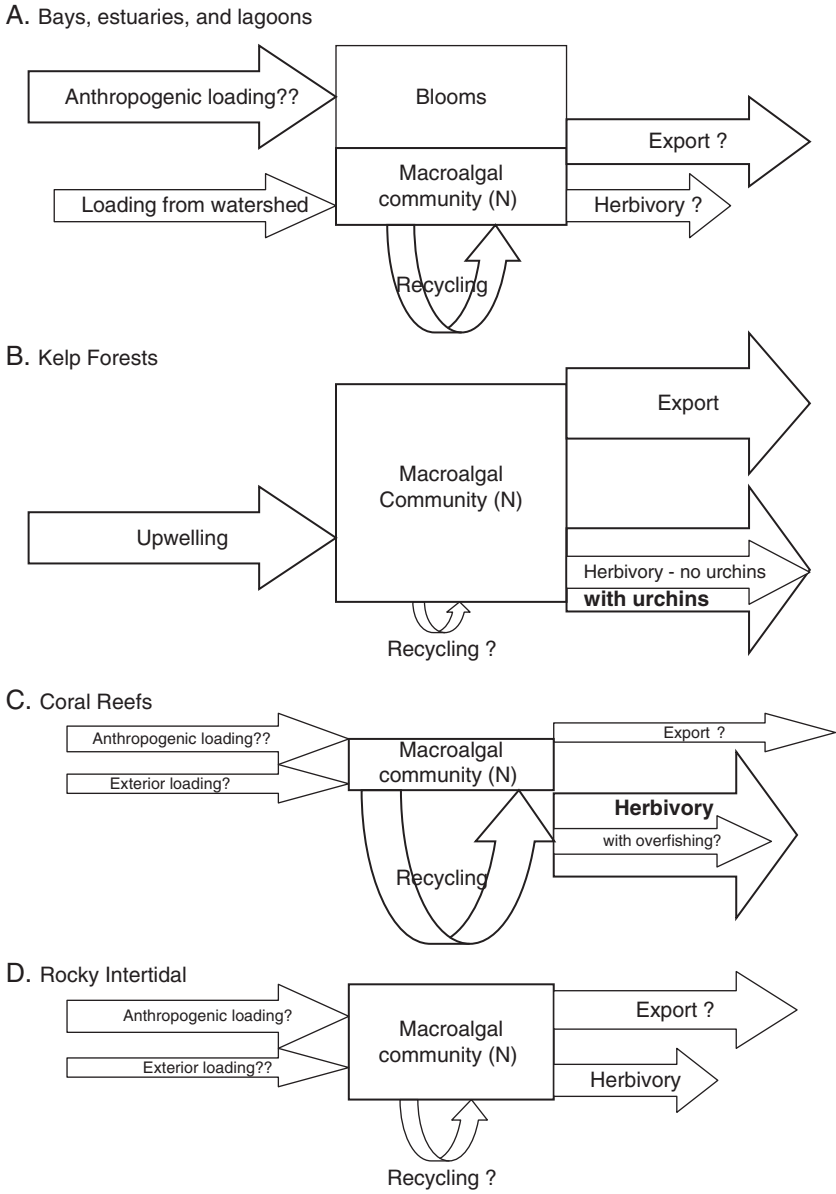
Figure 20.5 Sources, sinks and cycling of N in macroalgal dominated ecosystems. Width of arrows and size of boxes indicate relative magnitudes.

may vary along a N–resource gradient (Kamer *et al.*, 2004b) or may be a result of historical N enrichment (Stimpson and Larned, 2000). Macroalgal interception of N fluxes is ecologically important as macroalgae may uncouple sedimentary and water column N processes (Valiela *et al.*, 1992, 1997), reducing supplies to other producer groups (Fong and Zedler, 2000). Thus, preemptive competition for

recycled N by macroalgae may partially explain why some blooms dominate year-round (Kennison *et al.*, 2003).

5.2. Macroalgae as a sink of nutrients: nitrogen uptake

Predicting uptake rates of N by macroalgae from water column sources is complex. For years, N uptake was thought to be a simple function of water column inorganic N concentration that could be described by Michaelis–Menten uptake kinetics (e.g., Fong *et al.*, 1994b), an approach proven successful for phytoplankton (Droop, 1983; Sommer, 1991). More recently, the recognition that macroalgae are often both relatively stationary in a dynamic flow environment and have significant N storage capacities that reflect past nutrient supplies has focused current research into the physics of dissolved solute transport across boundary layers as well as the influence of the biological condition of algal thalli on uptake (for a review see Hurd, 2000). Both of these must be incorporated into our understanding of N availability to and uptake by macroalgae.

Uptake of N from the water column depends on many factors, including the nature and velocity of water flow, water nutrient concentrations of biologically useable substrates, and algal metabolic demand. The nature (turbulent vs laminar) and velocity of water flow affects the depth of the diffusion boundary layer surrounding an algal thallus while the nutrient flux across this boundary layer is controlled by the water nutrient concentration at the top and the metabolic demand which drives the rate of removal at the bottom (Hurd, 2000). If algal metabolic demand is low because storage is high or other factors are limiting, then mass transfer of nutrients across the diffusion boundary layer is not limiting. Theory predicts that mass transfer will be limiting most often when both flow and water column N concentrations are low. However, the importance of mass transfer limitation may be influenced by several factors, including other sources of nutrients such as the sediments or closely associated animals, the flexibility or flapping of algal thalli generated by water motion, and transient surge due to surface wave or tides (for a review see Hurd, 2000). In addition, for small filamentous or crustose algae completely within the benthic boundary layer, current speeds may be much reduced compared to those that extend into the overlying faster water flow (Carpenter and Williams, 1993; Nepf and Koch, 1999).

Algal morphology affects uptake of nutrients by controlling the accessibility to uptake sites on the algal thallus. As discussed above, morphology controls the algal height with respect to the benthic boundary layer and thallus structure may enhance uptake by flexibility in flowing water (Hurd, 2000). In addition some algal growth forms, such as densely packed mats, may reduce uptake (Fong *et al.*, 2001). Morphology also determines the physical surface area of an algal thallus containing uptake sites. Several studies have identified a positive relationship between an alga's surface area to volume ratio and uptake of nutrients (e.g., Hein *et al.*, 1995).

Once N crosses the boundary layer and contacts the macroalgal thallus surface, it must be transported across the cell membrane and then assimilated into organic compounds, followed by incorporation into proteins and macromolecules for

growth (Cohen and Fong, 2004b; McGlathery *et al.*, 1996). These processes vary among commonly co–occurring forms of N in coastal marine waters, including both inorganic forms (NH_4^+ and NO_3^-) considered most readily taken up by macro-algae. For example, some algae have strong preferences for NH_4 while others take up either inorganic N source (e.g., Hanisak, 1983; Lotze and Schramm, 2000; Naldi and Wheeler, 2002). Uptake of NH_4^+ is less energetically costly, as NO_3^- must first be reduced to NH_4^+ by nitrate reductase before assimilation (Hurd *et al.*, 1995); thus, energetics may explain preference for NH_4^+ by some macroalgae. However, NH_4^+ storage capacity may be limited due to toxicity (Haines and Wheeler, 1978; Lotze and Schramm, 2000; Waite and Mitchell, 1972), and therefore assimilation rate into inorganic molecules may limit maximum uptake rate of this form of N. Opportunistic macroalgae's ability to take up both forms of N simultaneously may be one mechanism that results in algal blooms (Cohen and Fong, 2004b; Thomas and Harrison, 1987).

Algal demand, a function of algal tissue nutrient status, also influences nutrient uptake rates of macroalgae. Tissue nutrient status reflects the history of nutrient supply to an alga as algae subject to excessive or pulses of nutrients may store nutrients for future growth (Fong *et al.*, 1994a,b; Lapointe and Duke, 1984; Wheeler and North, 1980). Several investigators (e.g., Fong *et al.*, 2003a; Fujita, 1985; McGlathery *et al.*, 1996) found that algae with nutrient enriched tissues always took up N more slowly than nutrient depleted algae. These studies demonstrate that algae with higher internal nutrient content will have lower metabolic demand and therefore slower N uptake rates.

Pedersen (1994) separated uptake of N into three phases. The first phase, surge uptake, is transiently enhanced nutrient uptake by nutrient-limited algae that may last only minutes to hours. Surge uptake has been documented in response to high-concentration pulses of N (500 µM N) for bloom species in southern California estuaries (Kennison *et al.*, in preparation) and tropical algae from nutrient-poor sites in Puerto Rico (Fong *et al.*, 2003a). Because water column nutrients in tropical systems are characteristically low, experimental nutrient pulses to macroalgae in these latter experiments were also relatively low ($\sim$20 µM N), yet the algal response was rapid and of equal magnitude to the bloom-forming species from temperate estuaries. This suggests that tropical macroalgae may be especially well adapted to take advantage of pulses of nutrients through surge uptake. During the second, internally controlled phase of uptake, the rate-limiting step is assimilation of N into organic compounds (Fujita *et al.*, 1988; Rees *et al.*, 1998). This occurs when external N is maintained at a relatively high concentration for enough time that storage pools within algal tissue begin to fill (Cohen and Fong, 2004b; Fujita *et al.*, 1988; Lotze and Schramm, 2000; McGlathery *et al.*, 1996). The third phase of uptake, externally controlled uptake, occurs at low substrate concentrations and is regulated by the rate of nutrient transport across the alga's surface (Pedersen, 1994). This is a function of mass transport to the thallus surface as well as diminishing water column nutrient concentration.

The complex and interacting factors and processes affecting N uptake rates of marine macroalgae result in a wide range of rates in the field, which may mask increasing N supplies to these systems. N uptake rates of estuarine macroalgae have

been well studied, as these systems have been long at risk from excessive N loading (Fig. 20.5A). In mesotrophic estuaries and lagoons still dominated by seagrasses, uptake by seagrass epiphytes may exceed that of seagrass despite low biomass levels (Dudley *et al.*, 2001). As algae have faster N uptake compared to seagrasses, phase shifts to algae may change N dynamics by increasing internal sequestering and by recycling pulses of nutrients to these systems (Valiela *et al.*, 1997). Overall, maximum values of N uptake have been found for bloom-forming green macroalgae in highly eutrophic estuaries and lagoons. The limited data available for kelp suggest uptake rates are also relatively high (Hurd *et al.*, 1996; Probyn and McQuaid, 1985), presumably to sustain the typically high levels of algal biomass (Fig. 20.5B). In coral reefs, algae respond quickly to even low levels of N enrichment by enhanced uptake rates (Fong *et al.*, 2001, 2003a), though rates most likely never approach those in systems characterized by high water column availability (Fig. 20.5C). Although little data are available, nutrient uptake rates of macroalgae from the rocky intertidal may rival those of kelp forests and estuaries, as many genera are shared among these habitats (Kim *et al.*, 2004). Thus, high N uptake in macroalgal-dominated systems may mask increasing supplies by maintaining low water column concentrations, the usual metric to assess increasing nutrient supplies. This has significant implications for managing water quality in coastal ecosystems.

5.3. Macroalgae as a sink of nutrients: nitrogen storage and retention

The capacity to store N, represented by standing stock or biomass, varies tremendously across macroalgal-dominated ecosystems. As an example consider the tremendous differences in biomass between one study of coral reef algae (e.g., 0.03–0.6 kg wet wt m^{-2} based on a wet:dry wt ratio of 10:1; Foster, 1987) and another in kelp forests (e.g., 3.5 kg wet wt m^{-2}; Manley and Dastoor, 1987). Although both are highly productive in terms of gross primary productivity, they clearly occupy different ends of a spectrum in terms of biomass accumulation and therefore N storage capacity. Although there are exceptions, in general opportunistic species with simple thallus forms such as those that dominate coral reef turfs often have low levels of biomass despite relatively rapid growth rates due to short life spans, susceptibility to removal by physical disturbance, and grazing by herbivores. In contrast, persisters such as kelp often have high standing stocks due to longevity of individual thalli, investment in structure to withstand physical disturbances, and chemical defenses as protection from herbivores. One exception to this generalization is blooms of opportunistic macroalgae in shallow estuaries and lagoons that can also reach very high biomass levels (Fig. 20.5). Estimates from these systems range from 1 to 10 kg wet wt m^{-2} (Kamer *et al.*, 2001; Maroli *et al.*, 1993). Blooms in these systems have been clearly linked to increased N loading (e.g., Boyle *et al.*, 2004; Valiela *et al.*, 1997).

Reservoirs of N stored in macroalgae often undergo cyclical and/or seasonal patterns. Kelp forests have lower standing stocks in stormy seasons, when there is ice, or in response to ENSO warming (Witman and Dayton, 2001). Intertidal macroalgae on rocky shores, especially in higher tidal zones, respond to interactions among monthly and seasonal changes in tidal amplitude, temperature, and light by changing

biomass (Menge and Branch, 2001). Even some coral reef algae demonstrate marked seasonal variation in biomass (e.g., Lirman and Biber, 2000). Macroalgae in lagoons and estuaries have dramatic seasonal cycles, usually in response to changes in nutrient supply, temperature, and light, especially in eutrophic systems where seasonal blooms occur (Kamer *et al.*, 2001). Valiela *et al.* (1997) found that the N stored in seasonal blooms of macroalgae in eutrophic estuarine systems can equal the total annual N-load.

Estimates of standing stocks or biomass alone may not be an adequate measure of N retention in macroalgal-dominated systems, as some macroalgae are able to store excess supplies of N that occur seasonally or episodically in pulses within tissues. This results in high spatial and temporal variability in tissue N content. For example, tissue N content of *Enteromorpha intestinalis* ranged from 1.6% to 4.2% N seasonally and 1.8% to 4.2% N spatially within a single season in Newport Bay, CA (Kamer *et al.*, 2001). The range of tissue N in *E. intestinalis* within estuaries increased with latitude along the California Coast (Fong *et al.*, in preparation) attributed to greater oceanic supplies from upwelling coupled with slower growth due to colder water temperatures. These patterns demonstrated that storage capacity for excess N in algal tissues and algal growth are interrelated. For example, opportunistic green macroalgae may store enough N to sustain high growth rates for about 10–14 days (Fong *et al.*, 1994a; McGlathery *et al.*, 1996) while kelp may be able to store enough N to support slower growth over a period of weeks to months. Arctic kelp stored considerable amounts of N (as NO_3, protein, and chlorophyll) that was used to maintain growth over 9 months of N starvation (Korb and Gerard, 2000; Pueschel and Korb, 2001). Thus, calculations of N storage across macroalgal-dominated systems must take into account standing stock, growth and removal processes, and tissue N content.

5.4. Macroalgae as a source of nitrogen: turnover rates

Turnover rates of N stored in algal biomass in macroalgal-dominated systems depend on rates of consumption by grazers, recycling due to death and decomposition, and export (Fig. 20.5). Macroalgal turnover rate can affect different processes and storage compartments of the N cycle, including rates of microbial transformations and sediment and water column pools. Overall, for systems dominated by opportunists that bloom, there is a more rapid turnover of N compared to systems dominated by persisters like kelp (e.g., Worm and Lotze, 2006).

On a global scale, direct consumption by grazers was estimated as 33.6% of total macroalgal net primary productivity, demonstrating the general importance of macroalgae as the base of gazing food webs in coastal ecosystems (Duarte and Cebrian, 1996). However, the importance of grazing varies greatly across ecosystems, depending, in part, on the abundance and nature of the resident herbivores and relative nutrient supply rates. While seagrass ecosystems with low N loading have historically been thought to support detritus-based food webs, recent evidence demonstrates macroalgae contribute significantly to a grazing-based web (e.g., Tomas *et al.*, 2005). In a shallow lagoon (Hog Island Bay, USA) where macroalgae proliferate, grazing snails may consume up to 88% of growth daily, functioning to

accelerate turnover of large amounts of N stored in tissue and enriching sediments (Fig. 20.5A; Giannotti and McGlathery, 2001). In contrast, in a highly eutrophic lagoon in California, snails release N bound in sediments, fueling macroalgal blooms (Fong et al., 1997). In sub-estuaries of Waquoit Bay, USA, subject to different N loading, the relative importance of top–down forces (grazing by herbivores) decreased as nutrient supplies increased (Hauxwell et al., 1998).

Evidence has begun to accumulate that this negative relationship between the relative strength of N supply and herbivory may be applicable across macroalgal-dominated systems (Valiela et al., 2000). For example, in rocky intertidal habitats, grazing is important in controlling algal blooms and maintaining diversity, but cannot overcome the effects of increased N loading across the whole nutrient gradient (Fig. 20.5D; Worm and Lotze, 2006). Also, on pristine, low-nutrient coral reefs, there are a multitude of large herbivorous fishes and sea urchins, and experimental and historical evidence abounds to show that herbivores consume a large proportion of macroalgal productivity (Fig. 20.5C; for a review see Jackson et al., 2001). The exception to this pattern is kelp forests, where nutrients are in high supply and grazing can also be so important that the abundance of herbivorous sea urchins determines whether an area supports a kelp forest or a ''barren'' dominated by grazer-resistant crustose calcareous algae (Fig. 20.5B; reviewed by Graham, 2004; Steneck et al., 2002). Clearly the generality of a negative top–down/bottom–up relationship warrants further research.

Death and subsequent decomposition of macroalgal detritus results in release and recycling of stored N (Fig. 20.5). Processing of N through detrital pathways comprises about a third of macroalgal net primary productivity globally (Duarte and Cebrian, 1996). However, like grazing, the relative importance of recycling varies across habitats. For example, high-energy zones like kelp forests recycle much less detritus within the system compared to lagoons, while the estimates for recycling within coral reef algae is very high (Duarte and Cebrian, 1996). When algae decompose, they release organic N to the water and, in soft-sediment systems, to the sediments (for a review see McGlathery et al., 2004). Recent studies demonstrated that substantial dissolved organic N also ''leaks'' from healthy thalli (Fong et al., 2003b; Tyler et al., 2003); healthy macroalgae in bloom phase may release 39% of gross primary productivity, including N, which is processed via detrital pathways. Some organic compounds in the water can be taken up directly or are quickly remineralized to inorganic forms that may fuel productivity of other primary producers. If burial in the sediment occurs, remineralization may be slower; thus, sediments may act as a slow release fertilizer and enhance algal productivity when external supplies are low (Boyle, 2002; Kamer et al., 2004b). N released from algae and stored in sediments may also be used by vascular plants (Boyer and Fong, 2005). Alternatively, sediment N may be denitrified, a net loss of N from marine ecosystems.

In shallow estuaries and lagoons subject to macroalgal blooms, the collapse of blooms and subsequent turnover of N can have major ecosystem level effects. Release of organic N may alter food webs toward heterotrophic bacteria pathways (Valiela et al., 1997), depleting water and sediments of oxygen. If severe, the result is die-off of resident fish and invertebrates. In addition, sediment anoxia may cause

direct release of N stored in sediments, a secondary or indirect effect on N cycling. Sediment anoxia may decrease denitrification in the sediment, or bring the denitrification zone (the anoxic–oxic interface) up into the decomposing mats on the surface (Krause-Jensen *et al.*, 1999; Trimmer *et al.*, 2000). Clearly blooms of macroalgae in shallow semi-enclosed systems cause dramatic changes in N processing in these systems.

N export from macroalgal-dominated habitats varies tremendously across eco-system types (Fig. 20.5). Duarte and Cebrian (1996) calculate a global average of 43.5% of macroalgal net primary production is exported; however, the range is perhaps the more important metric, varying from ∼0% to 85% across macroalgal–dominated habitats. Over all ecosystems, export is a function of standing stock, water motion, and algal morphology. Kelp forests export a far larger amount of N than coral reefs despite vigorous wave action in both systems due to their vastly larger standing stock. In contrast, pristine lagoonal systems export less N compared to kelp forests as there is both less physical disturbance and lower water exchange with other systems to detach and remove biomass. Thus, seagrasses in lagoons represent a large and relatively stable reservoir of N. In contrast, in eutrophic systems there is often accumulation of large floating algal rafts. In this case, phase shifts to macroalgae result in faster turnover and potentially more rapid net export of N to the ocean (Flindt *et al.*, 1997; Salomonsen *et al.*, 1999). Thus, phase shifts in lagoons and estuaries may have "downstream" effects on other nearshore macroalgal-dominated habitats such as coral reefs, kelp forests, and rocky intertidal zones.

6. MACROALGAE AS INDICATORS OF SOURCES AND MAGNITUDE OF NITROGEN SUPPLY

Because N loading to coastal macroalgal–dominated systems is rapidly accel-erating with resultant phase shifts, it is increasingly important to develop more accurate and affordable techniques to quantify the magnitude and sources of N available to primary producers (e.g., McClelland *et al.*, 1997). Water sampling can quantify N concentrations and species (NO_3^-, NH_4^+, DON), but can be costly as sampling must be spatially and temporally intensive in order to make representative conclusions (Fry *et al.*, 2003). This is especially true in macroalgal-dominated systems where nutrient concentrations have high temporal and spatial variability, such as estuaries and coral reefs (Fong *et al.*, 2001; Fry *et al.*, 2003; McGlathery *et al.*, 1996; Valiela *et al.*, 1992). In addition, changes in water quality parameters are often only measurable near points of discharge due to dilution, while effects may extend longer distances (Fong *et al.*, 2001; Jones *et al.*, 2001).

Macroalgae have been used successfully to identify changing N supplies to nearshore coastal areas (Costanzo *et al.*, 2000; Fong *et al.*, 1998, 2001; Horrocks *et al.*, 1995; Jones *et al.*, 2001; Lyngby, 1990). Macroalgal tissue N concentration reflects nutrient loading because N uptake and growth are often temporally decoupled, resulting in storage (Birch *et al.*, 1981; Hanisak, 1979; Martins *et al.*, 2001). Opportunistic green and red algae take up N very quickly (hours) in

proportion to changes in supply and retain the signal for up to 10 days (Fong *et al.*, 1998, 2001; Fujita, 1985; McGlathery *et al.*, 1996). Thus, tissue N content of macroalgae can be an effective bioindicator of the relative magnitude of N enrichment in coastal estuaries, lagoons, and nearshore open-coast ecosystems (Costanzo *et al.*, 2000; Fong *et al.*, 1998, 2001; Horrocks *et al.*, 1995; Lyngby *et al.*, 1999).

Stable isotopes of N (δ^{15}N) sequestered in algal tissue provide an integrative measure of relative supply of stable isotopes to a system and thus have been linked to ratios in nutrient sources (Costanzo *et al.*, 2000; Cohen and Fong, 2006). δ^{15}N values of algae, usually phytoplankton, have indicated not only whether N was from a terrestrial or a marine source (Page, 1995; Rogers, 1999; Van Dover *et al.*, 1992), but also whether the N came from fertilizer, processed sewage, soil, atmospheric sources, or groundwater (Heaton, 1986; Kendall, 1998; Page, 1995). However, these average δ^{15}N ratios can vary greatly depending on the type of N (NH_4^+ or NO_3^-), the origin of the material (e.g., inorganic vs animal waste fertilizer), and the amount of biogeochemical processing (denitrification, volatilization, mineralization, nitrification) (Kendall, 1998), making interpretation complex. Despite these limitations, sources of N have been identified based on numerical comparisons of isotopic ratios (Heaton, 1986; Kendall, 1998).

There are two important assumptions underlying the use of macroalgal tissue δ^{15}N as an indicator of nutrient sources that must be more fully investigated to validate the approach. The first is that δ^{15}N values of the water reflect δ^{15}N values that result from simple mixing of sources. However, there are complex interactions of source mixing and chemical and biological modification once the source N enters the system (Owens, 1985) that must be considered. One possible solution is the use of a suite of complementary indicators that may resolve multiple sources (Cohen and Fong, 2006). The second assumption is that macroalgae take up ^{15}N in proportion to availability in a predictable manner (Cifuentes *et al.*, 1989). Selection for ^{14}N versus ^{15}N atoms, or fractionation, has been documented for phytoplankton (Cifuentes *et al.*, 1989; Owens and Law, 1989). Theoretically, some level of fractionation will always occur in any alga since ^{15}N is heavier than ^{14}N and therefore kinetics predict differences in diffusion rates and processing of the two isotopes (Kendall, 1998). However, if fractionation over a range of natural conditions is predictable, macroalgal tissue δ^{15}N values may be used to indicate corresponding water column δ^{15}N values. Cohen and Fong (2005) found accumulation of ^{15}N in the macroalga *E. intestinalis* was predictable over a wide range of δ^{15}N values and N concentrations in microcosm experiments. In one experimental field study, *Ulva lactuca* converged on signature ratios adjusted for 3–4‰ fractionation within 2 days (Teichberg *et al.*, 2007). In addition, a limited number of field tests have used δ^{15}N in macroalgae to distinguish N sources to coastal areas (Cole *et al.*, 2005; Costanzo *et al.*, 2000; McClelland *et al.*, 1997; Cohen and Fong, 2006). While caution must be exercised when interpreting δ^{15}N data due to differential *in situ* processing of sources and fractionation effects, the relative importance of various N sources have been successfully identified in these studies using macroalgal tissue δ^{15}N ratios.

One recent study suggested that a suite of macroalgal indicators may be most effective in assessing N sources in coastal ecosystems subject to multiple sources

(Cohen and Fong, 2006). Tissue N concentration was used to quantify the magnitude of nutrient supply while $\delta^{15}N$ in conjunction with tissue potassium (K^+) concentration was used to differentiate N sources. K^+ functions as an osmolyte in some euryhaline macroalgae (Black and Weeks, 1972; Karsten *et al.*, 1991; Ritchie and Larkum, 1985a,b; Young *et al.*, 1987), and varies predictably with salinity across a wide range of natural salinity fluctuations (Cohen and Fong, 2004a). Thus, tissue $\delta^{15}N$ and K^+ concentration were used together as indicators to resolve complexity associated with multiple N sources that may be difficult to distinguish (Cohen and Fong, 2006). For example, within-estuary processes such as denitrification can lead to high $\delta^{15}N$ values in residual available N (Cifuentes *et al.*, 1989; Horrigan *et al.*, 1990) that may be similar to high $\delta^{15}N$ values usually associated with waste–derived N in groundwater (Fry *et al.*, 2003; Kendall, 1998). In this situation, high $\delta^{15}N$ values in association with low K^+ concentration could be used to distinguish between watershed sources and enrichment resulting from *in situ* processes (Cifuentes *et al.*, 1989). Further investigation into the use of a suite of macroalgal indicators to obtain simultaneous and integrated measures of nutrients and salinity for identifying N sources to estuaries is promising.

7. Knowledge Gaps and Future Directions

We have made tremendous progress over the last 20 years in advancing our understanding of the role of marine macroalgae in N processing and cycling in shallow seas and coastal ecosystems. Macroalgal-dominated ecosystems are the third most productive when considering global ocean productivity, and, when calculated on an areal basis, are the most productive habitats in the ocean. Macroalgae are important sinks, sources, and reservoirs of N within habitats, and often function as sources of N to other marine systems. The role of N in fueling productivity of macroalgae has been well established in some marine systems, especially semi-enclosed ecosystem such as lagoons, estuaries, and bays. In these systems, clear linkages have been established from these bottom–up processes through all levels of ecosystem functioning. Understanding is further advanced in these systems at least partially because their semi-enclosed nature makes experimental and modeling approaches more tractable. Most importantly, however, their proximity to and close relationship with terrestrial watersheds makes them most vulnerable to anthropogenic changes in N supply. Because these habitats are at risk from eutrophication due to excess N loading, with many already undergoing losses in diversity, changes in biogeochemical cycling, and even phase shifts, much effort has been expended in understanding N processes in these systems.

There are still many challenges to be met in the future. Clearly the progress made in semi-enclosed ecosystems must be continued, as these habitats are so gravely at risk from increasing anthropogenic sources of N. In addition, as lagoons and estuaries continue to degrade, their role in N retention and processing may also be compromised and effects may be translated "downstream" to open-coast habitats. Therefore, just as clearly, more study of nutrient supply and effects in other

macroalgal-dominated ecosystems such as coral reefs, rocky intertidal zones, and kelp forests is essential as N loading to the coastal ocean is most certainly going to increase. If the role of changes in N supply in controlling macroalgal productivity, abundance, and diversity remains understudied in some systems due to methodological complexity, or is overlooked due to possible threshold rather than continuous responses, these systems may be facing a very important threat in the future. We know that ''hysetersis'' or abrupt phase shifts occur in response to N enrichment in semi-enclosed systems such as estuaries and lagoons, where effects of N are predictably stronger due to greater retention times. Therefore it is very possible that increases in N loading may have to be larger or of longer duration in open–coast systems, but that the effects may ultimately take the same path. One approach to maximize future progress is more comparative work among the different macroalgal-dominated systems.

One important challenge to furthering our understanding of the role of N in open–coast macroalgal-dominated systems is to solve some very difficult methodological issues. As discussed earlier, it is difficult to scale up from microcosm experiments, yet equally challenging to effectively enrich *in situ* areas typified by high wave energy and water flow. Some promising approaches have emerged, including comparing similar areas with differing oceanography (e.g., upwelling vs downwelling, Menge *et al.*, 2003; Smith, 2005), enriching tidepools or tidally isolated coral micro atolls (Koop *et al.*, 2001; Nielsen, 2003), or taking advantage of ''natural'' experiments where N supply changes over space (Lapointe *et al.*, 2004) or time (Smith *et al.*, 1981). Others have successfully enriched experimental plots or transplanted macroalgae on coral reefs using slow release fertilizer (Fong *et al.*, 2006; Smith, 2005; and others).

Another challenge is to continue to investigate the relative importance of different N sources. Steps have been made toward understanding the importance of groundwater and aerial deposition. Sediment regeneration has been identified as important in soft-bottomed systems, and other recycled sources such as pulses of N from closely associated animal populations have begun to be investigated in other systems. This may be especially key in coral reef ecosystems that harbor a multitude of cryptic and/or territorial fauna. The traditional method of sampling the water column to identify these types of sources may not be an adequate tool as they may be extremely temporally and spatially patchy. Therefore, more research on macroalgal bioindicators of differing sources is essential.

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Nitrogen Cycling in Coral Reef Environments

Judith M. O'Neil *and* Douglas G. Capone

Contents

1. Introduction

Nitrogen cycling on coral reefs is a multifaceted process operating from the micro-scale of prokaryotes to ocean biogeochemistry cycles. Coral reefs are known for their high productivity despite low concentrations of ambient nutrients in the clear, well lit tropical and sub-tropical waters where they exist. Most of the productivity associated with coral reefs is benthic (Crossland, 1983; D'Elia and Wiebe, 1990; Webb *et al.*, 1975) and water column nutrient dynamics over the reef largely reflect net benthic metabolism. Most of the organic matter produced on the reef is recycled and retained in living organisms, or sediments within the reef system (Ayukai, 1995; Suzuki *et al.*, 1995).

Studying the changes in nutrient concentrations in waters before and after they flow over reefs was an approach first utilized to study coral reef nitrogen dynamics

(Sargent and Austin, 1949). These studies were followed by the landmark work of Odum and Odum (1955) and subsequently the Symbios project in Eniwetok Atoll, Marshall Islands in the Pacific, (Webb and Wiebe, 1975; Webb et al., 1975; Wiebe et al., 1975) which enabled a "big picture" idea of the net fluxes of nutrients cycling on reefs.

The results of these early experiments framed our view of coral reefs as areas of very high gross productivity, but low net productivity, which was hypothesized to be due to the efficient recycling of nutrients (D'Elia and Wiebe, 1990; Odum and Odum, 1955; Smith and Marsh, 1973). Research in the 1970s showed that reefs could be a source of nitrogen, particularly dissolved organic nitrogen (DON). Sources of nutrients to coral reef environments vary based on factors including proximity to the coast, ocean topography and latitude, amongst others. Whereas open ocean reefs can exist in extremely oligotrophic regions, reefs in fact exist along a gradient of nutrient regimes in coastal and mid-ocean regions (Szmant, 2002). Nitrogen sources include upwelling, lateral advective transport, coastal runoff, groundwater seepage, nitrogen fixation and in situ regeneration (D'Elia, 1988), as well as atmospheric inputs (Barile and Lapointe, 2005; Szmant, 2002) and localized guano inputs (Albert et al., 2005; Smith and Johnson, 1995). Wet deposition of inorganic nitrogen in episodic rainfall events is estimated to provide up to 20% of the "new" nitrogen to meet the metabolic demands of macroalgae on coral reefs in the Bahamas (Barile and Lapointe, 2005). Of primary interest in the last few decades is elucidating the anthropogenic components of groundwater seepage, coastal runoff and atmospheric deposition.

Understanding nitrogen cycling on coral reefs is paramount to understanding the "paradox of the coral reef": the anomalously high productivity in such low ambient nutrient waters (Szmant, 2002; Webb et al., 1975). Coral reefs have been described as "oases" in an otherwise oceanic desert (Hoegh-Guldberg, 1999). Another appropriate metaphor is perhaps that corals act as "cacti," effectively conserving precious limiting resources. The two most important underlying mechanisms to explain the coral reef paradox are: (1) the recycling of nutrients between algal symbionts and invertebrate hosts and (2) new nitrogen (Dugdale and Goering, 1967) introduced through nitrogen fixation by cyanobacteria and heterotrophic bacteria. The closely coupled nutrient and community dynamics that creates the circumstances for "something from nothing" (Hoegh-Guldberg, 1999) reveals a very complex interrelationship between habitat, hosts and symbionts with a true dependency between species (obligate mutualism).

How the species assemblages, and the delicate interchanges and balance among species may react to environmental changes is currently being tested as anthropogenic influences increase across the world's coral reef environments (Bellwood et al., 2004; Brodie, 1995; Hoegh-Guldberg, 1999, 2004a,b; Hughes et al., 2003; Knowlton, 2001; Pandolfi et al., 2003; Gardner 2003; Mora 2008). Increased concern over the fate of the world's coral reefs due to pronounced declines in the areal extent of coral reefs, their health and biodiversity, has been a central theme and impetus of much of the coral reef research carried out in recent decades (Birkeland, 1997, 2004). In addition to studying nutrient inputs, research has focused on the effect of other anthropogenic influences including over-fishing, coastal development and global climate change (Pew Oceans Commission, 2003).

1.1. New developments

Recent research on nitrogen cycling on coral reefs ranges from the further elucidation of the structural and functional properties of the reef that increase regeneration of nutrients leading to enhanced retention of nutrients within the reef system (Szmant, 2002), to the finer details underlying cycling of nutrients and the emerging importance of microbes interacting with the entire coral ''holobiont.'' In addition to the coral itself, this includes the zooxanthelleae, fungi, endolithic algae, >30 species of bacteria as well as the recently discovered coral-associated Archaea (Wegley et al., 2004). Oceanic Archaea have recently been found to play an important role in nitrification in the marine N cycle (see Chapter 5 by Ward, this volume).

Other new developments reveal that heterotrophic bacteria in the coral mucus layer (and other areas of the coral colony) may be critical to the health, nutrient cycling and resilience of the coral holobiont (Knowlton and Rohwer, 2003; Rohwer et al., 2002). The importance of both coral mucus (Wild et al., 2004a, 2005, 2008) and products from mass spawning (Wild et al., 2004b; Patten et al., 2008) as nutritional sources back into the reef system has also been documented. The significance of bacteria and viruses to sedimentary biogeochemical cycling have also been recognized (Hewson et al., 2007; Patten et al., 2008). These new insights all underscore the point that we have not yet discerned all the important nutrient controls and interactions in the reef ecosystem.

The fact that symbiotic associations on reefs are more complex than we have realized to date, is further demonstrated by work in the Caribbean using $\delta^{15}N$ values, which demonstrated that in some Montastrea cavernosa coral colonies nitrogen is obtained by zooxanthellae from cyanobacterial endosymbiotic nitrogen fixers. This ability appears to increase with depth, and dependency on heterotrophy. Greater depth also translates to less photosynthetically active zooxanthellae in the coral colonies and therefore less oxygen evolution which benefits the oxygen-sensitive diazotrophic symbionts (Lesser et al., 2007). This raises multiple questions about nutrient feedback loops between the coral host, symbiotic dinoflagellates and the cyanobacteria in terms of what role nitrogen ultimately plays as a "limiting and regulatory element" in these symbiotic associations (Lesser et al., 2007). This is just a small indication of how much more is yet to be unraveled in terms of the intricate mutualistic relationships involved in coral reef ecosystem functioning. It may prove to be that the symbiotic relationship between bacteria and corals, as well as other invertebrate hosts, is just as integral to the coral as a whole, as their relationship with symbiotic dinoflagellates (zooxanthellae).

Other new insights gained recently include the potential importance of atmospheric deposition, including the role of dust, as bearer of nutrients (Barile and Lapointe, 2005), as well as pathogens and metals (Garrison et al., 2003; Prospero et al., 2005). Another previously under-valued component in nutrient cycling in reef environments is the ''secret-garden'' of benthic microalgae (or microphytobenthos) which have emerged as major primary producers in coral reef systems and major mediators of nutrient flux at the sediment interface (Heil et al., 2004; MacIntyre et al., 1996; Werner et al., 2008). The one theme unifying all recent aspects of work on nutrient cycling on reefs is the incredible complexity of nutrient dynamics in these

systems. Recent results also underscore the need to further elaborate the details of these interactive dynamics, as we bring to bear the tools of molecular biology, remote sensing and geochemistry, as well as other technologies to these questions. By doing so, we may gain a better understanding of the diverse mutualistic and symbiotic relationships that are integral components in the coral reef nitrogen cycle.

2. NITROGEN CYCLE PROCESSES

Coral reefs are areas of intense nitrogen cycling and may contribute to the larger biogeochemical cycling of C and N in the ocean (Capone, 1996a,b; Crossland *et al.*, 1991; see also Carpenter and Capone, Chapter 4, this volume) (Fig. 21.1). Nitrogen fixation appears to satisfy much of the demand for new N in these systems and may provide a substantial input to the marine N cycle beyond the immediate reef area. High rates of nitrification also occur and likely contribute to N_2O fluxes to the atmosphere, although more research is needed in this area (Capone, 1996b). All of the major features of the marine N–cycle are mediated by biological, rather than chemical processes (Webb, 1981), which is in contrast, for instance, to phosphorus cycling (D'Elia, 1988). In addition to its role as a nutrient, because of its potential to be
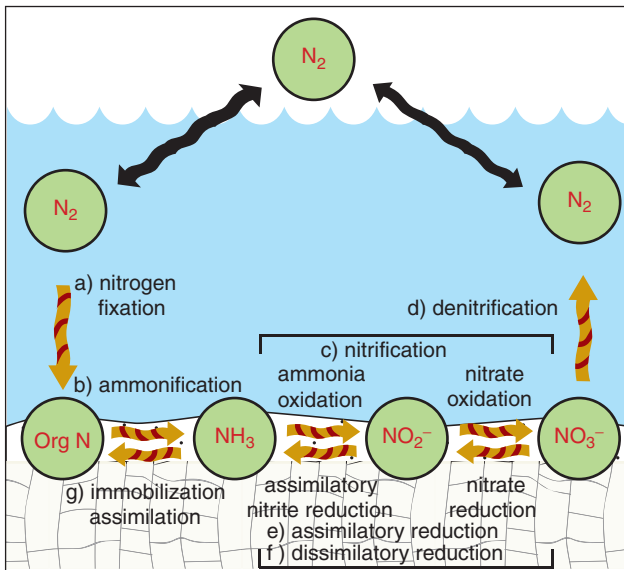
Figure 21.1 Microbial nitrogen cycling processes in sedimentary environments on a coral reef: (A) nitrogen fixation; (B) ammonification; (C) nitrification; (D) dissimilatory nitrate reduction and denitrification; (E) assimilatory nitrite/nitrate reduction; (F) ammonium immobilization and assimilation. Adapted from D'Elia and Wiebe (1990). Anammox (the anaerobic oxidation of NH_4^+ with NO_2 yielding N_2^-) is not represented, as it has not yet been shown to occur on coral reefs, but may be found to be important in reef sediments.

transformed among various oxidation states, species of nitrogen can act as a reductant or oxidant in energetic reactions (D'Elia and Wiebe, 1990). Aspects of the major pathways of nitrogen on coral reefs which include: nitrogen fixation (Fig. 21.1A); ammonification (Fig. 21.1B); nitrification (Fig. 21.1C); denitrification (Fig. 21.1D); uptake and regeneration (Fig. 21.1E and F), have been reviewed previously (D'Elia and Wiebe, 1990 and references therein), and are summarized in Fig. 21.1.Coral reef sediments can contain relatively high nitrogen concentrations with values generally 10-fold or greater than in the water column (Capone *et al.*, 1992; Corredor and Capone, 1985; Corredor and Morell, 1985; D'Elia and Wiebe, 1990; Hatcher and Frith, 1985; O'Neil and Capone, 1989).

Nitrogen occurs in a range of forms in the reef environment. Concentrations of nitrate and ammonium in reef environments vary by an order of magnitude in the water column ranging from ~0.05–0.5 μM. Nitrite is usually below 1 μM. DON is usually the form of nitrogen in highest concentration in the reef water column ranging typically from 5–50 μM (Atkinson and Falter, 2003; Crossland, 1983; Table 21.1). Nitrogen concentrations in porewater can be several orders of magnitude higher than the water column. Reef porewaters can be anoxic within a matter of cm in many cases and values of nitrate range from 1–6 μM, with ammonium concentrations ranging from 2–80 μM (Atkinson and Falter, 2003; Capone *et al.*, 1992), although higher values can be seen in altered systems (e.g., values up to 300 μM, see Corredor and Morrell, 1985; Entsch *et al.*, 1983; Hines *et al.*, 1992).

Since nitrogen concentrations in pore water are higher than in the water column, there is often a net flux of nutrients out of the sediments (Williams *et al.*, 1985). This may be of particular importance in areas of low flushing and pooling in reef areas (Szmant-Froelich, 1983; Szmant, 2002). However, these processes are also quite variable in space and time due to various factors including sediment type, hydrodynamics and loading (Corredor and Morrell, 1985).

In most marine ecosystems, the largest N fluxes occur between inorganic and organic pools of nitrogen. The net incorporation of inorganic nitrogen into organic nitrogen is generally attributed to photoautotrophs. Regenerative processes, including the production of NH_4^+, are usually associated with heterotrophic processes although depending on the C:N ratio of the organic matter being consumed, heterotrophs can also account for net N immobilization (Kirchman, 1994). For comparative purposes, areal rates of net phytoplankton productivity in oligotrophic waters typically account for 0.3–0.7 mol N m^{-2} year^{-1} (Berger *et al.*, 1987; DeVooys, 1979), whereas coral reef net production may account for areal demands of N in excess of 43 mol N m^{-2} year^{-1} (Capone, 1996a; Smith, 1984).

Regeneration within the sediments as well as a variety of sites within the coral reef structure itself are all extremely important (Capone, 1996a; DiSalvo, 1971; Szmant-Froelich, 1983). This efficient regeneration of nutrients fuels the intense productivity of the coral reef system and results in a tendency to export a smaller percentage of total production than other marine systems (D'Elia and Wiebe, 1990). Therefore, regeneration of N in coral reefs must meet demands (Charpy-Roubaud and Larkum, 2005).

Table 21.1 Nitrogen concentration in tropical coral reef sediments and overlying water columns

Nitrogen type	Range of conc (μM)	Typical conc (μM)	References
NO_3^- (sed)	1–6	<5	Atkinson and Falter (2003), Capone *et al.* (1992)
NO_3^- (water)	0.05–9.8	0.05–0.5	Crossland and Barnes, (1983), Crossland and Barnes, (1974), Falter and Sansone, (2000), Furnas *et al.* (1995), Johannes, *et al.* (1972), Johannes, *et al.* (1983), Kinzie, *et al.* (2001), Lapointe, (1997), Tribble *et al.* (1990), Webb, *et al.* (1975), Szmant and Forrester, (1996)
NH_4^+ (sed)	2.3–300 80	20–80	O'Neil and Capone, (1989), O'Neil and Capone, (1996)
NH_4^+ (water)	bd-2.4	0.05–0.5	Crossland and Barnes, (1983), Crossland and Barnes, (1974), Falter and Sansone, (2000), Furnas *et al.* (1995), Johannes *et al.* (1972), Johannes *et al.* (1983), Kinzie *et al.* (2001), Lapointe, (1997), Tribble *et al.* (1990), Webb *et al.* (1975), Szmant and Forrester, (1996)
DON (sed)	Na	na	na
DON (water)	bd-22	<5	Crossland and Barnes, (1983), Furnas *et al.* (1995), Johannes *et al.* (1972), Johannes *et al.* (1983), Kinzie *et al.* (2001), Lapointe, (1997), Webb *et al.* (1975), Alongi *et al.* (1996)

Source: Atkinson and Falter (2003).
bd = below detection limits. na = not available.

2.1. Nitrogen fixation

Nitrogen fixation (see also Chapter 4 by Carpenter and Capone, this volume), the process by which certain prokaryotes can reduce atmospheric N_2 gas to ammonium via the nitrogenase enzyme pathways has long been thought to be important linchpin of nitrogen cycling and productivity in coral reef systems (D'Elia and Wiebe, 1999; Larkum *et al.*, 1988 Fig. 21.1A). The first direct demonstration of the importance of nitrogen fixation in reef environments by cyanobacteria (originally hypothesized by Odum and Odum, 1955), was in the Symbios project (Crossland and Barnes, 1976; Hanson and Gundersen, 1977; Wiebe *et al.*, 1975). Indeed, nitrogen fixation has been subsequently posited as "*a prominent component of the nitrogen-cycle on coral reefs which may relieve N limitation and make a globally significant contribution to overall marine N inputs*" (Capone, 1996a).

Many studies since the 1970s found high rates of nitrogen fixation (see also Chapter 4 Capone and Carpenter, this volume). In fact, nitrogen fixation on coral reefs has been reported at rates that at times exceed that of intensive legumous crops (Szmant, 2002; Webb et al., 1975). This energetically demanding process in cyanobacteria is powered by the high light flux and recycled phosphorus. Heterotrophic diazotrophic activity is supported by a combination of recycled P and organic matter (D'Elia, 1988).

In addition to nitrogen fixation associated with the extensive sediments in and around reef flats (Capone et al., 1992; Corredor and Morrell, 1985; O'Neil and Capone, 1989; Wilkinson et al., 1984; Mayajima et al., 2001; Hewson and Fuhrman, 2006; Werner et al., 2008), nitrogen fixation activity has been detected associated with live corals (Williams et al., 1987; Lesser et al., 2007), and coral skeletons (Crossland and Barnes, 1976; Larkum, 1988; Shashar et al., 1994a,b; Davey et al., 2008) as well as with epiphytes on macroalgae (Capone et al., 1977; France et al., 1998; O'Neil and Capone, 1996) and by cyanobacterial mats. Nitrogenase activity has also been measured on limestone reef surfaces (Charpy-Roubaud et al., 2001), including exposed atoll rim areas of various sorts, largely attributable to cyanobacteria. In Tikehau Lagoon, French Polynesia, for instance, nitrogen fixation on these surfaces accounted for 25–28% of the total nitrogen demand for benthic primary productivity (Charpy-Roubaud et al., 2001; Charpy-Roubaud and Larkum, 2005). Rates of nitrogen fixation in sediments, while lower than rates in localized areas or in mats of cyanobacteria (Iizumi et al., 1990; Charpy et al., 2000; Bauer et al., 2008), when integrated over the large areal extent of sedimentary environments can make a significant contribution to the overall coral reef nitrogen economy (Capone, 1996a; Capone et al., 1992; O'Neil and Capone, 1989). Low $\delta^{15}N$ signatures noted in reef macrophytes and coral tissue provide further evidence that much of the nitrogen in reef systems is derived from nitrogen fixation (France et al., 1998; Yamamuro et al., 1995). Nitrogen fixation can also occur in the lagoon of coral atolls and may contribute to nitrogen economies in these systems.

Nitrogen fixation also occurs in the lagoonal waters of coral reefs and this may contribute to the nitrogen economy of these systems as well. Biegala and Raimbault (2008) recently reported on the relatively high densities of diazotrophic coccoid cyanobacteria in coral lagoon waters of New Caledonia.

Rates of nitrogen fixation in reef environments are variable and can be affected by the presences of grazers. Grazing by Acanther plancii, the crown of thorns starfish on corals resulted in high rates of nitrogen fixation on the coral skeletons after the outbreak of this starfish (Larkum, 1988). Similarly, the sea urchin Diadema antillarum was shown to significantly increase nitrogen fixation when grazing on "algal turf" in reef environments, as compared to areas where no sea urchins were present. The "diminutive" tightly cropped "algal turf" assemblage contained a significant proportion of cyanobacteria in addition to benthic diatoms and dinoflagellates (Williams and Carpenter, 1997). Grazers also aid in nitrogen cycling by excreting nitrogenous waste which enhances algal turf, while simultaneously cropping it (Williams and Carpenter, 1997 see below). Fish grazing may also be important in maintaining rates of nitrogen fixation on reefs, by keeping other benthic algae in check. Some diazotrophic cyanobacteria are less palatable than other algae, as they often have chemical deterrents, and also tend to be less nutritionally complete, lacking some essential fatty acids, and hence macroalgae are often preferred by grazers (Capper et al., 2005; O'Neil, 1999). Therefore, cyanobacteria may have less competition for growing space and expand into areas where the more palatable species have been removed by grazers (Wilkinson and Sammarco, 1983).

A major fraction of total benthic nitrogen fixation on a global scale may come from shallow coral reef environments (Capone, 1983). However, whereas rates of pelagic nitrogen fixation have been steadily refined by surveys of the filamentous cyanobacterium *Trichodesmium* (Capone *et al.*, 2005), novel geochemical approaches (Mahaffey, 2005), and the emergent recognition of other important contributors such as unicellular nitrogen fixers (Montoya *et al.*, 2004), nitrogen fixation in coral reef environments remains under-sampled and likely underestimated. Nitrogen fixation by benthic or symbiotic cyanobacteria (Charpy *et al.*, 2007; Lesser *et al.*, 2007; Bauer *et al.*, 2008; Davey *et al.*, 2008) as well as heterotrophic bacteria including those epiphytic on macroalgae and coral surfaces (Davey *et al.*, 2008), have yet to be fully elucidated (Koop *et al.*, 2001; O'Neil and Capone, 1989, 1996; Hewson *et al.*, 2007; Werner *et al.*, 2008). The diazotrophic community may in fact be changing as a consequence of coastal eutrophication. Nitrogen fixation could either decrease due to nitrogen loading, or increase in response to inputs of limiting factors such as phosphorus or micronutrients such as Fe, decreases in N:P loading ratios, or as a result of organic loadings and stimulation of heterotrophic N_2 fixation directly through provision of organic substrates or indirectly through the increase in local anoxic zones.

Black band disease (BBD), which is caused by a consortium of cyanobacteria and microbes may be diazotrophic (Frias-Lopez *et al.*, 2002, 2003). This hypothesis was recently suggested by a molecular study of bacterial communities associated with BBD which yielded a 16sRNA sequence 97% homologous with the nitrogen fixing pelagic cyanobacterium *Trichodesmium tenue*. This led the authors to speculate that nitrogen fixation might be carried out in the oxygen depleted microzones of the BBD assemblage (Frias-Lopez *et al.*, 2002). More work is needed to test this hypothesis in a rigorous manner as subsequent work on isolated *Phormidium corallyticum* (formerly *Oscillatoria corallinae*) from Florida corals with BBD tested negative for nitrogen fixation (acetylene reduction) activity (Richardson and Kuta, 2003).

Invertebrate diazotrophic symbioses have been reported from reef systems including reef sponges that acquire fixed nitrogen via associated cyanobacteria (Wilkinson and Fay, 1979). Symbiotic cyanobacteria and bacteria are found in almost all marine sponges (Mohamed *et al.*, 2006; Thacker, 2005) and may form mutualistic associations with hosts especially if the symbiont provides fixed C or N. The cyanobacterium *Oscillatoria spongieliae* has been found within several species of sponges including the reef sponge *Dysidea herbacea* on the Great Barrier Reef (GBR) (Flowers *et al.*, 1998; Ridley *et al.*, 2005). *Synechococcus* has also been described from several species of sponge, which are genetically distinct from free living planktonic species of *Synechococcus* (Thacker, 2005). In some instances metabolic products (e.g., fixed carbon) are translocated from symbiont to host (Arillo *et al.*, 1993). However, in most cases the relationship between host and symbiont are not well defined (Ridley *et al.*, 2005).

Potential translocation of N from nitrogen fixers to hosts has also been investigated. Nitrogenase activity was reportedly associated with an intact *Procholoron*-Ascidian association (*Lissoclinum* sp.) (Paerl, 1984), although activity could not be directly detected by the isolated symbiont (Odintsov, 1991). It was subsequently determined that at least a portion of the cellular N was derived (directly or indirectly) from N_2 fixation, based on the low isotopic ratio of $^{15}N/^{14}N$ in *Lissoclinum* sp. associations (Kline and Lewin, 1999). Diazotrophs have also been found associated with corals (Frias-Lopez *et al.*, 2002; Rohwer *et al.*, 2001, 2002; Shashar *et al.*, 1994a,b)

with nitrogen fixation measured in some live corals (Shashar *et al.*, 1994a,b; Williams *et al.*, 1987). More recently the presence of a diazotrophic coccoid cyanobacteria has also been reported in the tissues of certain species of the coral *Montastrea* (Lesser *et al.*, 2004). Interestingly it has been further demonstrated through δ N analyes that the zooxanthellae from Caribbean colonies of *Montastraea cavernosa* colonies which contain the endosymbiotic cyanobacteria acquire N from the nitrogen fixing cyanobacteria rather than the coral host (Lesser *et al.*, 2007).

2.2. Nitrification

Nitrification has been conventionally viewed as the two step process by which certain groups of chemoautotrophic bacteria oxidize ammonium to nitrite and subsequently, another group of chemoautotrophic bacteria, oxidize nitrite to nitrate (Fig. 21.1C) (see Chapter 5 by Ward, this volume). Nitrification generally occurs in aerobic zones throughout the marine environment, and can occur at high rates in coral reef ecosystems (Capone *et al.*, 1992; Corredor and Capone, 1985; Corredor *et al.*, 1988; Johnstone *et al.*, 1990; Wafar *et al.*, 1990; Webb *et al.*, 1975). Nitrification in reef lagoons can oxidize ammonium recently released from nitrogen fixation or regenerative processes (Webb and Wiebe, 1975). Nitrification is also thought to be a major source of N_2O in marine systems, and may contribute to the overall weak marine N_2O flux to the atmosphere (Capone, 1996b) (see also Chapter 2 by Bange, this volume).

Nitrification on coral reefs was first noted when net nitrate production was measured at Eniwetok Atoll. This, in conjunction with the isolation of a species of nitrifying bacteria, *Nitrobacter agilis*, was taken as strong evidence for nitrification as a significant process in the reef system (Webb and Wiebe, 1975). Ammonium oxidation and nitrite oxidation were presumed to be closely coupled as there was not a build up of the intermediary product nitrite (D'Elia and Wiebe, 1990). Coral reef sediments in Puerto Rico showed nitrification rates that were highest in the upper few centimeters of sediment, with a second peak at $\sim$20 cm (Corredor and Capone, 1985). Nitrifying bacteria have also been identified as sponge symbionts on reefs in the Caribbean (Corredor *et al.*, 1988). Later studies investigating the relationship between four sponge species including the Caribbean coral reef species *Chondrilla nucla* found that highest rates of nitrate release were associated with sponges that had cyanobacterial endosymbionts (Diaz and Ward, 1997) suggesting that sponge-mediated nitrification may be very common in tropical benthic communities and may constitute a large input of oxidized nitrogen in these systems (see Chapter 27, Foster and O'Mullin, this volume). However, further research is needed to elucidate the details of these pathways.

Recently research has revealed that nitrification is not exclusively associated with chemoautotrophic bacteria of the β and γ proteobacteria, but occurs in many Crenarcheaota as well (Francis *et al.*, 2005; Konneke *et al.*, 2005; Wuchter *et al.*, 2006) who may in fact may dominate this process in seawater (Wuchter *et al.*, 2006) (see Chapter 5 by Ward, this volume). The extent that archael nitrification occurs in coral reef habitats remains to be determined. Ammonium oxidation has also been shown to occur anoxically in some marine sediments at the expense of NO_2^- (Thamdrup and Dalsgaard, 2002) (see Chapter 6, Devol, this volume).

This process is termed ''Anammox'' for ''anaerobic ammonium oxidation'' and is catalyzed by a specialized group of planctomycetes bacteria first discovered in sewage reactors (Strous and Jetten, 2004). Again, the importance of Anammox in coral reef environments has not yet been considered.

2.3. Dissimilatory nitrate reduction and denitrification

There are two pathways of dissimilatory nitrate reduction, generally thought to be mediated by anaerobic, or facultatively anaerobic bacteria, using NO_3^- as a terminal electron acceptor in respiration (Fig. 21.1D and F) (see Chapter 6, Devol, this volume). One pathway leads to production of ammonium, and may act as an internal cycling loop within the system (D'Elia and Wiebe, 1990). The other pathway, denitrification, ends in production of N_2O and/or N_2 gas, which can then be lost from the system to the atmosphere.

Denitrification was originally dismissed as an important process on reefs as it was thought that anaerobic processes were unlikely to occur in a seemingly aerobic environment and, additionally, high amounts of nitrate export had been observed (D'Elia and Wiebe, 1990; Wiebe, 1976). However, denitrification does indeed occur within these systems and has been measured in a number of reef environments with highly variable rates obtained (Capone et al., 1992; Corredor and Capone, 1985; Johnstone et al., 1990; Koop et al., 2001; O'Neil and Capone, 1996; Seitzinger and D'Elia, 1985; Smith, 1984; Williams et al., 1985). This may be related to differences in substrate availability as well as differences in sediment organic and nutrient content (Koop et al., 2001; O'Neil and Capone, 1996). Thus, the quantitative importance of dentrification within reef sediments and the reef matrix after the deposition of particulate organic matter remains a large unknown (Furnas, 2003).

Additionally, the anammox reaction mentioned earlier results in the reduction of NO_2^- with the production of N_2 gas. Recent direct tracer evidence from both marine sediments (Thamdrup and Dalsgaard, 2002) (see Chapter 6, Devol, this volume) and several anoxic water column systems (Hamersley et al., 2007; Kuypers et al., 2005) has shown that the anammox pathway, rather than conventional denitrification, can be a significant and, at times, predominant source of N_2 production. Future studies of combined N loss from reefal environments should also consider this pathway.

2.4. Nitrogen acquisition and uptake

Uptake of combined N by organisms for growth is an aspect of the nitrogen cycle that has been relatively well studied for the coral/algal symbiosis (D'Elia and Webb, 1977; Webb and Wiebe, 1978; Blythell, 1990a,b). Other organisms in the reef environment, including macroalgae, benthic microalgae and phytoplankton as well as many heterotrophic prokaryotes have the capacity to take up combined nitrogen.

Oxidized forms of nitrogen such as nitrate, need to first be reduced to ammonium before their incorporation into biomass. Nitrate is first reduced by the enzyme assimilatory nitrate reductase to nitrite. Assimilatory nitrite reduction subsequently reduces the nitrite to ammonium (D'Elia and Webb, 1977) (Fig. 21.1E) (see Chapter 7, Mulholland and Lomas, this volume).

In most marine ecosystems primary productivity is concentrated in water column phytoplankton, but the bulk of coral reef productivity occurs in the benthos and can be attributed to coral zooxanthellae, turf algae and benthic micro and macroalgae (Larkum, 1983; Taylor, 1983a,b). Benthic microalgae (also called microphytobenthos, Macintyre et al., 1996) are a diverse assemblage of diatoms, dinoflagellates, and cyanobacteria which are poorly understood with regard to the influence of their productivity and abundance to nutrient dynamics relative to other nutrient processes on coral reefs (Heil et al., 2004). Benthic microalgae are ubiquitous and abundant in the clear well lit environments of many coral reefs, and can have chlorophyll values $100\times$ that of the integrated water column above them. Areal rates of photosynthesis by these turfs rival terrestrial systems and imply substantial N demand (Heil et al., 2004; Werner et al., 2008).

Benthic microalgae in and on reef sediments may derive some of their nutrients from the sediments as appreciable ammonium levels have been reported (Capone et al., 1992; Heil et al., 2004; Johnstone et al., 1990). Increases in benthic microalgae with nutrient enrichment suggest that these populations can also access nutrients in the water column, which result from regenerative processes within the water column. Benthic microalgae are also net O_2 producers which may influence nutrient dynamics, particularly nitrification and denitrification by changing redox profiles as well as the nutrient exchange across the sediment water interface.

2.5. Invertebrate/symbioses

Perhaps the most significant evolutionary adaptations for sustained high productivity in oligotrophic seas are the symbiotic relationships that lead to efficient retention and recycling of nutrients in these systems (Hoegh-Guldberg, 1999; Miller and Yellowlees, 1989). The multitude of algal–invertebrate symbioses that have evolved in reef environments to efficiently recycle nutrients is a testament to this strategy (Muscatine and Porter, 1977). Nitrogen transformations are the centerpieces of these associations (Taylor, 1983a) with the symbiotic dinoflagellates (zooxanthellae) providing C for translocation from photosynthesis to coral animal host, and the transfer of N from animal waste to the symbiotic algae (Taylor, 1983b; Wilkerson and Trench, 1986) (Fig. 21.2). Other, diverse but less well characterized symbioses occur in coral reef environments including microbes associated with sponges (e.g., Garson et al., 1998; Mohamed and Hill, 2006; Thacker, 2005; Wilkerson and Fay, 1979; see also Chapter 27, Foster and O'Mullin this volume), giant clams (e.g., Ambariyanto and Hoegh-Guldberg, 1999; Fitt et al., 1995; Summons et al., 1986; Wilkerson and Trench, 1986), anemones (e.g., Cook et al., 1988, 1992; Wang and Douglas, 1997); scyphozoan jellyfish (e.g., Wilkerson and Kremer, 1992), and Ascidians as well as N transfer from endosymbiotic, diazotrophic cyanobacteria to symbiotic dinoflagellates within coral (Lesser, 2007). Aspects of each of these symbiotic associations revolve around interactions with nitrogen.

Animals in general are not able to take up inorganic nitrogen directly (Muscatine et al., 1979), and usually excrete reduced nitrogenous wastes into the surrounding water (Rahav et al., 1989). Algal–invertebrate symbioses of coral reefs are an exception to this
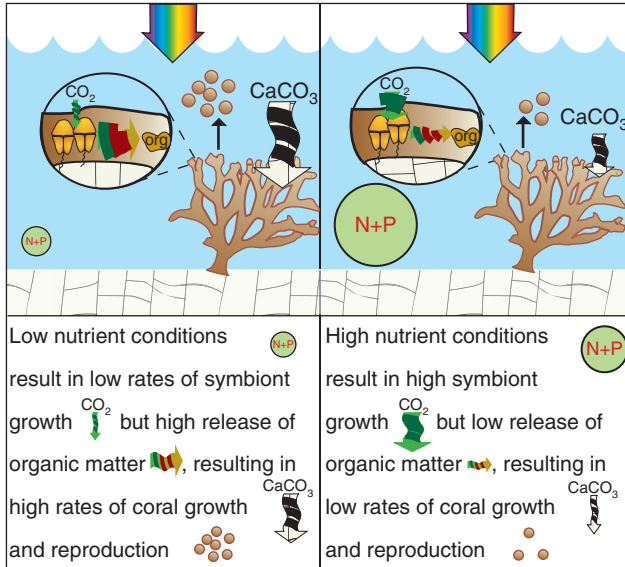
Figure 21.2 Nutrient availability changes the relationship between microalgal symbionts and coral hosts (adapted from Dubinsky and Stambler, 1996; Furnas, 2003). Under low nutrient conditions zooxanthellae translocate photosynthate to corals for growth, calcification and reproduction. Under higher nutrients, zooxanthellae increase in abundance and keep more of the photosynthate for themselves; translocating less to host coral for metabolic needs.

generalization (Cook *et al.*, 1987, 1988; Cook *et al.*, 1992; Miller and Yellowlees, 1989). In particular, the algal–anthozoan symbioses have been termed the ''physiological chimera between alga and animal'' (Furla *et al.*, 2005). Corals can take up both dissolved inorganic (e.g., Badgley *et al.*, 2006; Crossland and Barnes, 1977; Grover *et al.*, 2003a,b; Tanaka *et al.*, 2006) and organic nitrogen (e.g., Hoegh-Guldberg and Williamson, 1999; Grover *et al.*, 2006), as well as ingesting particulate nitrogen (e.g., Anthony, 2000; Lawn and McFarlane, 1991; Mills *et al.*, 2004; Ribes *et al.*, 2003).

The amount of nitrogen available to corals is a function of both particulate host feeding as well as inorganic N uptake which can be effected by nutritional history (Piniak and Lipshultz, 2004). Corals and other sessile organisms can acquire necessary nutrients by harvesting zooplankton, microbes and detritus from the water column directly (Erez, 1990; Lawn and McFarlane, 1991; Porter, 1974, 1978) through mucus-netting strategies, or indirectly by capture of protozoa that graze on bacteria. These trapping processes are so efficient it has been termed ''the wall of mouths'' (Yonge, 1930) and are one of the matrix of factors that permit coral reefs to support high biomass concentrations in oligotrophic waters. Corals can gain up to 70% of their nitrogen budget from particulate sources and microbes which account for 30–45% of the incorporated particulate matter (Bak *et al.*, 1998).

A second pathway is by the uptake up dissolved inorganic nitrogen (DIN) by the symbiont. The inorganic nitrogen may itself derive from recycling of animal wastes which are then retained by the symbionts in exchange for photosynthate released back to the host (Muscatine *et al.*, 1989; Piniak and Lipschultz, 2004; Wang and

Douglas, 1998, see below). Alternatively, the symbiont may obtain inorganic nitrogen either in reduced (e.g., ammonium) or oxidized forms (e.g., nitrate) directly from seawater (Bythell, 1990a,b; Ferrier-Pages *et al.*, 2001; Marubini and Davies, 1996; Wilkerson and Trench, 1986). The latter is often a light dependent process (Grover *et al.*, 2003a,b). It has further been demonstrated that both intact corals (D'Elia and Web, 1977), as well as isolated zooxanthellae, can take up both nitrate and ammonium (Bythell, 1988; Grover *et al.*, 2003a,b). This net uptake can account for approximately 30% of the daily nitrogen requirement for tissue growth, gamete production and DON production (Bythell, 1988, 1990a,b).

Summons and Osmond (1981) first demonstrated light dependent uptake of ^{15}N labeled nitrate, ammonium and urea by zooxanthellae which they attributed to the glutamine synthetase–glutamate synthase (GS-GOGAT) pathway. Anderson and Burris (1987) directly confirmed GS activity in symbionts isolated from corals and in pure culture (see also Miller and Yellowlees, 1989). In contrast, in giant clams Yellowlees *et al.* (1994) reported ammonium assimilation in zooxanthellae was catalyzed by the glutamate dehydrogenase enzyme (GDH). Exactly how this mechanism works has not been fully elucidated as both the symbionts and host appear to contain the enzyme GDH (Wilkerson and Muscatine, 1984; Yellowlees *et al.*, 1994). The model proposed by D'Elia *et al.* (1983) suggested a "depletion-diffusion" model for ammonium uptake, where the symbiont takes up ammonium at a rate that depletes the concentration in the tissue and allows for diffusion of nutrient through the animal tissues. In sea anemones with zooxanthellae there is evidence of uptake and translocation of ammonium derived nitrogen using ^{15}N tracers (Lipschultz and Cook, 2002).

The symbiotic dinoflagellates (zooxanthellae) are responsible for the uptake of the inorganic nitrogen, subsequently synthesizing organic compounds that are translocated to the host (Wang and Douglas, 1999). Whereas early workers focused on the release of glycerol by the dinoflagellate symbionts, Muscatine first showed that alanine was also an important metabolite (Muscatine, 1980). The symbionts thus allow coral systems to retain and incorporate inorganic nitrogen at micromolar, and down to nanomolar, concentrations (Muscatine and D'Elia, 1978; Grover *et al.*, 2002). Translocation of the amino acid aspartate from symbiotic dinoflagellates to their anemone hosts can occur (Swanson and Hoegh-Guldberg, 1998). Other studies have revealed the incorporation of aspartate into proteins that are deposited rapidly as skeletal organic matrix (Allemand *et al.*, 1998). This may be indicative of pathways of protein synthesis that are unique for the skeletal matrix and different from the general coral protein metabolism, or due to endolithic algal communities within the coral skeleton (Hoegh-Guldberg, 2004a,b).

Experiments with nitrogen additions have given some insight into the role of nitrogen in the algal-coral symbiosis relationship. Additions of ammonium and ammonium + phosphate increased zooxanthellae density, their protein synthesis rates (Muscatine *et al.*, 1989), as well as the amount of chlorophyll and nitrogen per symbiont (Snidvongs and Kinzie, 1994). More recently, Tanaka *et al.* (2006) demonstrated that 50% of $^{15}NO_3{}^-$ and ^{13}C delivered to the coral *Acropora pulchra* was taken up and appear in the host in organic forms demonstrating translocation from the zooxanthellae. The C:N ratio of the translocated organic matter appeared to change

based on the N availability for the zooxanthellae. The incorporated organic ^{15}N seems to be recycled within the coral-algal symbiotic system, with high C:N compound such as lipids and carbohydrates being more rapidly utilized than lower C:N compounds such as proteins and nucleic acids (Tanaka et al., 2006).

Yet other pathways of nitrogen acquisition may exist. Symbionts are able to take up dissolved free amino acids (DFAA) (Ferrier, 1991; Hoegh-Guldberg and Williamson, 1999). In the coral Pocillopora damicornis, DFAA uptake from seawater can account for ~11% of the nitrogen demands in the coral. Uptake of DFAA has also been shown by the giant clam Tridacna maxima (Ambariyanto and Hoegh-Guldberg, 1999). This may be an important episodic source of nitrogen for corals during times of local fish migrations and the activity of other grazers that result in algal cell breakage or exudation which increase DON concentration on the reef (Hoegh-Guldberg and Willamson, 1999). More recently, concentration dependent uptake of ^{15}N-urea was demonstrated in the coral Stylophora pistillata (Grover et al., 2006). Uptake kinetics suggested adaptation of the corals to low seawater concentrations of urea and, when compared to uptake rates of ambient ammonium and nitrate concentrations, urea was preferred to nitrate. Urea therefore may also be an important episodic source of nitrogen as well.

Zooxanthellae provide much of the coral's energy, transferring up to 95% of the symbiotic algae's photosynthate to the host (Fine and Loya, 2002; Muscatine, 1990). In the absence of zooxanthellae (e.g., in cases of coral bleaching and the loss of algal symbionts due to stress, Hoegh-Guldberg, 1999), translocation of photosynthetic carbon can also take place between algae living in the coral skeleton (endoltihic algae), and the coral host (Fine and Loya, 2002; Schlichter and Kerisch, 1995). Thus, endolithic organisms (e.g., bacteria, fungi and algae) living within the coral skeleton may also be potential nutrient sources (Fine and Loya, 2002; Le Campion-Alsumard et al., 1995; Kushmaro and Kramarsky-Winter, 2004). Ferrer and Szmant (1998) estimated that 55–60% of the nitrogen required by the coral can be satisfied by these sources. Recent work indicates that these symbiotic and mutualistic relationships are even more varied, complex and dynamic than previously thought (Fine and Loya, 2002; Furnas, 2003).

Reef sponges (Wilkinson and Fay, 1979) and some corals may acquire fixed nitrogen from associated diazotrophic cyanobacteria (Frias-Lopez et al., 2002; Lesser et al., 2004; Rohwer et al., 2001, 2002; Shashar et al., 1994a,b, see also Chapter 4, Capone and Carpenter, this volume). The nutritional significance of these alternative pathways remains to be fully evaluated.

2.6. Microbial populations

Recent research has shown the diversity and potential significance of microbial communities living in and on coral reefs (Kushmaro and Kramarsky-Winter, 2004; Rohwer et al., 2002) with an implicit role in nitrogen uptake, packaging and regeneration Bacterial productivity within cavities in the reef framework is higher than that in open water surrounding the reef (Scheffers et al., 2005) (see Chapter 8, Bronk and Steinberg, this volume), and the ecological role of bacteria living in coral mucus and skeletons is just now being elucidated. Cyanobacteria have

also been detected in the mucus layer of corals (Ritchie and Smith, 2004; Rohwer et al., 2001) and specialized bacteria may also protect the coral animal from opportunistic pathogens by having antibacterial or toxic properties. However, the role of the microbial populations of the coral surface mucopolysaccharide layer (SML) in terms of nitrogen dynamics has not yet been fully studied. One might hypothesize possible roles involving N immobilization, regeneration, nitrification and nitrogen fixation, amongst others.

Microbial populations in reef ecosystems may serve directly as nutrient sources for higher organisms. They are known to have a low C:N ratio in general and the use of the SML bacterial community in particular as a food source has been demonstrated (Ritchie and Smith, 2004; Sorokin, 1973). With respect to nutrient acquisition, microbes are effective in sequestering nutrients due to their high affinity transport systems and large surface to volume ratios. In nutrient poor waters, prokaryotes scavenge nutrients at much lower concentrations than eukaryotes, and assimilate most of the limiting nutrients thereby restricting primary productivity (Kirchman, 2000; Rohwer and Kelly, 2004). Coral-associated microbes may also be in direct competition with their host for water column nutrients (Rohwer et al., 2002). Given the ability of prokaryotes for nutrient uptake, corals may use microbes to ''fish'' for nutrients i.e. cultivation gardening for certain beneficial microbes by excreting organic matter in the form of mucus (Ducklow, 1990; Saffo, 1992).

Microbes may in some cases also act as pathogens causing harm to corals. Recent work has shown that reefs that are more eutrophied, often have denser stands of macro- and micro-algae (see below). The algae produces carbon substrates, which in turn then enhance the proliferation of bacteria on live coral surfaces which can cause the corals to die, enabling more substrate for algal growth (Smith et al., 2006).

2.7. Ammonification and regeneration

Given the high gross productivity of reefs, the majority of this production must be supported by the retention and internal recycling of nitrogen (Szmant-Froelich, 1983 Fig. 21.3). The pathway of ammonium production from organic matter is often referred to as ammonification (Fig. 21.1B). Ammonium is released through grazing processes in which fish, echinoderms, zooplankton, filter feeders (including sponges) as well as detritivores (such as worms), and bacteria recycle organic nitrogen and excrete it (D'Elia and Wiebe, 1990; D'Elia, 1988; Szmant-Froelich, 1983). Coral reef fish have excretory products very high in nitrogen (N:P = 48), with ammonium as the most important source of DIN and excretory products that are enriched in P (N:P = 8). Coral growth is higher in the presence of reef fish, particularly resting fish such as grunts, which forage and then return to the reef to rest in large aggregations (Meyer and Schultz, 1985).

Regeneration of organic nitrogen may occur in various components of the reef including within the diverse symbiotic associations (see Section 2.5). In addition to the surrounding sediments (DiSalvo, 1969; Entsch et al., 1983; Rasheed et al., 2002), structures on the reef proper may be important sources of regeneration of nutrients, including ''porewater'' within the coral skeletons themselves (Risk and
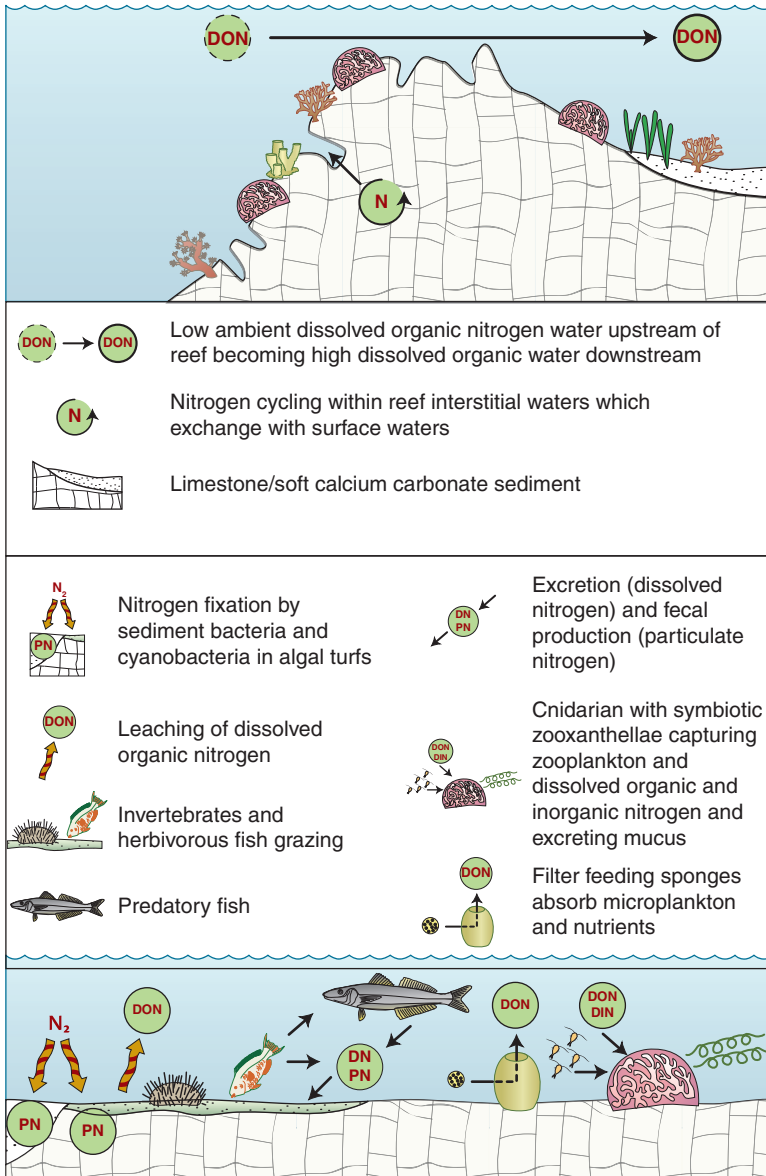
Figure 21.3 Generalized nutrient pathways and means of trophic cycling and regeneration on reefs (adapted from Szmant-Froelich, 1983); showing the importance of nitrogen cycling and regeneration within the interstices of the coral structure and from grazers.

Muller, 1983). All these loci serve important ''biocatalytic'' function in terms of regeneration of organic matter and release of nutrients to the coral reef (Rasheed *et al.*, 2002). Up to 70% of reef volume can be attributed to the myriad of nooks and crannies that trap sediments, fecal pellet and decaying organic matter (Ginsburg, 1983; Szmant-Froelich, 1983; Szmant, 2002). Regenerated inorganic species can

then percolate through the porous structures. Elevated nutrients have indeed been measured in reef cavities and interstices (DiSalvo, 1971; Andrews and Muller, 1983; Szmant-Froehlich, 1983, 2002), which can be introduced to the reef through "burps" (Szmant-Froelich, 1983) of nutrient-enriched water through holes and fissures that provide reef organisms with pulses of nutrients (Fig. 21.3). Cavities within the reef structure are areas of high bacterial activity and hence nutrient regeneration (Scheffers *et al.*, 2004, 2005). In a study in Curacao, open water bacterial productivity was limited by both N and P, whereas reef crevice bacteria were only limited by P (Scheffers *et al.*, 2005). N limitation appears to be circumvented in these interstices through remineralization processes which can, in turn, increase inorganic N concentrations in the overlying reef waters through exchange with the ambient water (Scheffers *et al.*, 2005).

Rasheed *et al.* (2002) determined that nutrient concentrations in these regenerative spaces within the reef framework were 1–3 times higher than surrounding water column values, corresponding to fluxes of 14.5 mmol m^{-2} day^{-1} for ammonium; 7.7 mmol m^{-2} day^{-1} for nitrate and 0.9 mmol m^{-2} day^{-1} for nitrite. Nutrients within the sediments were 15–80 times higher than the water column values, corresponding to a diffusive flux of 0.06 mmol m^{-2} day^{-1} for ammonium; 0.03 mmol m^{-2} day^{-1}; for nitrate and 0.01 mmol m^{-2} day^{-1} for nitrite; underscoring the importance of these sites in trapping organic matter and regenerating nutrients (Rasheed *et al.*, 2002).

Recycling of nutrients between coral hosts and symbiotic dinoflagellates (zooxanthellae) is a key reef process (see Section II above). Zooxanthellae take up the ammonium excreted by the coral animal, therefore this waste N is not released to the wider system (Muscatine and D'Elia, 1978; Wilkerson and Trench, 1986). Recycling of N by the coral host could satisfy all the nitrogen needs of the symbiotic algae (Crossland and Barnes, 1974, 1977).

Transfer of ammonium and other nitrogenous waste may aid in the process of coral calcification. Enhanced calcification was noted in experiments when ammonium and urea were added to corals (Crossland and Barnes, 1974). The authors suggested that excretory ammonia may combine with protons released when bicarbonate is converted to carbonate. Their model suggested that at the site of calcification, the hydrolysis of urea formed by the breakdown of allantoins yields CO_2 and NH_3. The CO_2 could provide a carbonate source for calcification and the NH_3 would then neutralize and remove protons from the calcifying milieu. However, Taylor (1983a,b) re-analyzing their study suggested it was more reasonable to attribute the enhancement of calcification by ammonium and urea to the stimulation of symbiont photosynthesis. Kinsey and Davies (1979) showed a decrease in coral calcification with the addition of nitrogen and phosphorus, but suggested this was more probably attributable to P effects rather than N (see Elevated Nutrients on Coral Reefs Experiment (ENCORE) case study below).

Much of the regenerated nitrogen on the reef ultimately is derived from nitrogen introduced to the system through grazing. Grazer interactions also help recycle nutrients with fish and invertebrates grazing benthic microalgae. Benthic microalgae can act as a nutrient trap for regenerated nutrients including N, and have been

shown to increase with increasing levels of ammonium (Uthicke, 2001; Uthicke and Klumpp, 1997). Benthic microalgae have been observed to increase in the presence of holothurian grazers (Uthicke, 2001), due to ammonium release. This constitutes an important component of a benthic retention mechanism akin to the pelagic microbial loop (Uthicke, 2001).

Recently there has been a growing awareness of the importance of coral mucus to the overall productivity and retention of nutrients in the reef system (Wild *et al.*, 2004a). Up to 50% of the carbon assimilated by zooxanthellae may be exuded as mucus, which protects the coral colony against fouling, dessication and sedimentation. A previously unrecognized aspect of mucus within these systems is that it efficiently traps organic particulate matter from the water column, including bacteria known to fix both N and C thereby increasing the nutritional quality in coral associated communities. In a study in Australia, Wild *et al.* (2004a) found that coral mucus could increase both its N and C content up to 3 orders of magnitude in 2 h. Bacteria are much better than corals at assimilating nutrients at low concentrations (Knowlton and Rower, 2003) and coral associated bacteria may scavenge limiting nutrients, including iron and vitamins, which are then harvested by the corals themselves. This is supported by the observation that some corals eat their own mucus (Coles and Strathmann, 1973). These enriched mucus-particle aggregates subsequently provided 10–20% of the total organic carbon supply to the sediments, maintaining nutrients for heterotrophs and remineralization processes within the coral reef system (Wild *et al.*, 2004a). Coral mucus and organic flux from mass spawning events back to the benthos from the overlying water column may affect sediment biogeochemistry in reef environments by enhancing the organic content of sediments and affecting microbial processes and nutrient processing (Wild *et al.*, 2004a,b,c; 2005, 2008).

3. NITROGEN PERTURBATIONS TO REEFS

The rapid decline in coral reefs over the last few decades clearly has resulted from multiple stressors from various sources including exposure to excess nitrogen which can have direct effects on corals and their symbionts (Stambler *et al.,* 1991; Dubinsky and Stambler, 1996; Furnas, 2003; Mora, 2008) (Fig. 21.2). Nutrients can have direct physiological effects on corals by reducing growth or reproduction rates. Nutrients can affect corals indirectly by: increasing susceptibility to bleaching and disease, affecting the coral-zooxanthellae symbiosis (Bruno *et al.*, 2003), or by causing phase shifts in community structure (Knowlton, 2001; Szmant, 2002). Food webs in coral reef systems can become highly altered with the removal of predators providing ''top–down'' control and increases in nutrients providing ''bottom up'' control. (Hughes *et al.*, 1999, 2007; Lapointe, 1997, 1999, 2004a,b; Szmant, 2002). With increased nutrients, coral reefs change from symbiosis-dominated systems with tight recycling of nutrients to regions with a higher proportion of macroalgae, with subsequent succession to heterotrophic filter feeders (Fabricius, 2005).

Coral reefs are not stable, static communities living in benign environments, but rather are very dynamic ecosystems which are subject to frequent natural physical disturbance on times scales which vary from minutes to years (Furnas, 2003; Furnas

et al., 2005). Despite their inherent resilience, coral reefs are nonetheless threatened world wide, with an estimated 140,000 km^2 lost in the last few decades and more than half of the world's coral reefs listed at high or medium risk with more than 80% of the reefs in SE Asia under medium to high threat from activities such as over-development and over-exploitation (Brown, 1987; Bryant *et al.*, 1998; Dubinsky and Stambler, 1996; Hoegh-Guldberg, 1999, Hughes *et al.*, 2003). The resilience of the system becomes weaker with more accumulated impacts (McClanahan *et al.*, 2002).

Impacts of overfishing, global climate change and the increase in coral disease (including coral-bleaching) (Bellwood *et al.*, 2006; Hoegh-Guldberg, 1999; Hughes *et al.*, 1999, 2003, 2007; Jackson *et al.*, 2001; Pandolfi *et al.*, 2003; Smith and Buddenmeier, 1992; Wilkinson and Buddemeier, 1994; Mora, 2008) are emergent areas of interest in coral reef research. There has been much debate in the literature over which factors (e.g., nutrients, loss of grazers, terrestrial runoff and sedimentation; climate change etc.) are the most deleterious. These factors may have synergistic or antagonistic effects, which may vary in differing regions and reefs (e.g., Aronson *et al.*, 2003; Boesch *et al.*, 2001; Fabricius, 2005; Hughes *et al.*, 1999, 2003; Jackson *et al.*, 2001; Lapointe, 1997, 1999, 2004a,b; Pandolfi *et al.*, 2003; Szmant, 2001, 2002). These problems may be further exacerbated by increasing urbanization and other changing land usages which can subsequently result in amplified runoff of nutrients, fertilizers contaminants and increased sediment loading (Furnas, 2003; Hutchins *et al.*, 2005) resulting in general coastal eutrophication (Bell, 1992; Costa *et al.*, 2000; Fabricius, 2005; Furnas and Brodie, 1996; Koop *et al.*, 2001).

Threats to reefs can be either acute or chronic (Edinger *et al.*, 1998). Acute is here defined as ''dramatic damage in a short period of time,'' which includes destructive fishing practices, anchor damage, ship groundings, cyclones or hurricanes, or disease or predation (e.g., *Acanthaster* outbreaks) (Brodie *et al.*, 2005). Chronic threats alter the physical or biological environment on a long term basis, and cause long term damage to coral reefs. Nutrient inputs including sewage; increased sedimentation (particulate nutrients), resulting in nearshore eutrophication (Tomascik *et al.*, 1993) can manifest themselves in both modes. Chronic stresses may be more detrimental to the long term health of coral reef ecosystem than acute stresses (Kuntz *et al.*, 2005). Combinations of stressors are associated with threshold responses, as well as ''ecological surprises'' which can include disease and pathogen breakouts (Brodie *et al.*, 2005; Bruno *et al.*, 2003; Knowlton, 2001).

Whether reefs will have the resilience to recover from multiple anthropogenic stresses (Hughes and Connell, 1999) is a major issue of concern to scientists and resource managers. Recent studies have highlighted the serious decline in water quality in coastal environments, including coral reef areas (Fabricius, 2005; Hutchins *et al.*, 2005; Kuntz *et al.*, 2005; Wilkinson, 2002; Barile, 2004).

3.1. Inorganic nitrogen increases

3.1.1. Effects on coral colonies and life cycle

Elevated nutrients (ammonium, nitrate and phosphate) may derive from multiple sources (see above) and can directly affect both adult coral colonies as well as reproduction and recruitment of larvae in a number of ways (Fabricius, 2005).

Nutrient induced perturbations include changes in biochemical composition of the corals (Marubini and Atkinson, 1996; Stambler *et al.*, 1991), skeletal growth (both positive and negative) (Ferrier-Pages *et al.*, 2000), calcification (Marubini and Atkinson, 1999), reproductive potential (Tomascik, 1991), and density of algal symbionts (Dubinsky and Stambler, 1996) (Fig. 21.2). When corals are in low nutrient environments, zooxanthellae produce photosynthate in excess of their capacity to take up nutrients. This excess organic carbon is then available to the host for growth, calcification and reproduction (Fig. 21.2. left panel). Under high nutrient conditions the zooxanthellae preferentially keep the organic carbon for their own growth, increasing symbiont numbers, but decreasing the transfer to the coral host, which decreases the coral's calicification and reproduction (Furnas, 2003). The long term ecological implications of these often subtle changes in symbiont and host interactions and translocation of organic matter and energy away from coral growth and reproduction, have not been fully elucidated (Furnas, 2003). Direct effects of DIN on both corals reproduction and coral adults has recently been summarized by Fabricius *et al.* (2005, and references therein).

3.1.2. Algal increases and grazer decreases

One manifestation of excess nutrients, and specifically N, on reefs is algal overgrowth (Barile, 2004; Lapointe, 1997; McCook, 1999, 2001; Reaka-Kudla *et al.*, 1997; Scheffers *et al.*, 2005; Szmant 2002). Excess nitrogen may also enable, or act synergistically, with other stressors to increase and expedite, the spread of disease within coral communities (Rosenberg and Loya, 2004, refs therein; Kuntz *et al.*, 2005). The determination of factors that lead to algal overgrowth on reefs has been an important, and at times controversial issue, as to whether increased nutrients (including N), or loss of grazers has given rise to the problem. In a special issue in the journal *Coral Reef* on the topic, Szmant (2001) summarizes the problem: *"It is clear from the studies in this issue that several factors are conspiring to change the dynamics of coral reef algal communities from those we observed (but did not study very well) in the early and middle decades of the past century, to those present now. These factors appear to differ in degree among localities."*

In the same special issue, McCook *et al.* (2001) provide an extensive review of coral—algal interactions which may be characterized as positive, neutral or negative. Positive effects include symbiotic algal–coral interactions; neutral interactions include situations where grazing and algal growth are balanced and negative effects of algae on corals which include both direct or indirect influences. Direct negative effects often involve competition or the direct overgrowth and/or invasion of coral by algae. An example of this is the algae *Coralliophila hurysmansii* which causes tissue swelling on the coral. Indirect effects include interactions such as those with the algae *Antrichium tenue* which has a mucus layer that traps sediments (and possibly microbes). This layer increases microbial processes which help turn the algal–coral interactions from a neutral relationship to a negative one, thereby taking on the characteristics of disease, with increased nutrients as the trigger (Willis *et al.*, 2004). Some other indirect affects include reduction in physical space for coral growth and recruitment of coral larvae due to algal coverage, and allelopathic activity (Kuffner and Paul, 2004; McCook *et al.*, 2001). Recent research suggests that overgrowth of algae can cause mortality of corals from enhanced microbial activity on coral

surfaces. This may in turn be a result of algal exudates, which then allows more free surface area for algal growth (Smith *et al.*, 2006). Other evidence indicates that algal exudates can also suppress fecundity, recruitment and survival (Hughes *et al.*, 2007).

Nutrient stimulation of algal growth may often only be visible in the absence of grazers (Hughes, 1994; Furnas, 2003). Concomitant with nutrient increase, reefs are experiencing a major reduction in grazers that help keep the system in balance (Hughes, 1994; Jackson *et al.*, 2001; Pandolfi *et al.*, 2003). In some instances this may promote a phase shift from corals to macroalgae, and a resultant loss of resilience in recovery to other perturbations such as storms and climate change and diseases (Bellwood *et al.*, 2004). For instance, Caribbean reef decline was preceded by a dwindling of fish stocks, along with simultaneous increases in nutrients and sediment runoff from the land (Hughes, 1994; Jackson *et al.*, 2001). Similarly, on the GBR, inputs of sediment and nutrients have increased by four times that of pre-European settlement (McCulloch *et al.*, 2003; Williams, 2002) and macro grazer populations of sea–turtles and dugong have decreased dramatically along with larger predator fish populations (Bellwood *et al.*, 2004). Additionally, some effects of nutrients and disease may be exacerbated by sea surface warming which can also lead to coral bleaching. What effect this will have on long term coral reef health is presently unknown (Buddemeier and Fauntin, 1993; Koop *et al.*, 2001; Smith and Buddenmeier, 1992; Szmant, 2002).

3.1.3. Effects of runoff and sedimentation

Although coral reef environments are generally characterized as low nutrient environments, there are some areas in which intermittent sources of nutrients are added by seasonal runoff reaching reef environments (Furnas, 2003; Gabric and Bell, 1993; Kinsey, 1991; Marsh, 1977). In the GBR region for instance, coastal river inputs of sediments and nutrients has increased fourfold over the last ∼150 years (Brodie *et al.*, 2003) and the dissolved nitrogen fraction of this runoff is widely dispersed in the GBR system (Devlin and Brodie, 2005). Land development including increases in impervious surfaces as well as processes that lead to increased erosion such as agricultural grazing can amplify runoff of sediments during rain events such as observed on Molokai, a high relief, heavily ranched portion of Hawaii that is an area of local concern and study currently (Chaston, 2006).

Terrestrial runoff is a major concern for areas with coral reefs. Nutrient enrichment, sedimentation, and turbidity can all degrade reefs at local scales (Fabricius, 2005). While there are some coastal coral reefs that can flourish in relatively high nutrient and high levels of particulate matter, they tend to be restricted in depth to ∼10 m; whereas in clearer offshore reefal systems they may extend to >40 m (Fabricius, 2005). Some corals can use the particulate matter for nutrition. However, there is a fine line, between increased nutrient availability for the coral for ingestion and the tipping point at which the corals are unable to cope with sedimentation and silting affects. (Anthony, 2000; Anthony and Fabricius, 2000; Fabricius and Wolanski, 2000; Mills and Sebens, 2004; Mills *et al.*, 2004).

Other anthropogenic, terrestrial sources of nitrogen to reefs include groundwater (D'Elia *et al.*, 1981; Littler *et al.*, 2006), and sewage (Hanson and Gundersen, 1976; Lapointe *et al.*, 2005a,b; Risk and Erdmann, 2000; Szmant Froelich, 2002). Since

1960, the use of nitrogen fertilizer globally on an annual basis has increased by a factor of six (Fabricius, 2005; Matson *et al.*, 1997). Fertilizer loss off the land is a major source of anthropogenic nutrients to coral reefs in many regions (Mitchell *et al.*, 2005). In some areas, roosting seabirds can be a major episodic contributor of guano and nitrogen input (Albert *et al.*, 2005; Furnas *et al.*, 2005; Smith and Johnson, 1995). Some of the effects of terrestrial runoff including sediment and nutrient enrichment on coral reefs have recently been summarized by Fabricius (2005).

Gradients of reef health, community structure, biodiversity, and ecological function in near-shore coral reef systems are often associated with environmental gradients influenced by terrestrial runoff (Fabricius, 2005; Fabricius and De'ath, 2004, Fabricius *et al.*, 2005). However, the exact cause of runoff related effects are controversial (e.g., nutrients vs. sediments vs. toxicants vs. fresh-water input) (Szmant, 2002).

Although coral growth itself may be positive in polluted reefs, increased nutrients can cause net reef erosion. Therefore the paradoxical combination of normal coral growth and net reef erosion needs to both be taken into consideration for any whole reef perspective approach to coral reef health (Edinger *et al.*, 2000).

In terms of nitrogen inputs, the effects of terrestrial run-off to marine systems may be better understood in a biogeochemical context and the gradients in microbial processing of nutrients (Alongi and McKinnon, 2005). For instance, denitrification in the coastal zone may help reduce the amount of N that is transported to offshore reefs (Seitzinger, 1988) and coastal habitats such as mangrove and intertidal zones may provide significant protection buffers for offshore coral reefs by trapping, transforming and storing sediments and organic matter. While intact coastlines may be able to mitigate some of the runoff effects, these vital areas are under increasing development pressure worldwide with large scale clearing of mangrove and coastal vegetation as well as the steady increase in impervious surfaces. These synergistic effects may therefore increase the effective load of nutrients reaching coral reef environments (Furnas *et al.*, 2005).

3.1.4. Sewage

Sewage pollution remains a concern in many coral reef regions, particularly in developing nations, and is widely considered as one of the major causes of the demise of coral reef health (Smith *et al.,* 1981; Brown and Howard, 1985; Edinger *et al.*, 1998, Furnas *et al.*, 2005; Ginsburg and Glynn, 1994; Hatcher and Frith, 1985; Johannes, 1975; Wilkinson, 1993). It is not only a concern from an environmental health perspective but for human health as well (Colwell, 2004).

Documented effects of sewage impacts on reefs include increased particulate and sediment load resulting in smothering of corals, lower photosynthetic rate by corals, increased bioerosion, hypoxia, algal overgrowth (see above) as well as changes in community structure and shifts towards filter-feeding heterotrophic organisms (Risk and Erdmann, 2000). In Indonesia, Edinger *et al.* (1998) found that reefs subjected to land based pollution (sewage, sedimentation and or industrial pollution) had a reduction in diversity of 30–50% at 3 m and a 40–60% reduction of diversity at 10 m, relative to unpolluted reefs.

Wastewater nutrients and animal wastes are generally enriched in ^{15}N, the heavy stable isotope of nitrogen (Sammarco *et al.,* 1999; Heikoop *et al.*, 2000).

^{15}N-signatures have been used as a tracer of fertilizer input to corals reefs in Bali (Marion *et al.*, 2005). New methods of tracking sewage plumes through using ^{15}N/^{14}N or δ^{15}N-signatures in bioindicator organisms such as stomatopods, macro-algae, mangroves, and corals (Heikoop *et al.*, 2001; Risk and Erdmann, 2000; Risk and Heikoop, 1997; Mendes *et al.*, 1997) have recently been implemented in some reef areas thereby providing a measure of integrated water quality over longer time scales rather than the snapshot of water quality obtained with discrete chemical measurements of nutrients (Costanzo *et al.*, 2001, 2005; Hoegh-Guldberg *et al.*, 2004; Muscatine *et al.*, 2005; Udy *et al.*, 2005). For instance, coral tissue δ^{15}N from *Porites lobata* was significantly higher in sewage affected reefs than reference sites in 70% of impacted reef sites in Indonesia (Heikoop *et al.*, 2001). Excess nitrogen can lead to increased zooxanthellae density and ultimately decreases in translocation of carbon and nitrogen to the coral host as the coral loses control of the symbiotic relationship, with the symbionts increasing in number and holding onto their photosynthate for their own growth (Fig. 21.2) (Heikoop *et al.*, 2001); this suggests that sewage derived nitrogen can disrupt normal coral–zooxanthellae physiology.

One very clear cut case where nutrient input, and in particular nitrogen, has had a serious and demonstrable deleterious effect on coral reefs is Kaneohe Bay, Hawaii (Smith *et al.*, 1981; Hanson and Gundersen, 1976; Szmant, 2002). A sewage outfall caused a major growth of the green "bubble" algae *Valonia* which overgrew the corals which then died. The sewage outfall was moved and some improvement in the system was seen. However the fragile balance had been disrupted, and the sediments contained a long memory of nutrients. Subsequently non-point sources became a problem at Kaneohe Bay as well and it is still not back to its original condition. This is partly due to organic matter accumulation, which is being observed in many reef systems (D'Elia and Wiebe, 1990; Furnas, 2003) as inputs of nutrients, particularly N, are exceeding losses. The impaired system of Kanehoe Bay was further damaged, due to loss of herbivores, and the introduction of the invasive algae *Dictyoshpaeria cavernosa* (Stimson *et al.*, 2001).

Increased nitrogen can also increase phytoplankton in the water column as well as macroalgal abundance, particularly in areas where over-fishing has occurred (Hughes *et al.*, 2003; McCook, 2001; McCook *et al.*, 2001; Szmant, 2002). The increase in BBD also appears to be increasing in prevalence which may also be associated with elevated nutrients input and or sewage outflows (Antonius, 1988; Bruckner *et al.*, 1997; Taylor, 1983a,b). Kuta and Richardson (2002) found a positive relationship between elevated nitrite and BBD incidence, but no positive correlation with nitrate, ammonium, or phosphate. BBD is, however, routinely observed on pristine reefs far from acute nutrient perturbations. Therefore, the exact relationship between BBD and water quality remains unknown.

3.2. Sea surface warming, coral disease and nitrogen dynamics

Global climate change is a major threat to coral reefs (Phinney *et al.*, 2006 and references therein), with reefs already exhibiting climate warming effects in terms of coral bleaching due to zooxanthellae expulsion (Brown, 1997; Hughes *et al.*, 2003). Thermal sensitivity of reef-building corals may prove to be the "Achilles

heel'' in terms of reef ecosystem resilience (Graham *et al.*, 2006). Climate change may further exacerbate population phase shifts in degraded reefs with bleaching coral mortality providing more space for macroalgae, and filamentous cyanobacteria and less space for coral recruitment to help recovery on over-fished and/or eutrophied reefs (Hughes *et al.*, 2007; Kuffner and Paul, 2004).

What direct effects climate change will have on various aspects of nitrogen cycling on reefs including coral bleaching events and zooxanthellae expulsion, remains to be seen. The subsequent consequences for nitrogen conservation and nitrogen acquisition of the corals and other symbioses, on the reef will undoubtedly be affected as the balance between symbionts and host interaction is very sensitive to environmental conditions (Knowlton, 2001).

Rising average sea surface temperatures resulting from global climate change may increase the amount of nitrogen fixed globally (Karl *et al.*, 2002) and in reef environments specifically (Paul *et al.*, 2005) due to both increased physiological rates of nitrogen fixation as well as increasing cyanobacterial populations (Paerl and Husiman, 2008). Recent research has also demonstrated an increased rate of nitrogen fixation on coral skeletons that have been subjected to bleaching events, which in turn may increase the availability of nitrogen in these systems for an extended period beyond the initial bleaching event. The scale of these events are predicted to become more prevalent, this may increase suitable substrate for diazotrophy as a result of climate change (Davey *et al.*, 2008). Similarly, increases in macroalgal populations (Lapointe *et al.*, 2005a,b; McCook *et al.*, 2001) may also increase the surface area available for both diazotrophic heterotrophic bacteria and cyanobacteria.

Warming conditions could also lead to the increase in the prevalence of harmful cyanobacterial assemblages such as the toxin producing genus *Lyngbya* (e.g., Albert *et al.*, 2005; Paul *et al.*, 2005), as well as species that have been linked with BBD in corals including *Phormidium* and others (e.g., *Oscillatoria submembranaceae; Beggiatoa; Desulfovibrio; Phormidium corallyticum*) (Rosenberg and Loya, 2004 and references therein). The prevalence of BBD in the Caribbean increases during the warmest months of the year when temperatures are above 30°C (Richardson, 2004).

4. Elevated Nutrients on Coral Reefs Experiment (ENCORE)

A replicated *in situ* fertilization experiment was conducted on the GBR at One Tree Reef Lagoon, Australia during 1993–1996 (Larkum and Koop, 1997). This experiment (ENCORE) was specifically designed to address nutrient effects *in situ* based on previous smaller scale fertilization studies in micro-atolls in One Tree Island lagoon (Kinsey and Davies, 1979; Kinsey and Domm, 1974). Laboratory results, and small scale mesocosm experiments had, to that point, indicated that nutrients additions could perturb the natural physiology of corals and some other invertebrate reef organisms (Belda *et al.*, 1993; Ferrier-Pages *et al.*, 1998; Hoegh-Guldberg and Smith, 1989; Jokiel *et al.*, 1994; Muller-Parker *et al.*, 1994; Muscatine *et al.*, 1989), but evidence directly from the field and simultaneous assessment of

different reef organisms and processes were largely lacking (Hoegh-Guldberg, 2004a,b; Koop *et al.*, 2001).

While increasing nutrient loads were perceived as deleterious, what the precise responses that different reef organisms would have was unknown. Therefore, part of the aim of the ENCORE study was to identify organisms or community response factors which could be used as indicators of ecosystem degradation (Steven and Larkum, 1993). Twelve micro-atolls, or patch reefs were fertilized with N (ammonium chloride), P (sodium phosphate) and N + P over the course of 3 years. Dosing with nutrients occurred to the internal ''lagoons'' of each micro-atoll during the ∼4–5 h of each low tide when they were physically isolated from the larger lagoon. During the first ∼ year of the experiment the atolls were dosed at N levels that were approximately 2 times ambient (average ∼0.7–11 μM). During the second year the dosage was increased to approximately 6 times ambient (∼0.7–36 μM) (Steven and Atkinson, 2003).

The results of these experiments are summarized in Fig. 21.4. Overall the parameters that were most responsive to nutrient enrichment were coral fertilization, benthic microbial processes, stomatopod recruitment and symbiont numbers (Koop *et al.*, 2001). In terms of direct effects to nitrogen cycling, nitrogen fixation increased in P fertilized plots and decreased in the N treatments, whereas denitrification increased in the N and N + P treatments and macroalgae showed rapid uptake of N (Koop *et al.*, 2001; O'Neil and Capone, 1996). Other effects that were seen in the +N treatments included reduced fecundity, fertilization and settlement in corals as well as reduced lipid content in the corals (Harrison and Ward, 2001; Koop *et al.*, 2001; Ward and Harrison, 2000). Increased number of zooxanthellae in corals and the giant clam *Tridacna* were also observed; as well as increased N:P and reduced NH_4^+ uptake capacity in the giant clams. In the +P treatments; coral reproduction was affected with reduced settlement of larvae and smaller eggs, as well as increased mortality in adult corals. Corals also had increased linear extension, but were more fragile. Some species of coral had more zooxanthellae and increased lipid content. *Tridacna* had decreased N:P. Macroalgae showed reduced alkaline phosphatase activity. In the +N+P treatments, there was a mixed result, with coral reproduction again affected in terms of reduced fecundity, and settlement as well as increased mortality. Corals had increased linear extension but were more fragile; zooxthanthellae numbers stayed the same, but corals showed a reduction in lipids. There was rapid uptake by macroalgae of both N and P and reduced alkaline phosphatase activity. There was reduced stomatopod recruitment. *Tridacna* had no change in N:P but increased zooxanethellae (Fig. 21.4).

What was not observed, however, was a massive overgrowth of algae. Overgrowth of algae in response to nutrients may in fact only be detectable in the absence of grazers in over-fished areas (McCook *et al.*, 2001). As a protected research area within the Great Barrier Marine Park, One Tree Island has an abundant fish population; therefore underscoring the probable importance of top-down control with a healthy grazing fish population for the health and resilience of a system under nutrient stress (Hughes *et al.*, 2007). The increased +N and P inputs increased denitrification in the sediments, while P inputs increased sedimentary nitrogen fixation. Increased denitrification with P enrichment may have been due to
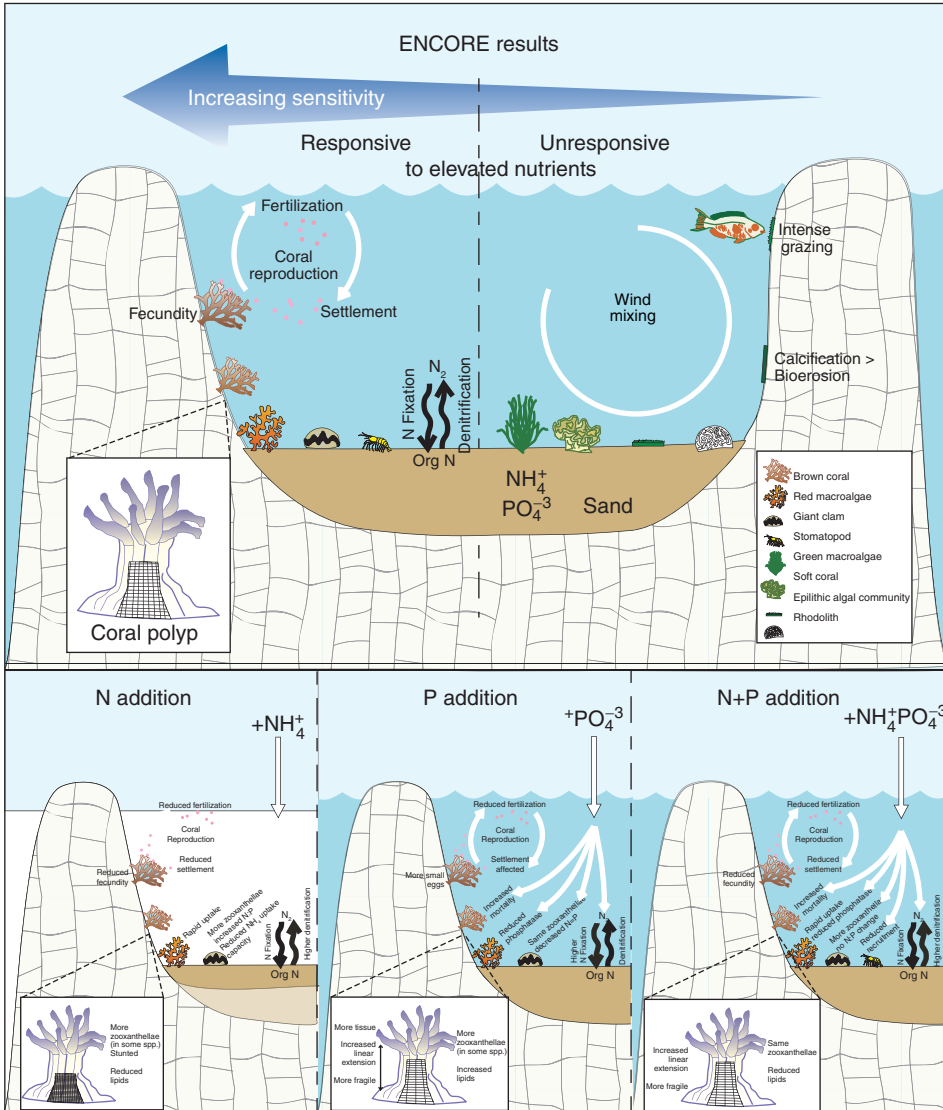
Figure 21.4 Summary of the major effects of elevated nitrogen on coral reef processes from the ENCORE project, showing a gradation in responsiveness to nutrient additions; as well as the individual effects of +N; +P as well as combined effects of +N+P.

concomitant increases in nitrogen fixation providing substrates for denitrification (Fig. 21.4). This suggests a very dynamic feed–back capacity of the reef system which allows it to respond to changes in nutrients (Capone, 1996a). While the potential for denitrification on reefs has been demonstrated (Johnstone *et al.*, 1990; O'Neil and Capone, 1996), the information on its quantitative significance is poorly constrained (Capone, 1996a). Part of the missing picture in the ENCORE study was that changes in the sedimentary benthic microalgal (BMA) community, and nutrient

uptake by this important component of the benthic community were not fully quantified (Koop *et al.*, 2001).

δ^{15}N isotope signatures in two species of corals from +N treatments provided evidence that 50–100% of the overall nitrogen budget of enriched corals could come from ammonium suggesting that dissolved nutrients can have a significant effect on nitrogen uptake and dominated both the δ^{15}N signature of both the symbiont and the host. It also suggested that the nutrient history of the reef may be recorded within the organic components of the symbiotic dinoflagellates, as well as the corals and the skeletal associates which may therefore possibly be used as indicators of the chronology of nutrient concentrations surrounding a coral during its lifetime (Hoegh-Guldberg, 2004a,b; Marion *et al.*, 2005).

5. CONCLUSION

The nitrogen cycle is key to high productivity and healthy ecosystem functioning on coral reefs. By trying to unravel the complex interaction and specific roles of each component of the reef ecosystem and coral holobiont with regard to the uptake, exchange and transformation of nitrogen, insight may be gained into "how corals have come to dominate tropical near-shore systems and how they may be able to adapt to changing environmental conditions" (Rohwer and Kelley, 2004).

As threats to coral reefs worldwide have grown, the urgency to better understand the basic natural functioning in these systems has also grown in order to be better able to assess these impacts on reefs. All aspects of nutrient cycling are undergoing change with increases in anthropogenic nutrification, and multiple perturbations including increased sedimentation, runoff and levels of pollutants. Therefore it is important to continue to gain more insight into nitrogen cycling in coral reefs ecosystems as the ability of coral reefs to "adapt to these perturbations in the past is no guarantee of resilience in the future" (Hughes, 1994). We must, as Bellwood *et al.* (2004) suggest, confront our global coral reef crisis worldwide and reassess our current management practices (Hughes *et al.*, 2003; Phinney *et al.,* 2006 and references therein).

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Nitrogen Dynamics of Coastal Salt Marshes

Charles S. Hopkinson *and* Anne E. Giblin

Contents

1. Introduction

Salt marshes are among the most productive ecosystems in the world—rivaling that of intensively cultivated agriculture (Odum, 1971). This high production is attributable to several factors, including nutrient enrichment from watershed runoff and tidal mixing (Day *et al.*, 1989). These are important components of estuarine systems because they provide a food source to both estuarine and coastal ocean consumers, serve as habitat for numerous young and adult estuarine organisms, provide refuge for larval and juvenile organisms, and regulate important components of estuarine chemical cycles. Nitrogen (N) dynamics have been well studied since N is the most limiting element of salt marsh primary production. However, their location, at the land–sea interface, places salt marshes in the path of ever-increasing N loads from land, raising concern about their susceptibility to eutrophication and interest in their potential for removing the N before it enters estuarine and coastal ocean waters.

In this review we provide a very brief background on salt marshes and then focus on: (1) the role of N in regulating primary production, plant zonation, and community structure, (2) the input and output N budget of salt marshes, and (3) the cycling of N in the plant-sediment system. We also include a brief discussion of marshes and

eutrophication and place marshes in the context of the global N cycle. We did not discuss the potentially important role of salt marsh microalgae in N dynamics and in support of secondary production, focusing instead on marsh macrophytes.

2. BACKGROUND

Estuarine salt marshes are intertidal wetlands vegetated by salt tolerant, non-woody, rooted, vascular plants. They are found in temperate, boreal and arctic biogeographic provinces worldwide and have an areal extent of 3.8×10^5 km^2 (Maltby, 1988). In tropical provinces, salt marsh vegetation is replaced by mangrove forests, which are not included in this review. Worldwide, over 600 species of plants grow in salt marshes (Chapman, 1974). Species diversity is lowest in the arctic (Jefferies, 1977) and generally increases toward the tropics. The most extensive salt marshes occur in estuarine systems with abundant rainfall or river runoff, muddy to sandy coasts, and moderate climate (Day *et al.*, 1989). Atlantic and Gulf of Mexico salt marshes (including oligohaline, tidal marshes) are floristically rich with 347 vascular plant species in 177 genera in 75 families (Duncan, 1974). Pacific coast marshes are less rich with 78 species between Barrow, Alaska and Baja California (MacDonald and Barbour, 1974). Only 28 vascular plant species are reported for a Hudson Bay salt marsh (Kershaw, 1976). Although salt marshes are rich in flora, they are dominated by only a few species. Atlantic and Gulf Coast marshes of the US are dominated mostly by *Spartina alterniflora* Loisel., *Spartina patens* (Ait.) Muhl., *Salicornia* sp., and *Juncus* sp. Salt marshes of the US west coast are dominated by *Spartina foliosa* Trin., *Salicornia virginica* L., and *Distichlis spicata* (L.) Greene. *Puccinellia phryganodes* (trin.) Scrib. and Merr. and *Carex subspathacea* Wormsk dominate in the Hudson Bay region. *Puccinellia maritime, Halimione portulcoides, Suaeda maritime*, and *Limonium vulgare* historically dominated salt marshes in northern Europe. Over the last several decades the hybrid *Spartina anglica* has become more common and in some cases dominant in northern Europe (e.g., Morris and Jensen, 1998). Non-native species of *Spartina* have also taken over salt marshes or mud flats on US Pacific coasts, and in New Zealand, Tasmania, and China (Valiela, 2006).

Salt marshes are exposed to a unique combination of environmental variables, including strong salinity gradients, fluctuating water levels and water tables, and anaerobic, waterlogged sediments. There are distinct patterns of species distribution and biogeochemical zonation in salt marshes that arise from gradients in elevation and porewater drainage (Haines and Dunn, 1976). Elevation typically increases with distance away from tidal creeks and bays, which results in differences in tidal flooding frequency and duration. In expansive marshes, elevation sometimes plateaus or even decreases in interior marsh areas, far from tidal creeks (e.g., Louisiana, DeLaune *et al.*, 1976). Drainage decreases away from creekbanks (Harvey *et al.*, 1987; Howes and Goehringer, 1994; Nestler, 1977; Riedeberg, 1978), which can result in hypersaline conditions when flooding is infrequent. Probably the most important effects of flooding and drainage are in the sediment chemical environment (Osgood and Zieman, 1998). Flooding and porewater drainage affect sediment oxygen

availability and redox potential. These in turn affect solubility of various elements both beneficial and toxic to plant and animal life (Patrick and DeLaune, 1977). Redox potentials vary from $>+400$ mV in the upper few centimeters of frequently flooded and drained sediments to <-200 mV at greater depths and poorly drained sediments (Howes *et al.*, 1981). There are important interactions among the ionic forms of N, Mn, Fe, and S along the redox potential gradient. These strong horizontal and vertical depth gradients also change seasonally with the growth and senescence of the dominant macrophytes. This rich biogeochemical diversity leads to a wide array of possible microbial N transformations but also greatly complicates the full quantification of the marsh N cycle.

3. Nitrogen Cycling Processes in Salt Marshes

3.1. Nitrogen and salt marsh plants and communities

Nitrogen plays a major role in controlling the dynamics of marsh plants and plant communities. Two excellent reviews on the physiological ecology and effects of N-enrichment on intertidal marsh plants include Haines and Dunn (1985) and Morris (1991). Supply of N affects: (1) plant production and biomass, (2) plant architecture, resource allocation, and tissue N content, (3) plant species composition, and (4) marsh community structure.

3.1.1. Plant production and biomass

It has long been observed that there are strong spatial gradients in marsh plant shoot density, annual productivity and standing stock of roots and rhizomes in salt marsh systems (Gallagher, 1974; Gallagher *et al.*, 1972). Highest levels of biomass and productivity are found in creekbank marshes or in areas with good porewater drainage. It has also been observed that the differences in biomass and productivity are not due to genetic differences between short and tall forms of *S. alterniflora* (Shea *et al.*, 1975; Valiela *et al.*, 1978) and that differences can be reversed by N fertilization (Broome *et al.*, 1975; Gallagher, 1975; Patrick and DeLaune, 1976; Sullivan and Daiber, 1974; Valiela and Teal, 1974).

The strong growth response of marsh plants to N fertilization suggests that porewater inorganic N concentrations are limiting primary production. Under laboratory conditions with aerated sediments, however, those same concentrations of inorganic N (50–200 μM) can support maximal uptake rates. Comparisons of V_{max} and K_m determined from aerobic culture experiments with field measurements of available NH_4^+ suggest no lack of the inorganic N necessary for *S. alterniflora* to grow well (Morris, 1984). NH_4^+ concentrations in short *S. alterniflora* marshes are often 1–2 orders of magnitude higher than K_m values determined in aerobic solution culture experiments. Thus N limitation is not due to scarcity of available N per se, but is instead a secondary effect of factors that restrict uptake of limiting nutrients.

Other laboratory culture experiments on NH_4^+-uptake kinetics in *S. alterniflora* and *S. patens* showed that anaerobic conditions, salinity, and H_2S concentrations decrease the ability of marsh plants to take up N (Bradley and Morris, 1990; Morris, 1984).

Reductions in $V_{\max}$ under anaerobic conditions are even greater with increased salinity (3 vs 32 ppt). Dissolved sulfide concentrations as low as 0.25 mM inhibit NH_4^+ uptake and stop uptake at concentrations greater than 2.0 mM. N uptake by *S. patens* is more sensitive to salinity and anoxia than is uptake by *S. alterniflora*. High salinity influences N availability to marsh plants in two ways. Haines and Dunn (1976) suggested that competitive inhibition of NH_4^+ uptake by Na^+ could result in increased energy expenditure for NH_4^+ uptake at high salinities, thus reducing the energy available for plant growth (Jefferies, 1981). Marsh halophytes also change internal N allocation in response to saline conditions. Salt is actively excluded from the transpiration stream as H_2O passes through the plant (Antlfinger and Dunn, 1983). Many halophytes, including *S. alterniflora, S. patens*, and *Limonium* sp., have salt excreting glands (Anderson, 1974). NaCl that is taken up is sequestered in vacuoles in most halophytes, thereby protecting the cytoplasmic metabolic machinery. To balance the low osmotic potential in the vacuoles, the free amino acid proline and the quaternary ammonium compound, glycinebetaine, are produced and accumulate in the cytoplasm (Cavalieri and Huang, 1981). Cavalieri and Huang estimated that up to a third of the total leaf N could be in the form of proline and glycinebetaine. The higher the sediment salinity, the greater the proportion of the already limited N supply that is allocated for osmoregulation. Accumulation of these compounds by salt stressed plants can significantly reduce the N available for growth.

Anaerobic respiration in marsh plant roots contributes to an apparent N limitation. Many halophytes respond to oxygen deficiency in plant roots by switching to fermentation as a means of producing ATP. The presence of alcohol dehydrogenase activity is a measure of alcoholic fermentation in *S. alterniflora* and *S. patens*. Concentrations of ethanol, lactate, alanine, and malate increase under conditions of decreased redox potential (Burdick *et al.*, 1989; Mendelssohn *et al.*, 1981). Reduced plant growth may be ascribed to a decrease in carbon (C) available for growth resulting from the increased consumption of glucose during fermentation and subsequent diffusion and loss of ethanol from the roots. Field data suggest that H_2S concentrations further reduce energy yield in plant roots (Koch *et al.*, 1990). Apparently, sulfide limits the potential for root anoxic energy production, which further reduces the energy available for root N uptake.

The current general explanation for local patterns in salt marsh primary production is that sediment drainage, such as that found in streamside locations of the marsh, creates a less reduced sediment substrate and prevents the accumulation of toxic levels of hydrogen sulfide and high levels of salinity. Sediment aeration also reduces the potential for root anaerobic fermentation, which is stimulated by oxygen deficiency. By allowing air entry, drainage also influences the production rate of H_2S and soluble iron concentrations (King *et al.*, 1981; Mendelssohn and Seneca, 1980). This combination of factors helps to alleviate apparent N limitation and results in creekside zones being more productive than the less drained, interior sites. By experimentally enhancing marsh porewater drainage, Wiegert *et al.* (1983) demonstrated that plant productivity can be enhanced and the effects of water logging reversed.

The much lower level of apparent N limitation in creek banks also explains why fertilization has a much greater effect on inland plants than on creekside plants,

which may show no response at all to fertilization (Valiela *et al.*, 1978). An indirect effect of N fertilization and increased plant growth is greater evapotranspiration, which leads to increased air entry and higher sediment redox potentials (Howes *et al.*, 1981). Oxidation in the rhizosphere increases nutrient availability, which further increases nutrient uptake by the plants.

3.1.2. Plant architecture

Nitrogen availability, as mediated by edaphic factors that limit plant NH_4^+ uptake, also influences plant morphology, tissue N concentrations and the allocation of plant resources. Fertilized short *Spartina alterniflora* comes to resemble tall form *S. alterniflora* in leaf width, stem diameter and general appearance (Rogers *et al.*, 1998). Tissue N is dramatically increased in short form *S. alterniflora* receiving N fertilizer (Covin and Zedler, 1988; Gallagher, 1975). Fertilization increases branching in *S. virginica* (Boyer *et al.*, 2001). N availability also influences root topology and diameter. Bouma *et al.* (2001) suggested that herringbone root systems are most efficient in terms of nutrients gained per C invested and that dichotomous root systems offer a greater potential for exploring the sediment, which contributes to potential competitiveness of plants growing in N limited sediments.

NH_4^+ supply influences the allocation of C resources between rhizomes and shoots (Haines and Dunn, 1976; Rogers *et al.*, 1998; Valiela *et al.*, 1976). Morris showed that rhizome–shoot ratios ranged between 0.35 at a high rate of N supply to 0.93 at a low rate of N supply. Although aerial and rhizome production were stimulated by increased N supply, total root production did not differ significantly between treatments (Morris, 1982). Nitrogen supply affects plant developmental processes as opposed to the specific rate of photosynthesis. Root to shoot ratios increase as plant growth becomes limited by nutrients (Mooney, 1972), and this response decreases plant production independently of any changes in weight-specific rates of photosynthesis by decreasing the growth rate of leaves. In Morris's experiments with *Spartina*, there was an insignificant effect of N supply on specific growth rate, but a 4-fold difference between treatments in total net production, and the major determinant of this difference was the effect of N supply on plant development.

3.1.3. Marsh plant community structure

Numerous studies have demonstrated an effect of N supply on marsh plant succession, the distribution of high and low marsh species, and plant invasions. Many of these studies have illustrated the linkage between these changes and N-enrichment due to human development adjacent to marshes or urbanization (Bertness *et al.*, 2004; Chambers *et al.*, 1998; Wigand *et al.*, 2001). N-enrichment promotes the invasion of *Phragmites* into the salt marsh. By reducing the N competitive ability of high marsh plants, *Phragmites* can invade these areas and then by shading short plants typical of the high marsh become a monoculture. A long-term fertilization experiment identified the interactions between marsh elevation, disturbance and N availability in creating mosaics of short and tall forms of *S. alterniflora*, bare patches, *Salicornia europaea, S. patens*, and *D. spicata* (Valiela, 1995).

In southern New England, an effect of N-enrichment is to shift the boundary between low marsh, *S. alterniflora,* and high marsh, *S. patens*. Thus *S. alterniflora* occupies an increasingly large area of the marsh relative to *S. patens*. The distribution of *S. alterniflora* and *S. patens* is a product of both plant competition for nutrients and stress tolerance (Bertness *et al.*, 2002; Emery *et al.*, 2001; Levine *et al.*, 1998). The low elevation border for *S. alterniflora* is set by the physical stress of flooding, while the high elevation border, which marks the boundary between *S. alterniflora* and *S. patens,* is set by plant competition for nutrients. Competitively superior plants dominate the high marsh habitats, whereas stress-tolerant plants dominate the low marsh (Bertness *et al.*, 2002; Emery *et al.*, 2001; Levine *et al.*, 1998). With N-enrichment, the border between low and high marsh plants moves up in elevation; *S. alterniflora* displaces *S. patens*, which in turn competitively displaces *Juncus gerardi*. Similar changes have been observed in other salt marsh systems (Rogers *et al.*, 1998), including southern California, where *S. virginica* is superior to *S. foliosa* in competing for N (Covin and Zedler, 1988) and in European low marshes, where fertilization promotes the growth of *Suaeda maritima*, which then displaces *P. maritima* by shading it (Kiehl *et al.*, 1997). Likewise, N-enrichment can accelerate plant succession in salt marshes, with displacement of short, early successional plants, such as *L. vulgare*, with taller, more mature plants, such as *Elymus pycnanthus* and *Artemisia maritima* (van Wijnen and Bakker, 1999).

3.1.4. Salt marsh community structure

When N supplies are increased, salt marsh communities behave similarly to more widely studied terrestrial grassland and forest communities (Southwood, 1973; Tilman, 1988). Nitrogen enrichment can structure marsh communities by mediating both plant competitive and facilitative interactions and plant-herbivore interactions. For example, Levine *et al.* (1998) showed that nutrients mediate *Iva frutescens* and *J. gerardi* competitive and facilitative interactions, which has an indirect effect on aphid populations. Other studies showed the plant-herbivore effect to be propagated even to a third trophic level (Gratton and Denno, 2003). Community affects can be mediated by changes in plant architecture, plant N content or shifts in plant community structure. For example, studies have shown that nutrients can have positive effects on the abundance and distribution of insect herbivores by increasing the nutrient content of host plants (e.g., Bowdish and Stiling, 1998; Onuf *et al.*, 1977; Vince *et al.*, 1981). Changes in plant community structure, such as occur with *Phragmites* expansion can in turn lead to a reduction in total plant and animal biodiversity as many native species of plants are replaced by the cosmopolitan species (Chambers *et al.*, 1999).

There is less information on how salt marsh community structure affects N cycling within a marsh. Clearly the partial or complete loss of salt marsh vegetation by disturbance can lead to dramatic changes in the N cycle. In the Canadian arctic, intense grazing by geese reduces plant N uptake, total N stocks and increases the rate of N fixation by cyanobacteria (Wilson and Jefferies, 1996; Walker *et al.*, 2003). When areas are invaded by non-native *Spartina* species, there can be significant changes in the uptake and loss of N from sediments mediated by plants, and changes in sediment redox chemistry (Hacker and Dethier, 2006; Lee, 2003). These changes also likely

affect N cycling although this has not been well studied. Studies of N budgets in different vegetation zones of marshes show considerable differences between sites but it is difficult to separate out the role of the vegetation from physical factors (Bouchard and Lefeuvre, 2000). Because marsh plants change the physical structure of the habitat where they live, there are dynamic feedbacks between plant growth, organic matter deposition and nutrient availability (Tyler and Zieman, 1999).

3.2. Nitrogen budgets for salt marshes

There is tremendous geographic variability in the spatial orientation and connections of salt marshes within estuaries and of estuaries with adjacent uplands and the ocean (Fig. 22.1). This paper focuses on the salt marsh ecosystem and associated tidal creeks and not the larger estuarine ecosystem, which may include deeper and extensive bays and sounds. However, it should be recognized that some marshes, especially those that do not have freshwater inputs from rivers, have no true open estuarine area and directly exchange with the ocean. Salt marshes are linked to adjacent terrestrial environments through water and material inputs from rivers, groundwater and precipitation. In cases where the majority of these inputs first pass though the open
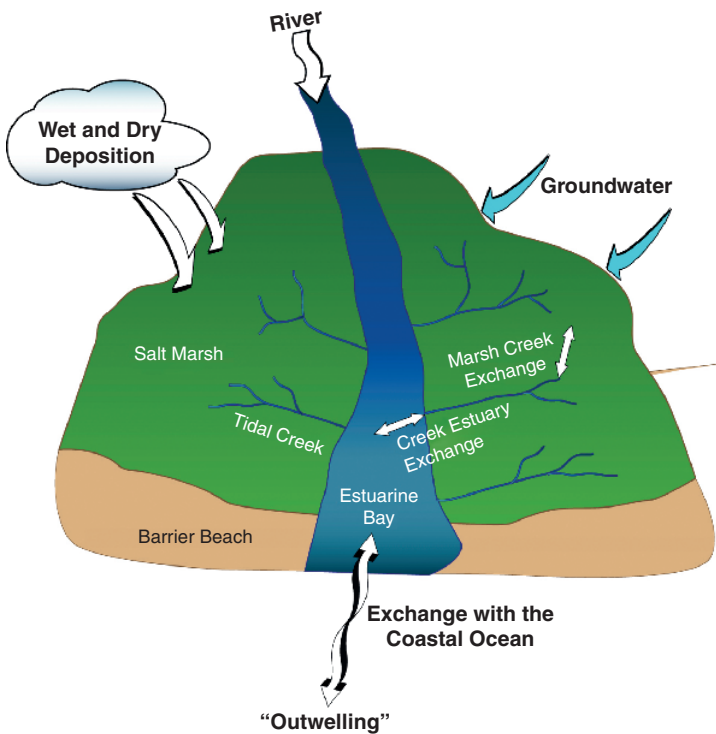
Figure 22.1 Geography of a salt marsh ecosystem showing linkages to atmosphere, terrestrial run-off via rivers and groundwater and the coastal ocean. See text for a description of boundaries used in this review of nitrogen dynamics.

estuarine area it can be quite difficult to quantify the portion that actually reaches the marsh. Quantifying the role of salt marshes in exchanging nutrients with the coastal ocean is similarly difficult and comparisons across systems further complicated by various studies defining system boundaries differently. Exchanges even between the marsh platform and the adjacent tidal creeks can be very difficult to quantify because flow paths change over a tidal cycle. The marsh surface exchanges dissolved and particulate materials with creeks during high tides, while at low tide, porewater drainage tends to export materials from the marsh platform (Fig. 22.2).

In the following four sections we present the major components that must be considered in constructing whole ecosystem N budgets for a salt marsh: (1) Internal standing stocks of N in organic and inorganic pools, (2) abiotic and biotic sources of N from the atmosphere and terrestrial systems, (3) sinks of N either as long-term accretion in marsh sediments or gaseous losses to the atmosphere, and (4) abiotic and biotic exchanges of N with adjacent estuarine systems.

3.2.1. Plant and sediment stocks

The amounts of the various forms of N in salt marshes vary considerably geographically (Table 22.1). Percent N by weight in marsh sediments ranges from about 0.15% in U.S. southeastern marshes to 2% in organic rich, New England marshes. Differences are primarily attributable to the organic content, which varies inversely with bulk density. Peaty marshes have low bulk density, while sediments with low organic content, such as those in Georgia salt marshes, have a higher bulk density. Total N content ranges from about 420 to 1260 g N m^{-2} integrated over 30 cm depth. Most N is in particulate form (organic and fixed N). Dissolved stocks are more than 2 orders of magnitude lower. Of the dissolved stocks of N, the largest stock is dissolved organic N, followed by NH_4^+, and then NO_3^-. Nitrate is only present in the thin, surface oxidized layer and perhaps immediately adjacent to roots in the rhizosphere. NH_4^+ is present both in dissolved (free) and extractable forms (e.g., 1 N KCl), with the dissolved form increasing with increasing salinity (Gardner et al., 1991). At seawater strength salinity, free and extractable forms are roughly
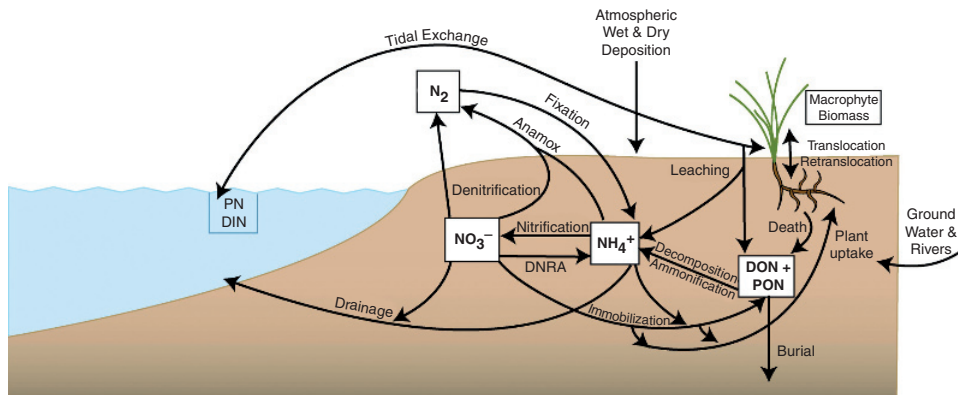
Figure 22.2 Diagram of major nitrogen stocks, fluxes and transformations in a salt marsh–tidal creek system.

Table 22.1 A comparison of nitrogen standing stocks between salt marshes along the Gulf of Mexico (Louisiana) and the US Atlantic Ocean coast (Georgia and Massachusetts). Stocks integrated to 30 cm.

	New England	Southeast	Gulf of Mexico
Bulk Sediment			
% N,	1.2–2.0%,	0.15–0.50%,	0.46–0.88%,
g N m^{-2}	500–840,	462–490,	580–1300,
Bulk Density (g/cc)	0.06–0.14	0.4–0.8	0.12–0.46
Porewater			
NH_4^+ μM,	45–615 μM,	40–70 μM,	20–928 μM, extr
g N m^{-2}	1.0–10.0	0.014–.018	0.15–11.2
NO_3^- μM,	0–12 μM,	2–18 μM,	NA
g N m^{-2}	0.01–0.06	0.03–0.04	
DON μM,	50–400 μM,	157–192 μM,	NA
g N m^{-2}	1.0–6.5	0.56–0.70	
Plants			
Live culms %N,	0.6–2.1%,	0.59–2.02%,	0.7–2.15%,
g N m^{-2}	0–8.4	1.00–12.88	1.4–11.06
Live roots %N,	NA,	0.35–0.57%,	0.4–0.6%,
g N m^{-2}	2.8–22.4	2.52–6.86	8.4–12.6
Dead culms %N,	NA,	0.38–0.90% N,	0.4–0.8%,
g N m^{-2}	0–22.4	1.4–5.6	1.4–7.0
Sources	Valiela, (1983), Chambers, (1997), Henry and Jefferies, (2002), Windham and Meyerson, (2003), Bowden, (1984), Clarke, (1985)	Haines, (1979), Chalmers (1979), Hopkinson and Schubauer, (1984), Gallagher *et al.* (1980), Stribling and Cornwell (2001)	DeLaune *et al.* (1979, 1981), Casselman *et al.* (1981), DeLaune and Pezeshki, (1988), DeLaune and Patrick, (1990), Mendelssohn, (1979)

Note: "extr" refers to extractable ammonium concentration.

equivalent. Free NH_4^+ concentrations typically increase with depth and can approach 1 mM in concentration. Porewater DON is measured infrequently, even though it generally makes up 45–80% of total dissolved N.

Live and dead plants are additional major stocks of N in salt marshes (Table 22.1). Tissue N concentrations are higher in aboveground tissues than roots and rhizomes and range from about 0.6% N in mature culms to >2% N in rapidly growing shoots. The total stock of N in aboveground plant tissues ranges from 0 (winter) to about 14 g N m^{-2} at the time of peak live standing crop (late summer). Root and rhizome concentrations of N are lower than in culms but because of the much larger standing crop of belowground tissues, the total mass of plant N is higher (up to 22.4 g N m^{-2}). Following the death of roots and rhizomes, a large percentage of N is rapidly leached. However, N content of dead plant material increases upon further decomposition as microbes immobilize inorganic N (see later section on decomposition).

3.2.2. Inputs

The major sources and pathways by which allochthonous sources of N enter salt marshes are from their watersheds including surface water (rivers) and groundwater. Additional N is delivered from airsheds via atmospheric deposition (wet and dry deposition, see Chapter 9 by Seitzinger and Harrison, this volume), from adjoining seas via tidal exchange, and N$_2$ gas in the atmosphere via biological N fixation (see Fig. 22.2 and Chapter 2 by Carpenter and Capone, this volume). The magnitude and relative importance of the various sources seem site specific. As salt marshes are but components of the larger estuarine system and connections with adjacent waters are tied to water level fluctuations, it is difficult to scale inputs to the entire estuary to salt marsh portions of an estuary. For example, riverine inputs to an estuarine bay may pass through to the ocean with little exposure to salt marshes on the bay flanks. Ground water inputs may be more effectively processed, as marshes often border upland regions where groundwater enters (Teal and Howes, 2000).

Riverine Inputs The magnitude of N loading to estuaries from rivers has received considerable attention over the past several decades due to the estuarine eutrophication problem, which is largely driven by excessive riverine and groundwater N inputs (NRC, 2000, see Chapter 9 by Seitzinger and Harrison, this volume). Generally this attention is at the scale of entire estuaries and does not partition loading to various subsections of an estuary, such as salt marshes. On an areal basis estuaries are among the most heavily "fertilized" ecosystems on Earth, because of the their small size relative to the large size of N-leaky watersheds entering them (see Chapter 3 by, Boynton and Kemp, this volume). Several reviews of Scott Nixon indicate that estuarine loading can exceed 40 g N m^{-2} year^{-1}. This rate is twice that typically applied to intensively managed agricultural systems (e.g., 20 g N m^{-2} year^{-1}). The percentage of riverine N inputs to an estuary that actually enters a salt marsh is not well known. Primary factors controlling this input would include the frequency and duration of tidal flooding, seasonal water saturation of marsh sediments, the efficiency of the marsh in "removing" N from floodwaters during inundation, the degree to which inputs are removed by other processes in the estuary (e.g., denitrification, algal uptake), and river water transit time in the estuary. Further discussion of this can be found in Chapter 1 by Boynton and Kemp, this volume.

Groundwater Inputs The majority of freshwater N runoff from land is from rivers with groundwater contributing 6–10% (Burnett *et al.*, 2003). However for salt marshes and estuaries adjacent to topographically low relief uplands, considerable N can be transported by groundwater flow (Valiela and Teal, 1979; Valiela *et al.*, 1978). The importance of groundwater N transport is likely to be greatest in long narrow marshes with a long upland border but small total area, such as the pocket marshes of southern New England and the fringing marshes of Chesapeake Bay and its sub-estuaries. Where urban and residential developments occur on uplands immediately adjacent to salt marshes, there has been a well-documented increase in dissolved inorganic N concentrations (primarily NO_3^-) in groundwater (Moore *et al.*, 2006; Valiela *et al.*, 1990; Weston *et al.*, 2006). Some of the most-studied salt marsh systems in the U.S. have nutrient budgets dominated by groundwater inputs, including Great Sippewissett Marsh in Massachusetts, Flax Pond in New York, North Inlet in South Carolina, and Tomales Bay in California (during summer) (see Chapter 9 by Seitzinger and Harrison, this volume).

Groundwater fluxes have been identified and quantified by a variety of approaches, including seepage chambers, thermal infrared photography, hydraulic modeling (Nuttle and Harvey, 1995), salt balances (Howes *et al.*, 1996; Valiela *et al.*, 1978), tracer additions and ^{228}Ra and ^{226}Ra isotope budgets (Krest and Harvey, 2003) (for overall review see Tobias *et al.*, 2001a). Employing the radium mass balance approach, Krest *et al.* (2000) estimated that groundwater and DIN inputs to the North Inlet salt marsh ecosystem were sufficient in magnitude to support the net primary productivity of the ecosystems and to account for the known export of nutrients from the marsh. Discharge from the marsh was 20–40 l m^{-2} day^{-1}, which represents 5% of the average tidal prism. Inorganic N flux from the marsh to tidal creeks was 12 g N m^{-2} year^{-1} over the annual cycle.

Groundwater inputs are often patchy and highly dependent on specific flow paths in relation to patchy wetland deposits, including silts, clays, and organic matter (Harvey and Odum, 1990; Hemond and Fifield, 1982; Hemond *et al.*, 1984; Shultz and Ruppel, 2002; Snyder *et al.*, 2004). Flow can be focused through highly permeable sands between silt and clay lenses. Portnoy *et al.* (1998) employed a combination of thermal infrared photography and seepage meters to quantify water and DIN inputs to Nauset Marsh, Massachusetts. Seepage meters were used to quantify flow rates and nutrient concentrations and photography was used to spatially extrapolate to the larger estuarine system. In Nauset marsh groundwater discharge occurred in high-velocity seeps immediately seaward of the upland-fringing salt marsh and thus did not constitute a direct input to the marsh ecosystem. This pattern has also been observed in the Virginia coastal plain, where low permeability tidal marsh peat forces most discharge through subtidal sediments (Harvey and Odum, 1990) or tidal creek bottoms (Howes *et al.*, 1996). In Sippewissett Marsh a combination of seepage chamber and salt balance approaches were used to determine the magnitude and location of groundwater inputs. As with Nauset marsh, there was little passage through salt marsh peats, but there was substantial input at the upland-marsh border of the overall system. However, in some cases groundwater N interacts more directly with the marsh (Tobias *et al.*, 2001b,c). The estimated N input from groundwater varies annually in relation to variation in groundwater levels of the

upland aquifer, and spatially with the degree of watershed development. Along the entire coast of South Carolina groundwater inputs to the coastal zone average about 5 g N m^{-2} year^{-1} (Krest *et al.*, 2000); in the more heavily populated coast of Massachusetts, average groundwater inputs to Sippewissett marsh were greater than 12 g N m^{-2} year^{-1} (Valiela *et al.*, 1978).

Atmospheric Inputs Dissolved N is deposited in precipitation (wet deposition) and as gases and particles (dry deposition). Wet deposition (rain, snow, sleet, cloud water) contains a variety of N compounds, most of which are available for biological utilization, including NO_3^-, NH_4^+ and organic (amino acids, urea) species (Peierls and Paerl, 1997; Seitzinger and Sanders, 1999). Dry deposition includes gaseous compounds or aerosols that are deposited on terrestrial or aquatic surfaces through sedimentation, interception, and diffusion processes. The most abundant gaseous N compounds are NH_3 and nitric acid vapor. Direct atmospheric N deposition (wet and dry deposition directly onto the system, as opposed to riverine transport of N deposited within the watershed) is recognized as a potentially important source of new N to salt marsh systems, but it is rarely quantified. To a large extent this is attributable to the difficulty of measuring dry deposition; dry deposition is often assumed to equal that of wet N deposition (Fisher and Oppenheimer, 1991; Paerl *et al.*, 2002). Paerl (1985) and Fisher and Oppenheimer (1991) first showed the potential importance of atmospheric deposition in their studies of the Neuse River estuary, Pamlico Sound and Chesapeake Bay. Available evidence indicates that direct deposition onto the marsh surface alone contributes 1–40% of the total N input to marshes, depending in large part on the relative area of the estuary and its watershed (Nixon *et al.*, 1996; Valigura *et al.*, 2000). In estuaries where the ratio of the area of the estuary to the area of its watershed is greater than 0.2:1, direct atmospheric deposition usually makes up 20% or more of the total N loading (Valigura *et al.*, 2000).

The magnitude of N deposition is geographically quite variable and depends primarily on the extent of the estuarine airshed and the level of industrial fossil fuel combustion in that airshed. At a more local scale, gaseous NH_3, which is directly emitted into the atmosphere from agricultural sources such as fertilizer application and confined animal operations (Paerl *et al.*, 2002), can be a major component of N deposition (Whitall *et al.*, 2003). In estuarine marshes of the Middle Atlantic States, where both agricultural and fossil fuel sources are high, wet and dry N deposition are about 2.2 g N m^{-2} year $^{-1}$. In the New England region atmospheric deposition ranges from about 0.5 to 2 g N m^{-2} year^{-1} (Driscoll *et al.*, 2003). In the Plum Island Sound marshes in northern New England, wet and dry deposition were estimated at about 1.7 g N m^{-2} year $^{-1}$ (Williams *et al.*, 2004); N deposition is about 1.1 g N m^{-2} year^{-1} in Cape Cod, Massachusetts marshes.

Several nationwide monitoring networks in the U.S. collect data that can be used to estimate N deposition. These include the NCDC for precipitation, NADP for precipitation chemistry and the Clean Air Status and Trends Network (CASTNet) for dry deposition. Few of these sites are in coastal systems, however, so caution must be taken when using these data.

Fixation The process of N fixation is carried out by an incredible diversity of organisms, including heterocystous and nonheterocystous cyanobacteria, aerobic, facultative anaerobic and anaerobic eubacteria, and by photosynthetic bacteria

(Bagwell *et al.*, 2001; Currin and Paerl, 1998a,b; Currin *et al.*, 1996; Gandy and Yoch, 1988; Lovell *et al.*, 2000; Piceno *et al.*, 1999). N fixation is conducted by heterotrophs, chemolithotrophs and photoautotrophs. It is conducted in marsh sediments, in surface marsh algal mats, in the plant rhizosphere and in the plant phyllosphere. N-fixing bacteria have been detected invading plant roots in the outermost and innermost cortical layer (Patriquin, 1978).

N-fixation activity typically is estimated indirectly as the rate of reduction of acetylene to ethylene. This approach avoids the difficulty of measuring changes in N_2 gas in an atmosphere with 80% N_2 content. While the method is sensitive, there are a number of assumptions made in its application, including the conversion factor from acetylene reduced to N_2 fixed. The conversion factor most commonly used is 3:1 or sometimes 3:2, but this has been directly measured in only two cases in salt marsh systems (Carpenter *et al.*, 1978; Currin *et al.*, 1996).

The distribution of N-fixation activity throughout the salt marsh is determined by several factors. N fixation is temperature dependent (Carpenter *et al.*, 1978; Patriquin, 1978) with Q_{10} values above 2 (Whiting and Morris, 1986). Lack of available light seems to restrict the importance of photoautotrophic N fixers in the vegetated marsh. By comparing light vs. dark incubations investigators found less than 10% (Teal *et al.*, 1979) to 25% (Tyler *et al.*, 2003) of the fixation due to autotrophs with the rest due to heterotrophic fixers. In contrast, in pans, creek banks, algal mats, and on standing dead plants, autotrophic fixation can dominate (Abd Aziz and Nedwell, 1986; Carpenter *et al.*, 1978; Currin and Paerl, 1998a,b; Joye and Paerl, 1994). Fixation can also be inhibited by high oxygen levels, which inhibit the nitrogenase enzyme, so fixation may be temporally separated from photosynthesis. This implies that autrotrophic fixation is underestimated using light vs. dark comparisons (Currin and Paerl, 1998b; Currin *et al.*, 1996). Hetero-trophic fixation requires labile organic matter as an energy source for reducing N_2. Some investigators have found a positive correlation between fixation activity and sediment primary productivity, detrital inputs, sediment C content and/or glucose additions (Hanson, 1983; Herbert, 1975; Langis *et al.*, 1991; Talbot *et al.*, 1988). But Hanson (1977a) also observed a long-term decrease in N fixation following glucose additions to sediments, presumably because microbes outcompeted plants for avail-able N, which in turn suppressed plant root production and organic C exudation. Finally, many investigators have found that high concentrations of inorganic N can inhibit fixation (Carpenter *et al.*, 1978; Hanson, 1977a; Teal *et al.*, 1979; Van Raalte *et al.*, 1974; Yoch and Whiting, 1986).

Vertical distribution of N-fixation activity is typically highest in the plant rhizosphere in the zone of maximum root production (Carpenter *et al.*, 1978; Casselman *et al.*, 1981; Hanson, 1977b), considerably lower at the sediment surface and lowest in the phyllosphere (Hanson, 1977b): up to 51 g N m^{-2} year^{-1} in rhizosphere and 0.5 g N m^{-2} year^{-1} in the phyllosphere. Surface cyanobacterial activity can be 3× higher for heterocystous cyanobacteria than for nonheterocystous cyanobacterial species (Currin *et al.*, 1996). Horizontal distribution of activity is also related to the pattern of plant productivity, being higher in the creekbank, tall *S. alterniflora* zone and lower in interior or high marshes (DeLaune and Patrick, 1990; Teal *et al.*, 1979). Fixation rates range from about less than 0.05 g N m^{-2} year^{-1} in

the Puccinellia/Spartina zone in Colne Point marshes (Abd Aziz and Nedwell, 1986) to more than 17 g N m^{-2} year^{-1} for a *S. anglica* marsh (Jones, 1974). Most rates of fixation in U.S. marshes fall between 2 and 15 g N m^{-2} year^{-1} (Table 22.2). Only a few investigators have attempted to calculate fixation rates integrated over the entire marsh system and these range from 0.33 g N m^{-2} year^{-1} for the Colne Point marsh, U.K. (Abd Aziz and Nedwell, 1986), to 6.8 g N m^{-2} year^{-1} for the Great Sippewissett marsh in Massachusetts U.S.A. (Valiela and Teal, 1979).

Nitrogen fixation rates in marshes are highest in salt marsh pannes (Valiela and Teal, 1979) and cyanobacterial mats where lack of vegetation allows higher light levels. Heavily grazed *Puccinellia* marshes in arctic Canada had three times the fixation rates as nearby ungrazed areas (Bazeley and Jefferies, 1989). Young marshes, and constructed marshes with more open canopies, also tend to have higher rates than closed canopy mature marshes. For example, fixation rates in a recently planted marsh in North Carolina were nearly 50 times greater than the adjacent, natural marsh (Currin *et al.*, 1996). However, light may not be the only explanation for differences in fixation between sites. Fixation rates in young marshes on Hog Island, Virginia, were three times greater than in the mature marsh, yet experiments did not indicate light limitation (Tyler *et al.*, 2003). The authors suggest that fixation decreases over the course of succession in marshes because the availability of N increases over time. Of course exceptions to the pattern of higher fixation in young marshes have been reported; fixation rates were uniformly low in both a natural and constructed marsh in San Diego, California, perhaps due to very low C availability (Langis *et al.*, 1991).

3.2.3. Exchanges

Tidal Exchange Beginning in the early 1960s there was a great deal of interest in determining the role that marshes might play in supporting coastal food webs through the ''outwelling'' of organic matter from extensive intertidal marshes to the sea (Teal, 1962, reviewed by Hopkinson, 1988 and Childers *et al.*, 2000). While the initial outwelling focus was on organic C, studies attempting to quantify organic matter flux soon included inorganic and organic forms of nutrients as well (Pomeroy *et al.*, 1967). Few studies attempted to quantify the exchange of material from marsh to the coastal ocean in the true sense of outwelling as described by Odum (1968), rather studies typically focused on quantifying fluxes between subsystems of an estuary (Fig. 22.1), for example between estuarine bay and ocean (Dame *et al.*, 1986), between tidal creeks and larger estuarine bays (Axelrad *et al.*, 1976; Heinle and Flemer, 1976), or between intertidal marsh and adjacent tidal creeks (Wolaver *et al.*, 1980).

Exchange of N between salt marshes and adjacent tidal creek waters follows two primary flowpaths: (1) Vertical flux (including diffusion, erosion, and deposition) between the vegetated marsh sediment and tidal water followed by horizontal move-ment (advection) across the marsh surface and (2) subsurface, lateral drainage (or seepage) of interstitial water from marsh sediments (advection) across the intertidal creek face (Fig. 22.2). Movements of water are key in calculating exchanges and care must be taken in interpreting water level dynamics (Gardner and Reeves (2002). Most often N exchange has been measured at the mouths of narrow, shallow channels connecting small marsh creeksheds to larger basins (Fig. 22.1, creek –estuary exchange). This approach measures the net balance of processes occurring both on the marsh,

Table 22.2 Nitrogen fixation rates in salt marshes

Site	Habitat type	Method	Rate mg N m^{-2} day^{-1}
Nova Scotia			
Patriquin and McClung (1978)	Short *S. alterniflora*	AR growing season -only Surface and subsurface	31.0
Massachusetts			
Carpenter *et al.* (1978) and Teal *et al.* (1979)	Tall *S. alterniflora*	AR 3:2 calibrated (algal + bacterial)	25.1
	short *S. alterniflora*		35.4
	high marsh		11.7
	creek bottoms		7.9
	pannes		6.5
	algal mat		6.2
Virginia-Hog Island			
Tyler *et al.* (2003)	*S. alterniflora* -7 year old marsh	AR 3:2	50.1
Tyler *et al.* (2003)	*S. alterniflora*-15 year old		24.7
Tyler *et al.* (2003)	*S. alterniflora* >150 year		16.7
Newport River NC			
Currin *et al.* (1996)	*S. alterniflora*	AR 3:1, calibrated; seasonal	2.0
Currin and Paerl, (1998a)	*S. alterniflora*		7.1
Georgia-Sapelo Island			
Hanson, (1977b)	*S. alterniflora* (control)	AR 3:1 min(max about 2×)	60.8
Hanson, (1983)	9 sites, including *Juncus & Spartina* AR3:1		40.5
Louisana			
DeLaune and Patrick, (1990)	*S. patens*		18.6
Baritaria Basin, LA			
Casselman *et al.* (1981)	*S. alterniflora* inland	AR 3:1, seasonal	12.3
	S. alterniflora streamside	(to 40 cm)	41.9
	mud bottom creek		4.4
Tomales Bay CA			0.0
Joye and Paerl, (1994)	microbial mat	AR 3:2	31.0

Table 22.2 Nitrogen fixation rates in salt marshes (*continued*)

Site	Habitat type	Method	Rate mg N $m^{-2} day^{-1}$
San Diego CA			0.0
Langis *et al.* (1991)	*S. foliosa*	AR reported as C2H2	<.1
England—Colne Point			
Abd Aziz and Nedwell, (1986)	salt marsh pannes	Assumed 3:2	4.8
	creeks		0.9
	Puccinellia/ Halimione		1.1
	Puccinellia/ Spartina		0.1
England—Bank End		Assumed 3:2	
Jones, (1974)	*S. anglica*		46.8
Jones, (1974)	*Puccinellia maritime*		14.0
Jones, (1974)	*Salicornia dolichostachya*		7.7
Canada-La Perouse Bay			
Bazeley and Jefferies, (1989)	Grazed Puccinella	AR growing season only	8.7
Bazeley and Jefferies, (1989)	Ungrazed Puccinella	AR growing season only	2.6

AR refers to the acetylene reduction method, and the ratio (3:2 or 3:1) refers to the conversion factor between acetylene reduced to N_2 fixed. In two cases where this ratio was measured using ^{15}N, the method is marked as calibrated.

creekbank, mudflats and in the channel (e.g., water column, sediments, reefs), and precludes the separation of specific flowpaths connecting surface and subsurface marsh sediments with adjacent creek waters. Marsh flume and creekbank hydraulic approaches (see Childers and Day, 1990b; Harvey and Odum, 1990; Hemond and Fifield, 1982; Nuttle and Portnoy, 1992) effectively separate surface and subsurface flux pathways (Fig. 22.1 "marsh –creek exchange; also see Childers, 1994).

Tidal range is a major factor regulating the relative strength of surface and subsurface flux pathways (Childers, 1994). Individual studies show considerable spatial and temporal variability in N fluxes: some show marshes to be net sinks of N (i.e., import from tidal waters greater than export to tidal waters, e.g., DeLaune *et al.*, 1989), others net sources (e.g., White and Howes, 1994b). Still other studies (Table 22.3) show marshes to import inorganic N and export organic N (e.g., Whiting *et al.*, 1989), while others import particulate N but export dissolved N (e.g., Jordan *et al.*, 1983). Jordan *et al.* (1983) concluded that the primary role of marshes is to transform particulate to dissolved forms rather than act as sources or sinks. Summarizing results from southeastern U.S. studies, Childers (1994) and Childers *et al.* (1999, 2000) concluded that tidal range was the primary factor

Table 22.3 Estimated net annual nitrogen exchange (g N m^{-2} year^{-1}) during inundation and drainage of the vegetated marsh at North Inlet, SC. PN and DON represent particulate and dissolved organic nitrogen.

	PN	DON	NH$_4^+$	NO$_x$	Total flux
Inundation	3.3	−5.9	2.9	0.62	0.9
Drainage	−2.4	−2.8	−0.18	−0.01	−5.4
Net Flux	0.9	−8.7	2.7	0.61	−4.5

A negative flux indicates an export of nitrogen from marsh to creek. From Whiting *et al.* (1989).

controlling the magnitude, direction and flowpath of exchange. Where tidal ranges are low, marsh-tidal creek water exchanges of inorganic and organic N are primarily intertidal (horizontal across marsh surface) and flume fluxes measured in these marshes are export-dominated. At higher tidal ranges, horizontal subsurface flow and subtidal benthic advection become important as marshes tend to take up nutrients and organic matter from the inundating water column while exporting these constituents to adjacent tidal creeks by subsurface flow and subtidal advection (Chambers and Odum, 1990; Harvey *et al.*, 1987; Whiting and Childers, 1989). Measurement of particulate fluxes in flumes is particularly problematic and there is anecdotal evidence that most particulate fluxes occur during extreme events, such as hurricanes or intense rains. Particulate fluxes can only occur via horizontal surface flowpaths, so do not fit the Childers schema, with respect to tidal range.

Another major control on tidal exchange appears to be marsh geomorphic age (Boorman *et al.*, 1992; Childers, 1994; Childers *et al.*, 2000; Tyler and Zieman, 1999; Valiela *et al.*, 2000). Young, immature marshes in estuaries are often lower in elevation and flood with greater frequency. With tidal ranges greater than about 1 m, immature marshes appear to take up nutrients and organic matter at rates 1.5 to 3 times higher than in old, mature marshes. Particulate N fluxes probably also follow this pattern, as Friedrichs and Perry (2001) conclude that particle (mineral and organic) deposition increases with increasing flooding frequency, depth and duration, which would tend to be greater in low elevation, immature marshes.

There are few estimates of total N exchange with salt marshes as most studies have not measured all forms of N. Valiela *et al.* (2000) estimated net exchange qualitatively by tabulating directional flux of individual forms of N for many salt marshes, regardless of whether all forms were measured. They took the aggregate results from all the marshes to represent overall export or import characteristics and found that a majority of salt marshes export N. For all forms of N, with the exception of nitrate, the aggregate data suggest that a majority of salt marshes exported N. That in aggregate, marshes export N to adjacent tidal creeks does not imply that they are net exporters to all adjacent systems or that they do not accumulate N *in situ*. Obviously there are other sources that enable marshes to both accumulate and export N to adjacent tidal waters.

Nutrient Transport by Fish Migration Many estuarine organisms can readily migrate between tidal creeks and the marsh platform and this migration can represent an appreciable flux of inorganic and organic N. Direct measurements of aquatic faunal

densities from a variety of salt marsh estuaries demonstrate that these animals utilize the marsh surface for feeding and to escape predation (see review by Kneib, 2000). The N flux pathway consists of larval or juvenile organisms residing on the marsh during early life stages, increasing in biomass by consuming marsh detritus or resident flora and fauna, and then migrating to estuarine waters at later stages in their lifecycle. Alternatively marsh fauna can be grazed by wading birds (Bildstein *et al.*, 1992), or consumed by large carnivorous fish during extreme high tides. These pathways represent a flux of particulate organic N from marshes to adjacent estuarine waters and in some cases the coastal ocean (Deegan, 1993; Deegan *et al.*, 2000). Deegan determined that migration of menhaden from Mississippi River deltaic marshes to the Gulf of Mexico represented a flux of 3.1 g N m^{-2} year^{-1}. While not all this N originated on marshes, many other species are involved in this pathway in addition to menhaden, and thus macrofaunal migration can represent a significant flux of N from marshes to estuarine waters. In a recent review of this process, Teal and Howes (2000) concluded that marshes as a whole do grow fish, but until more studies quantify their contribution to the N budget, we will not know if the magnitude of the flux measured in Louisiana marshes is typical.

3.2.4. Outputs

Denitrification and Gaseous N Losses Denitrification is of particular interest in estuaries because it represents the primary process reducing the effects of N-enrichment from anthropogenic sources (see Chapter 3 by Devol, this volume). The perceived low susceptibility of salt marsh estuarine systems to N-enrichment and eutrophication is often attributed to high rates of denitrification (NRC, 2000). It has been suggested that through denitrification and burial, fringing salt marshes also play an important role in intercepting land-derived nutrients and thereby helping to prevent eutrophication in downstream ecosystems, such as sea grass meadows (Valiela and Cole, 2002). However, in spite of the widespread belief that denitrification is an important process, we find relatively few studies of gaseous N loss from marshes.

The relative importance of various forms of N lost to the atmosphere has been shown clearly. N_2 is the primary form of N lost during denitrification in salt marshes (Cartaxana and Lloyd, 1999; Smith *et al.*, 1983). NO and N_2O losses are orders of magnitude smaller by comparison. Maximal rates of N_2O loss normally do not exceed 0.14 mg N m^{-2} day^{-1} (Smith *et al.*, 1983). NH_3 volatilization, while not a component of denitrification, is another form of gaseous N loss in salt marsh systems. It too is orders of magnitude lower than rates of N_2 loss due to the fairly low sediment pH values (<8) in most marsh sediments (Koop-Jakobsen, 2003; Smith *et al.*, 1983).

Measuring denitrification in any natural system has proved challenging in part due to the difficulty of measuring small changes in N_2 against a relatively large background concentration (Cornwell *et al.*, 1999; Groffman *et al.*, 2006, see Chapter 1 by McCarthy and Bronk, this volume). For a variety of reasons, marshes present especially difficult additional challenges. Tidal inundation and drainage, as well as oxidation of the plant rhizosphere, lead to a spatially complex and temporally dynamic redox environment in vegetated sediments, which may favor coupled nitrification and denitrification (Risgaard-Petersen and Jensen, 1997). In theory,

the marsh plant rhizosphere should be a *hot spot* of nitrification and denitrification due to plant roots, which provide oxygen and labile organic matter. This has been demonstrated in a laboratory setting for fresh water marsh sediments dominated by *Juncus effuses* using ^{15}N (Reddy *et al.*, 1989) and in submerged seagrass sediments (Caffrey and Kemp, 1992), but data from salt marsh systems are sparse.

Because of the difficulty of measuring denitrification in salt marshes, a wide variety of approaches have been taken. It is telling that of the 16 studies where we found denitrification rates reported for salt and brackish marshes, twelve different methods were employed (Table 22.4). In earlier studies, acetylene inhibition was the method of choice. Although this is one of the simpler and more sensitive methods of measuring denitrification, acetylene reduction also blocks nitrification. In systems where coupled nitrification/denitrification is important, the acetylene reduction technique can seriously underestimate denitrification if experiments permit NO_3 concentrations to drop significantly (Groffman *et al.*, 2006; Seitzinger *et al.*, 1993). In the few cases where direct and coupled nitrification/ denitrification have been separately quantified both are important. The relative importance of the two processes varies depending upon location in the marsh, the presence of benthic algae, and the availability of nitrate and organic matter (Hamersley and Howes, 2005; Tobias *et al.*, 2003). Four studies that used acetylene inhibition reported denitrification rates that cluster at the lower end of all reported rates for salt marshes, suggesting that this technique may not be optimum for these environments.

More recent improvements in denitrification measurement have relied on the use of stable isotopes or the direct measurement of N_2 fluxes. Direct N_2 flux techniques measure net denitrification (denitrification-N_2 fixation) and in some cases investigators corrected the reported values for fixation, while in others only net values were reported (Table 22.4). A variety of approaches have been tried to apply these techniques to the vegetated marsh platform. Some investigators have applied the techniques under exposed conditions (Kaplan *et al.*, 1979), while others have measured rates under submerged conditions (Davis *et al.*, 2004). Isotopic methods have ranged from short-term use of the isotope pairing method of Nielson (1992) (Ericksson *et al.*, 2003), to N_2O pool dilution (Anderson *et al.*, 1997), and to measurement of the short-(Hamersley and Howes, 2005) and long-term fate of ^{15}N added *in situ* (White and Howes, 1994b). Most studies have been conducted during the growing season; only a few were carried out frequently enough to realistically estimate annual averages. Due to the difficulty of dealing with tall plants in chambers, most studies have excluded live culms during the measurement period. Hamersley and Howes (2005) showed that measurements applied *in vitro* to sediments isolated from plants and other environmental factors may greatly underestimate the true rate of denitrification. Similarly Reddy *et al.* (1989) found almost no denitrification in freshwater marsh microcosms without plants. It should be noted that the measurements of Hamersley and Howes (2005) were made over a period of days. A comparison of their measured *in situ* values to those of other investigators using *in vivo* methods (Table 22.4) suggests that for short-term measurements, the methodological problems of removing the plants may not be as severe as they are in longer-term laboratory studies.

Table 22.4 Denitrification rates from salt marsh sediments and associated small tidal creeks. Rates are both from vegetated and unvegetated sediments. All rates in mg N m^{-2} day^{-1}

Site	Habitat	Method	Vegetated		Non–Vegetated	
			Gross★	Net★	Gross★	Net★
Massachusetts Plum Island estuary						
Tobias et al. 2003	creek high NO_3^-	whole system ^{15}N addition			161.0	
	creek lower NO_3^-	with ^{15}N gas flux and pool dilution			63.0	
Great Sippewissett Marsh						
Kaplan et al. 1979	tall *S. alterniflora*	*in situ* N$_2$ gas partitioning,	57.8	32.7		
	short *S. alterniflora*	annual	43.6	8.3		
	high marsh		22.2	10.4		
	creek bottoms				69.0	61.3
	pannes				62.7	56.3
	algal mat				35.3	29.2
White and Howes, (1994b)	*S. alterniflora*	Long-term ^{15}N budget	14.0			
Hamersley and Howes, (2005)	*S. alterniflora*	Short-term ^{15}N budget, annual	29.5			
Nauset Marsh						
Nowicki *et al.* (1999)	Sub- and intertidal creeks	direct N$_2$ flux				7.7
Mattapoisett Marsh						
Hamersley and Howes 2003	Sandy creek	direct N$_2$ flux				88.2
	Muddy creek					103.6
Rhode Island Estuaries						
Davis et al. 2004	*S. patens*/bare patches	porewater N$_2$ accumulation		16.8		50.4
	S. patens/bare patches			117.6		58.8
	S. patens/bare patches			67.2		−16.8
	S. patens/bare patches			50.4		−6.7
	Schoenplectus pungens/bare			−25.2		−84.0

North Carolina, Newport River

Thompson *et al.* (1995)	*Spartina* (natural sites)	acetylene block- cores	0.1–0.7	

Virginia, Virginia Coast Reserve

Anderson *et al.* (1997)	*S. alterniflora* /nearby creeks	^{15}N$_2$O Pool dilutions	1.6	1.9

Georgia, Sapelo Island

Haines *et al.* (1977)	*S. alterniflora*	N$_2$ gas profiles, annual	32.9	

Louisiana, Barataria Basin

DeLaune *et al.* (1989)	salt marsh	N$_2$O:N$_2$ ratios	11.0	3.5
	brackish marsh		17.0	7.4
Smith *et al.* (1985)	Salt marsh unvegetated	acetylene block	5.7	4.6

California, Tomales Bay

Joye and Paerl, (1994)	Microbial mat	acetylene block		4.1

England

Koch *et al.* (1992)	*Halimione portcuiacoides*	acetylene block, annual	14.0	
	upper mud flat			1.4
	lower mud flat			1.4

Colne Point

Abd Aziz and Nedwell, (1986)	salt marsh panes	short-term ^{15}N gas flux		0.8
	Creeks			0.4
	Puccinellia/Halimione		0.5	
	Puccinellia/Spartina		3.1	

Italy, Venice Lagoon

Ericksson *et al.* (2003)	Mud	isotope pairing, April–Oct		36.0
	Limonium		24.0	

The method used for each study is listed. Depending upon the method, the study may have measured gross denitrification (total production of N$_2$ from nitrate reduction) or the net rate of N$_2$ flux (the difference between fixation and denitrification). Negative numbers indicate a net uptake of N$_2$ by the sediments.

$\star$ Rates given are in mg N m^{-2} day^{-1}.

The published rates of denitrification in vegetated sediments range from 0 to more than 100 mg N m^{-2} day^{-1} (Table 22.4). Median values of 14–28 mg N m^{-2} day^{-1} are in general higher than those reported for other environments including estuaries and continental shelves (see Chapter 18 by Boynton and Kemp, this volume). Given the available data, it is difficult to determine if rates in sediments from the vegetated marsh are significantly different from rates measured in small creeks draining the marsh. Both sites can have very high rates. Kaplan *et al.* (1979) reported that rates were highest in creek bottoms and the tall *S. alterniflora* zone, while the lowest rates were found in the vegetated high marsh. Similarly Ericksson *et al.* (2003) found higher rates in creek sediments than vegetated sediments. Other studies found that rates were higher in vegetated sediments than in nearby creek bottoms (Abd Aziz and Nedwell, 1986; Davis *et al.*, 2004; DeLaune *et al.*, 1998; Koch *et al.*, 1992).

Denitrification may also be important in marsh sediments that receive nitrate-rich groundwater inputs. Tobias *et al.* (2001a,b) investigated a plume of groundwater in a mesohaline marsh using isotopic methods *in situ*. They estimated that denitrification rates could be as high has 504 mg N m^{-2} h^{-1} during times when groundwater discharge was high; at this rate, denitrification could remove up to 90% of the nitrate load to the marsh. Addy *et al.* (2002, 2005) found substantial denitrification activity in groundwater passing under a salt marsh. In contrast Nowicki *et al.* (1999) found that the majority of nitrate-rich groundwater passed under the marsh and that the nearby creeks were the major site of denitrification. In marshes, as in all systems, denitrification is a function of both reactivity and hydrologic conductivity (e.g., Bohlke and Denver (1995). Until there are more studies in marshes with different groundwater flow paths, the overall importance of denitrification in groundwater as it enters marshes cannot be evaluated, but it warrants further investigation.

Anammox is the anaerobic reduction of ammonium to N$_2$ using nitrite as the terminal electron acceptor. This process can contribute significantly to benthic N$_2$ production in some marine environments, but studies to date have found that the importance of anammox in organic rich coastal sediments is low (Engstrom *et al.*, 2005). Anammox may be of minor importance in organic rich salt marshes as well.

Observations and experiments show that availability of NO$_3$$^-$(Koch *et al.*, 1992; Tobias *et al.*, 2003), labile organic matter, and oxygen (required for nitrification) are the primary factors controlling the rate of denitrification (Cornwell *et al.*, 1999; Kaplan *et al.*, 1979; Koch *et al.*, 1992; Sherr and Payne, 1978; Thompson *et al.*, 1995). Although it would be expected that denitrification would increase with increased N loading, previous studies have not always found a clear relationship (Kaplan *et al.*, 1979), or found that net denitrification (net N$_2$ flux) decreased with loading (Davis *et al.*, 2004). However, Wigand *et al.* (2004) and Lee *et al.* (1997) found that potential denitrification increased along a eutrophication gradient. Hamersley and Howes (2005) found denitrification rates in fertilized salt marsh plots were more than an order of magnitude higher than nearby control areas. They suggested that under conditions of high N loading, competition for N between microbes and plants is reduced or eliminated resulting in very high denitrification rates. Understanding the effect of elevated nutrient inputs on denitrification is further complicated by two recent findings. First, high rates of

denitrification in marshes may be due in part to phosphorus limitation of microbial heterotrophs and that increased P can decrease denitrification (Sundareshwar et al., 2003). Some studies on the denitrification response to fertilization were done in sites receiving N+P while others were conducted in sites only receiving N. Second, the N composition of fertilizer (inorganic vs. organic) may determine the degree to which denitrification is stimulated (Enwall et al., 2005).

In spite of the difference of approaches, the overall picture that emerges is that denitrification typically ranges between 2–40 g N m^{-2} year^{-1} and is a significant loss term in salt marsh N budgets. Because some of these measurements are net fluxes, and because of methodological problems, we suspect that these rates may be underestimates (in some cases, large underestimates). At present there seems to be insufficient data to say with confidence how denitrification rates are altered by N-enrichment or eutrophication, but this is clearly a critical question.

Sediment Accumulation Marshes must keep pace with increases in sea level rise to survive. The net accretion of salt marsh sediments in conjunction with sea level rise is another potentially important flux of N in salt marsh ecosystems. Generally marsh elevation increases in parallel with sea level rise (Valiela, 2006). In most cases rates of N storage have been measured using sediment bulk density and N content with direct measurements of vertical accretion using ^{137}Cs or ^{210}Pb (Craft et al., 1993; Merrill and Cornwell, 2000) or calculated from independent measures of local relative sea level rise (Anderson et al., 1997; White and Howes, 1994b). These yield long-term averages for specific locations. Less frequently, short-term rates of N accretion have been directly measured using sedimentation plates or marker horizons on the marsh surface (see for example DeLaune et al., 1981) and flumes (Childers and Day, 1990a,b). Increasingly researchers are employing sediment elevation tables (SET; Boumans and Day, 1993; Cahoon et al., 2002) to estimate both short-and long-term changes in sediment elevation. Estimates of N inputs required to balance long-term accumulation of N in sediments span a wide range, 1–50 g N m 2–1 year^{-1} (Table 22.5), with median values between 2 and 4 g N m^{-2} year^{-1}. The wide range is due in part to the difficulty of designing a sampling scheme that realistically takes into consideration episodic events (storms, hurricanes) when rates are generally at their highest (Childers and Day, 1990a) and to finding truly representative sites. The majority of studies have been conducted in depositional areas and generally erosional sites are not considered (c.f. Chmura et al., 2003). For example, while DeLaune et al. (1981) report high rates of sedimentation on the surface of brackish marshes in Four League Bay, Louisiana, an overall budget of the entire area, which takes into account regions where marsh is being lost, demonstrates the N loss by burial is much less (17 g N m^{-2} year^{-1} vs <5 g N m^2 year^{-1} -DeLaune et al., 1989).

Although the data on N burial is somewhat sparse, Chmura et al. (2003) compiled an extensive data set on rates of C sequestration in tidal saline wetlands, which included more than 110 salt marsh sites. They concluded that on average salt marshes store 210 g C m^{-2} year^{-1}. Sediment C/N data for salt marshes has been compiled by Valiela (1983) and Craft et al., 1991 and while this data also comprises a wide range of values the majority of the sites have C/N values that fall between 14 and 30. Combining estimates of C accretion and C/N ratios, yields an overall

Table 22.5 Burial rates of nitrogen Rates were calculated using several methods, assuming accretion has kept up with relative sea level rise (SLR), a budget of organic matter inputs and losses (OM budget), mass accretion rates using radiometric dating techniques (^{137}Cs or ^{210}Pb), or net particulate nitrogen (net PN) budgets to the marsh or in tidal creeks. GSW stands for Great Sippewissett Marsh

Site	Habitat	Method	Rate ($g\,m^{-2}\,year^{-1}$)
Massachusetts GSW			
White and Howes, (1994b)	*S. alterniflora*	SLR	4.4
White and Howes, (1994c)	*S. alterniflora*	OM budget	3.9
Virginia			
Anderson *et al.* (1997)	*S. alterniflora*	SLR	4
North Carolina			
Craft *et al.* (1993)	*S. alterniflora* streamside	^{137}Cs	4.1
Craft *et al.* (1993)	*S. alterniflora* back marsh	^{137}Cs	1.3
Craft *et al.* (1993)	*Juncus*/other streamside	^{137}Cs	10.3
Craft *et al.* (1993)	*Juncus*/other back marsh	^{137}Cs	6.9
Louisiana			
Barataria Smith *et al.* (1983)	*S. alterniflora*	^{37}Cs	16.0
Smith *et al.* (1983)	*S. patens*	^{37}Cs	50.3
DeLaune *et al.* (1981)	Oligo/mesohaline marsh	^{137}Cs	21.0
DeLaune *et al.* (1981)	Streamside marsh	^{137}Cs	13.4
Sites in Four League Bay Smith *et al.* (1985)	Brackish	^{137}Cs	23.0
Smith *et al.* (1985)	Brackish	^{137}Cs	6.6
Smith *et al.* (1985)	Brackish	^{137}Cs	14.1
Smith *et al.* (1985)	Brackish	^{137}Cs	8.0
Smith *et al.* (1985)	Brackish	^{137}Cs	11.4
DeLaune and Patrick, (1990)	*S. patens* brackish	^{137}Cs	7.2
Maryland			
Choptank MD Merrill and Cornwell, (2000)	Oligohaline/mesohaline marsh	^{210}Pb	23.0
Monie Bay Merrill and Cornwell, (2000)	Oligo/mesohaline marsh	^{210}Pb	13.6
Netherlands Wadden Sea			
Rozema *et al.* (2000)	*Elymus athericus*	Net PN budget	11.2
	Elymus athericus	Net PN budget	51.5
England, Colne Point			
Abd Aziz and Nedwell, (1986)	Puccinellia, Halimione, Spartina	Net PN budget	1.2

average N accumulation rate of 7–15 g N m^{-2} year^{-1}. This rate compares reasonably well with N burial rates measured directly (Table 22.5): median -10.7 and average -13.9 g N m^{-2} year^{-1}.

Many factors interact to control wetland accretion and N plays a potentially interesting indirect role. There have been a number of studies and models examining how marsh plant species composition, production and allocation to above and belowground biomass and decomposition, sedimentation, erosion and compaction interact to determine accretion rates (Cahoon, 1994; Friedrichs and Perry, 2001; Morris and Bowden, 1986; Reed, 2000). How this balance is affected by increased N inputs to the coastal zone is still not well understood. Nutrient inputs stimulate plant production but in some cases also alter the relative abundance of marsh plants toward species with less belowground storage (Levine et al., 1998), which could possibly result in lower rates of peat formation. Nutrient enrichments increased sediment respiration and lead to some loss of peat in a fertilized marsh in South Carolina (Morris and Bradley, 1999) but 30 years of N addition increased accretion rates in Great Sippewissett marsh in Massachusetts (Valiela et al. unpublished).

3.3. Recycling

3.3.1. Decomposition

The primary fate of marsh macrophyte tissues is decomposition and incorporation into detrital food chains. Herbivory typically is not a major trophic pathway in salt marsh systems (but see Bertness et al., 2004 and Pomeroy and Wiegert, 1981). Nitrogen plays a critical role during decomposition and detrital processing. The quality of salt marsh detritus as a food source for consumers depends to a large degree on the availability of N for assimilation and growth (Tenore, 1984). Decomposition is primarily a microbial process and qualitatively similar to that of other aquatic macrophytes and terrestrial plants. It occurs in three phases: a short, rapid weight loss period with leaching of soluble compounds, a microbial degradation phase with concomitant leaching of hydrolyzed substances, and a final, slow phase either associated with enzymatic alteration of plant and bacterial biomolecules (Ogawa et al., 2001; Tremblay and Benner, 2006) or with humification (Rybczyk et al., 1996; Valiela et al., 1985a).

Nitrogen is essential to the microbial community decomposing litter and can limit the decomposition rate. The N needed in decomposition can come either from the litter or the surrounding soil and water. During decomposition both the mass of N and the C/N ratio of litter change. Initial N content of aboveground S. alterniflora litter ranges from about 0.6% to 0.9% (DW) and from 0.5% to 0.6% for belowground tissues (Hopkinson and Schubauer, 1984; Marinucci, 1982). C/N ratios increase during the first stage of decomposition and then decrease afterward (e.g., from 100–150:1 then down to 8–12:1). There is a net increase or immobilization of N during decomposition. Controlled experiments, ^{15}N tracers and the change in N compounds of bacterial origin reveal that by the midpoint in decomposition, 50–65% of the total detrital N pool is from external sources and a major part of the N incorporation is microbially mediated and of microbial origin (Tremblay and Benner, 2006; White and Howes, 1994a). The presence of bacterial organic matter

in decomposing litter has been confirmed by the occurrence of biomarkers unique to bacteria, muramic acid, only prevalent in bacteria (D-amino acids), or virtually absent from higher plants (galactosamine) (Tremblay and Benner, 2006). The increase in fungal and bacterial biomass on decomposing litter represents only a minor component of the increase in N over time, as several studies have found few recognizable microbial cells or living bacteria or fungi (White and Howes, 1994b). For aboveground decomposition, fungal biomass can contain 12–22% of the N in litter, while bacteria contribute about 1/10 that (Marinucci et al., 1983). Under subaqueous conditions, where fungi are of minor importance, 60–75% of the N and 20–40% of the C in highly decomposed detritus are not from the original plant tissues but are mostly from heterotrophic bacteria (Tremblay and Benner, 2006). The microbial contribution to N is mostly from the remains of bacteria, including exudates like mucopolysaccharides and exoenzymes (Lee et al., 1980; Rice and Hanson, 1984). Reactivity of bacterial detritus (that produced by bacteria during their decomposition of plant detritus) is similar to that of plant detritus. Only a minor fraction of bacterial detritus escapes rapid biodegradation and the relationship between bacterial activity and N immobilization is consistent with an enzymatically mediated preservation mechanism (Tremblay and Benner, 2006). The sequestration of external N into decaying *S. alterniflora* represents an important mechanism for retaining N in salt marsh ecosystems.

Lignin is one of the most resistant compounds in plant litter to decomposition and its content relative to total mass or ligno-cellulose content is a good predictor of decomposition rate. Up to 50% of the initial litter N is present as lignin. There is a rapid increase in relative lignin content during the first phase of decomposition (Benner et al., 1991; Klap et al., 1999), as the cellulose and hemicellulose fractions are preferentially degraded. While the lignin content stabilizes over the next two years of decomposition, there is continuous modification of the lignin polymer during this time. During a final humification stage, condensation reactions between quinines, derived from phenolics, and amines have been proposed as an important mechanism in N immobilization and preservation.

Lignin decomposition rates are substantially lower in the absence of molecular oxygen (Benner et al., 1991), which results in decomposition rates decreasing from the sediment surface through the partially oxygenated rhizosphere to deeper anoxic sediments. However, comparison of lignin production rates with estimates of accretion of organic matter in sediments suggests that most of the annual input to sediments of *S. alterniflora* lignin is removed through complete remineralization to CO_2 (Benner et al., 1991). One explanation for the decreased decomposition rate of lignin in anaerobic sediments is the great reduction in fungal activity. Fungi are the primary organisms responsible for lignin decomposition and they are obligate aerobes (Valiela, 1995).

The turnover of N in marsh plant litter is difficult to determine because of the immobilization that occurs during decomposition. Carbon loss cannot be used as a proxy for N turnover because C and N decomposition are decoupled and C is also immobilized during decomposition (Tremblay and Benner, 2006). Based on the decrease in mass of ^{15}N-labeled detritus from the White and Howes experiment (1994b), we calculate N turnover rates to exceed C during the leaching phase of

decomposition and then to be lower during the next two phases. Over an annual cycle, rates are similar (about 1.4 year^{-1}). As temperature is a primary control on decomposition rate, we can expect turnover rates to vary across geographic gradients.

3.3.2. Inorganic nitrogen dynamics

Ammonification, nitrification, and dissimilatory reduction of nitrate to ammonium (DNRA) are difficult processes to quantify, especially in the presence of high densities of marsh macrophytes. Conducting measurements in small cores introduces a suite of artifacts that are difficult to evaluate. Coring prevents further oxidation of the rhizosphere and production of labile C exudates by living plant roots. The process of coring results in roots being cut, which then leads to increases in acetate concentrations (Hines *et al.*, 1994; Howes *et al.*, 1985). Tracer approaches have been employed *in situ* and in the laboratory with and without macrophytes to assess the rhizosphere effect and to estimate plant N uptake and microbial N immobilization. These techniques are typically done with tracer level additions of ^{15}N ammonium or nitrate to sediment or overlying floodwater. Isotope dilution approaches can provide information on rates of gross production and uptake, and net production of various N species in sediments (Blackburn, 1979; Koike and Hattori, 1978) although care must be taken to meet the assumptions of isotope dilution techniques (Schimel, 1996).

Ammonification   Ammonification is the process whereby organic N is mineralized to NH_4^+. It is the first step in organic N decomposition and is often referred to as N mineralization Ammonification in combination with DNRA must balance plant uptake requirements, microbial immobilization, and NH_4^+ leaching to flood waters and creekbank drainage. Quantification of ammonification can be done by examining total ammonium accumulation in cores, by using harvest or litter bag methods and C/N ratios to calculate N turnover (Hopkinson and Schubauer, 1984; White and Howes, 1994b) or by $^{15}NH_4$ pool dilution (Anderson *et al.*, 1997; Bowden, 1984). Short-term pool dilution and ammonium accumulation yield data that represent gross mineralization while longer-term harvest methods approximate net values. Because salt marsh sediments are sites of high belowground production and decomposition, ammonification rates tend to be higher than those of unvegetated coastal marine sediments and similar to rates seen in seagrass beds (see rates in Herbert, 1999). In spite of the fairly large ammonium pool in salt marsh sediments, turnover rates are often short. Tobias *et al.* (2001b) reported a turnover time of 5–14 days for the ammonium pool in a Virginia salt marsh (based on NH_4^+ concentration of 500–1000 μM). Ammonification rates were highest near the surface and in the rhizosphere and decreased with depth, because of declining organic N quality. Anderson *et al.* (1997) found gross ammonification rates at Phillips Creek Virginia of 84 g N m^{-2} year^{-1}. Net values for Great Sippewissett Marsh, Massachusetts and Sapelo Island, Georgia were on the order of 15 and 20 g N m^{-2} year^{-1} respectively. Few investigators have examined controls. In a New England tidal marsh, Bowden (1986) estimated an annual rate of ammonification of 53 g N m^{-2} year^{-1} (to 10 cm), and found ammonification rates varied inversely with sediment lignin content.

Nitrification   Nitrification has been measured using isotope techniques similar to those used for gross ammonification but the much lower concentrations of nitrate in salt marsh sediments, and the more rapid turnover times make the techniques more difficult to apply (see Chapter 11 by Ward, this volume). Alternatively, non–isotopic techniques have been used that involve measuring ammonium accumulation with and without nitrification inhibitors but these are difficult to use if there are other substantial sinks of ammonium. Because of these difficulties many investigators have relied on potential nitrification measurements to examine spatial and temporal patterns and to examine how the microbial community correlates to rates (Bernhard *et al.*, 2005; Dollhopf *et al.*, 2005). In spite of the low concentrations of nitrate in salt marsh sediments, nitrification rates integrated below the rhizosphere have ranged from about 1/10 to 1/2 of the rate of ammonification (Anderson *et al.*, 1997; Bowden, 1986; Tobias *et al.*, 2001b). In tidal creek sediments the amount of ammonium nitrified spans a similarly large range (Tobias *et al.*, 2003). In many systems the fate of most of this nitrate appears to be denitrification. Even larger percentages of ammonium may be nitrified in fringing marshes receiving groundwater inputs from uplands where ammonium–rich porewater mixes with oxic groundwater and in sediments experiencing tidal infiltration and drainage (Tobias *et al.*, 2001a). Controls on nitrification are O_2 availability and NH_4^+ concentrations but elevated salinity (Seitzinger *et al.*, 1991) may play a role and sulfide levels (Joye and Hollibaugh, 1995) can severely inhibit nitrification.

Ammonium Uptake   Standard ^{15}N-NH_4^+ addition experiments have demonstrated, that in the presence of marsh plants, upwards of 95% of remineralized ammonium in marsh sediments is taken up by marsh plants prior to nitrification (Buresh *et al.*, 1981; Smith and DeLaune, 1985). In the absence of plants however, microbes decomposing high C/N plant detritus immobilize 15%. Once immobilized, further transformations or losses of this "organic associated fraction" are not detectable with current methods (Smith and DeLaune, 1985). These patterns are in agreement with bulk litter decomposition studies. NH_4^+ isotope dilution experiments conducted in the absence of plant roots show that a much larger percentage of remineralized ammonium is microbially immobilized. That plants secure a larger percentage of available NH_4^+ than decomposers or nitrifiers suggests that plants outcompete sediment microbes for inorganic ammonium. Another pathway of NH_4^+ uptake would be that by microalgae on the sediment surface and macrophyte culms. But as far as we know there has been little examination of this process in salt marshes.

DNRA has been identified as an important process under some conditions in estuarine sediments (Koike and Sorensen, 1988) Relatively few studies have been conducted in salt marshes. Abd Aziz and Nedwell (1986) could not measure the release of labeled nitrate as N_2 gas and concluded DNRA must greatly exceed denitrification. Tuerk and Aelion (2005) found that more than 30% of nitrate added to marsh sediments was quickly converted to ammonium. Tobias *et al.* (2001b) measured the relative importance of nitrate reduction to a marsh–aquifer ecotone and found that when groundwater discharge was low, nitrification rates were also low and the majority of the nitrate produced was denitrified. During high

discharge periods, nitrification rates greatly increased but while denitrification to N_2 increased two fold, 30% of the nitrate produced was reduced to ammonium (Tobias *et al.*, 2001c). In contrast, other studies have suggested that the importance of DNRA relative to denitrification is greatest when nitrate concentrations are low or the ratio of electron donors is high relative to NO_3^- (Herbert and Nedwell, 1990). Several studies have found that strongly reducing conditions favor DNRA (An and Gardner, 2002; Buresh and Patrick, 1981; Smith *et al.*, 1982). Because DNRA is a process that retains, rather than removes nitrate from salt marshes, the shunting of nitrate to DNRA under high nitrate loading tends to decrease the ability of marshes to remove anthropogenic N.

3.3.3. Marsh macrophyte nitrogen dynamics

Quantitatively some of the largest internal fluxes of N in the salt marsh ecosystem are associated with the growth and death of the emergent marsh macrophytes (Hopkinson and Schubauer, 1984; White and Howes, 1994c). All rooted macrophytes obtain inorganic N (NH_4^+ preferentially) from sediments and incorporate it into new plant biomass, both aboveground and belowground tissues. Upon senescence a portion of the aboveground N is translocated belowground where it is stored in roots and rhizomes for use the following season, while the remainder is either leached from the plant or remains in dead plant parts (Table 22.6).

Mass balance and isotopic tracer approaches have been used to quantify N fluxes associated with marsh plant growth and death. One study in a very productive, medium form of *S. alterniflora* from a Georgia salt marsh shows that total uptake of N by roots of *S. alterniflora* was 34.8 g N m^{-2} year^{-1}. Of this 43% was lost by death or leaching from aboveground plant parts, while the rest was lost by death of roots and rhizomes. Total transfer of N from below-to aboveground tissues was 33 g N m^{-2}

Table 22.6 Nitrogen fluxes associated with growth and death of marsh plants: short and medium forms of *Spartina alterniflora* and *Zizaniopis miliacea* (White and Howes, 1994c; Hopkinson and Schubauer, 1984)

	Short form *S. alterniflora*	Medium form *S. alterniflora*	*Zizaniopis miliacea*
New root uptake	21.8	34.8	57
Aboveground Retranslocation	3.7	33	39
Belowground Translocation	1.4	17.9	10.1
Root death	19.5	19.7	28.2
Aboveground death	2.9	14.4	14.9
Aboveground leaching	0.4	0.7	13.9

Zizaniopis is a fresh marsh plant and is included by way of comparison to illustrate the potential importance of N availability in controlling recycling efficiency, seen here as the profligate leaching of N from dead *Zizaniopis*. Units—g N m^{-2} year^{-1}

year^{-1}, 46% of which was new N taken up from the sediment. Of the N transferred aboveground, 14.4 g was lost to detritus upon culm death, 0.7 g was leached from the living culms, and 17.9 was translocated from senescing leaves to rhizomes for storage or immediate transfer to actively growing shoots. The high degree of N conservation in *Spartina*, low leachability, and retraction from senescent tissue are consistent with plant growth being N limited.

These results are in general agreement with patterns observed for a much less productive, short form of *S. alterniflora* in a New England salt marsh (White and Howes, 1994c). Using an isotopic approach, White and Howes (1994c) concluded that the fate of translocated N was not in support of next years aboveground production. While the relative percentage translocated was similar in the two studies (38% and 54% of aboveground uptake), the estimated importance of translocated N for next season's aboveground growth differed substantially. From the ^{15}N tracer, White and Howes (1994c) determined that none of the translocated N was retranslocated aboveground the following season. Hopkinson and Schubuaer assumed from mass balance considerations that it was all reused.

The high degree of N reuse in salt marsh macrophytes is consistent with them being N limited. By way of comparison, in a fresh water riverine system with high N loading, *Zizaniopsis* shows relatively little internal N recycling and high leaching loss upon death (Hopkinson, 1992). There is the indication that nutrients turn over more rapidly and nutrient cycles are less retentive and conservative as systems become more open and nutrient inputs increase. The degree of system closure and N availability appears to be a major factor controlling nutrient retention and cycling in wetland ecosystems.

3.3.4. Summary of salt marsh nitrogen fluxes

The magnitude of N fluxes internal to salt marshes and between salt marshes and adjacent systems are summarized in Table 22.7. The largest fluxes are typically internal to the system and involve the uptake of inorganic N by marsh macrophytes, plant death, organic matter decomposition and remineralization of organic matter in and on marsh sediments. The turnover of N in salt marsh sediments can be estimated by comparing these internal N fluxes to the standing stock of N in salt marsh sediments (460–1300 g N m^{-2} to 30 cm—see Table 22.1). Based on the annual rate of plant N uptake, the turnover rate of N ranges from about 0.03 year^{-1} to 0.09 year^{-1}. The turnover rate of inorganic forms of N is much higher, being as high as 54 year^{-1} for NH$_4^+$.

Inputs, outputs and exchanges of N with systems adjacent to salt marshes are generally much smaller in magnitude than internal fluxes (Table 22.7). The source and relative importance of various external inputs of N to salt marshes varies from system to system. While the input of N from rivers is potentially large, most of this N is probably not taken up by salt marshes but is processed in aquatic portions of estuaries or routed to the open ocean. On average, the largest input is from N fixation (2–15 g N m^{-2} year^{-1}), followed by atmospheric deposition (0.5–2.2 g N m^{-2} year^{-1}). Groundwater inputs are a major source of N in some smaller salt marshes with developed uplands such as found in the northeastern United States.

Table 22.7 Summary of the range of values found for major salt marsh nitrogen fluxes both internal to the system and with adjacent ecosystems

Category	Description	Flux $(g\,N\,m^{-2}\,year^{-1})$
Inputs	Rivers (estuarine average)	28
	Groundwater	0–12.6
	Atmosphere	0.5–2.2
	Fixation	2–15
Cross-system exchanges	Tidal (negative = export)	−4.5+12
	Fish	3.1 (export)
Outputs or accumulation	Denitrification	2–20
	Sediments	4–16 (accumulation)
Internal cycling	Decomposition	1.4 $year^{-1}$
	Ammonification	5–53
	NH_4^+ uptake/immobilization	15% of ammonification
	Nitrification	1/10–1/3 of ammonification
	Dissimilatory nitrate reduction	15% of nitrification
Plant cycling	Root uptake	21–57
	Aboveground uptake	4–39
	Aboveground death	3–15
	Aboveground leaching	0.4–14
	Translocation belowground	1–18
	Belowground death	20–28
	Retranslocation aboveground	0–18

Values for different categories often represent ranges from very different systems based on a variety of assumptions. Values should not be averaged.

Major outputs of N include denitrification, and burial (sediment accumulation). On average denitrification is similar to N fixation in magnitude (Table 22.5 and Fig. 22.3). In the few cases where both fluxes have been quantified for an entire marsh system, denitrification usually exceeds fixation (e.g., Great Sippewissett Marsh; Valiela and Teal, 1979). There are exceptions; in the Barataria Basin marshes of Louisiana, fixation exceeds denitrification by 6 g N m^{-2} year^{-1} (10 vs 4 g N m^{-2} year^{-1}). Some specific habitats, such as microbial mats (Joye and Paerl, 1994) and transplanted young marshes (Currin *et al.*, 1996) also have fixation rates that are much greater than denitrification. The burial of N can also be substantial, varying in importance in relation to rates of sea level rise. Burial rates are similar in magnitude to fixation (Fig. 22.3). Where studied, fish migration has been shown to be an important component of N exchange between tidal waters and salt marshes, but it has received little attention in most salt marsh studies. The flux with the greatest variability between systems is tidal exchange of dissolved and particulate forms of N.

The N budget of Great Sippewissett marsh published in 1979 is still the only one we have found that measured all of the major inputs and outputs on a seasonal basis
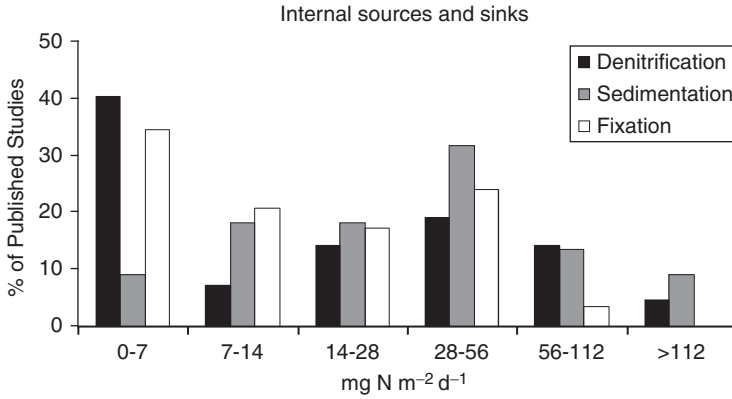
Figure 22.3 Summary of values of the internal sources and sinks of nitrogen in marshes from studies in Tables 22.2, 22.4 and 22.5.

in all of the major zones on the marsh platform and in small tidal creeks (Valiela and Teal, 1979 Fig. 22.4). This budget provides an example of the role of marshes in the coastal landscape, but the reader should keep in mind that this small, groundwater–dominated pocket marsh in the US northeast might not be representative of all marshes. For this system, marsh sinks (denitrification and burial) remove 11.21 g N m^{-2}
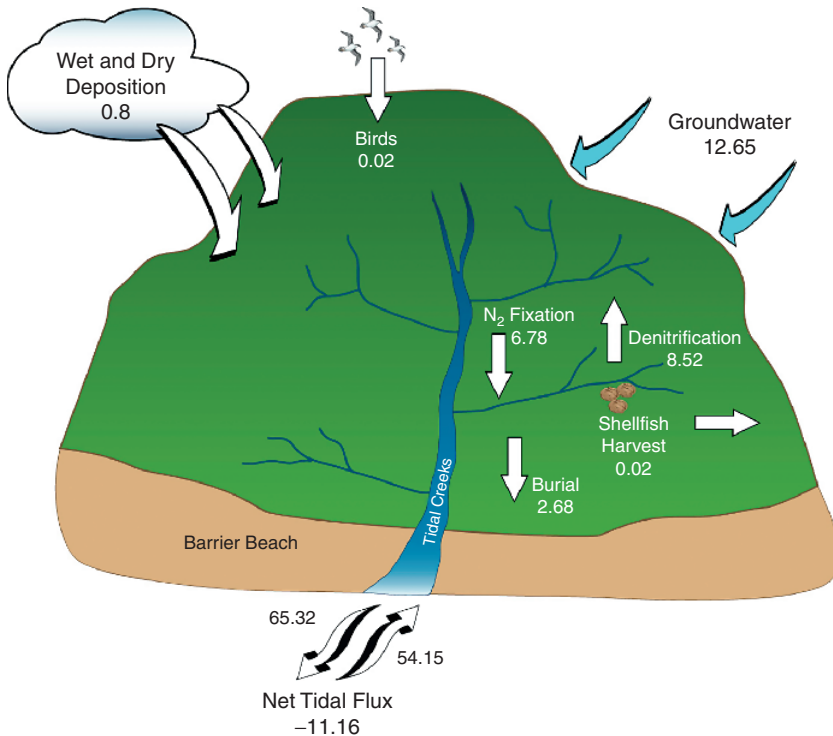
Figure 22.4 Summary of the nitrogen budget of Great Sippewissett Marsh, Massachusetts, USA (Valiela and Teal, 1979). All fluxes are in g N m^2 year^{-1}.

year^{-1}, which is a large percentage of the land based and atmospheric N inputs to the marsh (13.47 g N m^{-2} year^{-1}). However, fixation adds another 6.78 g N m^{-2} year^{-1} to the marsh, so the net removal of N from the land and the atmosphere is 4.43 g N m^2 year^{-1}, or 33%. This can be compared to the N exported at the mouth (net export of 11.16 g N m^{-2} year^{-1}), which would suggest a smaller removal of land and atmospheric inputs (2.29 g N m^{-2} year^{-1} or 17%). Approximately 55% of inputs (from land and fixation) are exported to the ocean, with the remainder stored or denitrified. These balances make it clear that marshes can be both sinks and sources within the larger landscape.

4. Eutrophication and Management

There have been at least two studies examining the effects of applying sewage to salt marshes. The reader is directed to papers by Haines, Sherr and Chalmers for a review of these studies in a Georgia salt marsh (e.g., Chalmers *et al.*, 1976) and to papers by Valiela, Teal, and Howes for a Massachusetts tidal marsh (e.g., Valiela *et al.*, 1973, 1976, 1985b). A number of studies have also examined how anthropogenic nutrient inputs may be affecting salt marshes (Bertness *et al.*, 2002; Rosensweig, 1999; Warren and Niering, 1993). Unlike non-tidal, freshwater marshes, tidal salt marshes are typically not used for the treatment of wastewaters but by virtue of their location are receiving ever increasing N loads. The estuarine research and management community is greatly concerned about N-enrichment and eutrophication of estuaries (NRC, 2000). There is the general feeling that salt marsh dominated estuaries are less susceptible to N-enrichment (NRC, 2000), but the data to rigorously defend this assumption is lacking and in need of substantial future research.

Comparative evidence suggests that estuaries with salt marshes protect adjacent seagrass meadows from land-derived N loads (Valiela and Cole, 2002). The specific role that marshes play in actually reducing total external loads (land, atmospheric, oceanic) can be determined by the net balance between new internal inputs (fixation) and the internal sinks (denitrification and burial). Our examination of the literature suggests that denitrification and burial are of similar magnitudes on a system basis. As each of these processes equals or exceeds published rates of N fixation it appears that marshes can in fact play a role in protecting downstream coastal ecosystems from land and atmospheric N inputs. Valiela *et al.* (2000) suggest that salt marshes can play an effective role in systems with external loading rates up to 10 g N m^{-2} year^{-1}. However, if fixation decreases with increased loading, and denitrification increases with loading to the degree seen in some studies (Hamersley and Howes, 2005), then marshes may be effective at even higher loading rates. An exception to this pattern may be young or constructed salt marshes where fixation appears to exceed denitrification. In addition, by transforming dissolved inorganic N to organic forms (most of which have extremely long turnover times relative to NO$_3$) and temporally buffering N loads to downstream systems, all marshes could

play a substantial role in reducing some of the adverse effects of nutrient loading on seagrass meadows and open water systems. However, research to support this hypothesis is currently lacking.

5. ROLE OF SALT MARSHES IN GLOBAL NITROGEN BALANCE

Anthropogenic alterations of the global N cycle have increased our interest in quantifying global N sinks and source. Here we ask what role salt marshes might play in the global cycle. Based upon a global salt marsh area of 3.8×10^{11} m^2 (Maltby, 1988) and a median denitrification rate of 21 mg N m^{-2} day^{-1} (after eliminating negative net numbers which include fixation), marshes return about 3 Tg N year^{-1} to the atmosphere. This level of denitrification is less than the amount of denitrification taking place in estuarine subtidal sediments worldwide, estimated at 8 Tg N (Seitzinger *et al.*, 2006). While there may be some overlap in the small creek areas considered in our estimate and the subtidal sediments used in the Seitzinger *et al.* (2006) calculations, the majority of the 3 Tg N year^{-1} we calculate for marshes would be in addition to their estimate for estuaries as a whole (including marshes). Even when added together however, coastal areas are minor sinks for N when compared to continental shelves and oceanic oxygen minimum zones, which together remove more than 300 Tg N year^{-1} (Seitzinger *et al.*, 2006). We expect there to be large variances associated with these global estimates. While the magnitude of the error is hard to quantify, an indication of its size can be seen merely from the range of values associated with N cycling rates in Table 22.7, and this does not include any potential error in the estimate of global salt marsh area.

The loss of N through denitrification is partially offset by N fixation. Using the median N fixation rate of 12.6 mg N m^{-2} day^{-1}, total N fixation introduces about 1.7 Tg N year^{-1} into marshes.

Estimates of natural global N fixation range from 190 to 370 Tg N year^{-1} but in either case, marshes represent only a small fraction of the global total (Galloway *et al.*, 2004). However, it does mean that there is a net loss of combined N as N$_2$ in marshes at a rate of about 1.3 Tg N year^{-1}.

Although marshes may be only a small global sink for combined N, marshes do play a significant role in removing N from the coastal zone. If we again use the global marsh area and assume a burial rate of 7–15 g N m^{-2} year^{-1} then 2.6 to 5.7 Tg N year^{-1} is sequestered in marshes. When this is added to gaseous losses, on net balance marshes remove approximately 4–6 Tg N year^{-1}.

When compared to the total amount of N entering the coastal zone from anthropogenic sources, which now exceed 150 Tg N year^{-1}, the global contribution of marshes in removing anthropogenic N is small globally but relatively large in proportion to the total marsh area. However, if this removal is compared to the amount of N entering coastal areas then it is locally quite significant. Riverine inputs averaged for all coastal temperate systems between 23.5° and 66°N and S are 16.8 Tg N year^{-1} (Chapter 9 by Seitzinger and Harrison, this volume). Not all of this load enters estuaries, and this does not include groundwater or direct deposition. However, salt marshes may remove as much as 1/3 of this load.

6. Summary and Future Research Directions

1. Our current state of knowledge on the controls, and even the quantity of net gaseous fluxes of N_2 in salt marshes is poor due to the problems of applying existing methods to vegetated intertidal sediments. Methods need to be improved to enable measures of N_2 flux across the salt marsh sediment—atmosphere interface including marsh macrophytes. However, even with the limitation of existing methods, the number of studies on gaseous N fluxes in salt marshes is quite small and seasonal data is lacking.

2. In spite of the limitations of the data, this review has shown that most salt marshes appear to serve as a net sink for N. Very young marshes, or constructed marshes appear to be exceptions to this pattern. Although on a global basis marshes do not play a major role in the global N cycle, areal rates of N sequestration are high enough that marshes can play a significant local role in removing N.

3. Marshes have experienced increased input of anthropogenic N loads over the last century. There is growing evidence that fixation, denitrification and even sedimentation rates are altered by increased external N inputs but these responses need to be much better understood and quantified.

4. There are large fluxes of N between marshes and tidal creeks and between marsh/creek complexes and estuarine and coastal waters. The net direction of the fluxes is not consistent and the rules governing exchange are not well understood. A review of a small number of studies has suggested that marshes generally export N to coastal waters, although some specific forms, such as nitrate may be imported. Marshes play a large role in altering both the timing and the form of N flowing from land to sea. The degree to which this interaction buffers coastal systems from land-based nutrient inputs and supports estuarine food webs needs to be better understood.

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SEAGRASS HABITATS

Karen J. McGlathery

Contents

1. INTRODUCTION

Seagrasses are marine flowering plants that comprise approximately 0.02% of the species of angiosperm flora, with >60 recognized species (den Hartog and Kuo, 2006; Hemminga and Duarte, 2000). They are distributed worldwide in the coastal zone of every continent except Antarctica, and are estimated to cover about 10% of coastal sediments (Charpy-Rouband and Sournia, 1990). Seagrasses are among the most productive ecosystems in the world, with annual rates of production similar to

that of salt marshes, coral reefs, and tropical rainforests (Valiela, 1995). As "foundation" species, seagrasses provide food resources and a three-dimensional habitat structure that supports a diverse array of algae, invertebrates, fish, and megafauna (e.g., turtles, dugongs). Many of these species are of high commercial value. Seagrasses are also tightly linked to adjacent ecosystems; they serve as both a nursery ground for juvenile fish that spend their adult lives in coral reefs or offshore (e.g., Jackson *et al.*, 2001) and export dissolved and particulate organic matter (e.g., Hemminga *et al.*, 1991). Finally, seagrass roots and rhizomes stabilize the seafloor, preventing sediment erosion/resuspension and promoting particle deposition (Gacia *et al.*, 2002; Kenworthy *et al.*, 1982; Koch, 1999). These ecosystem services make seagrasses a particularly valuable coastal resource. At the same time, seagrasses are vulnerable to environmental changes and recent studies have documented global declines in seagrass habitat, many of which are associated with anthropogenic stresses (Hauxwell and Valiela, 2004; see online list referenced in Orth *et al.*, 2006).

Seagrasses colonized the marine environment about 100 million years ago from either coastal land plant or freshwater plant ancestors (Den Hartog, 1970). To successfully adapt to marine conditions, these plants had to acquire characteristics for architecture in a high-energy environment, submarine pollination, and internal gas transport to shunt oxygen to the root zone (Hemminga and Duarte, 2000). Seagrass diversity is low compared to freshwater or terrestrial plant communities. There are 50 species in 12 genera representing only 2 families: Hydrocharitaceae and Potomogeonaceae. This number will likely change as new studies using DNA sequencing provide evidence of genetic diversity in seagrass meadows (Waycott *et al.*, 2006). Most seagrass meadows are monospecific; even the most speciose meadows, which occur in the Indo-Pacific and the Red Sea, have <12 species. Globally, there is a pattern of decreasing species diversity from the equatorial zone to higher latitudes. Most of the species diversity in seagrass meadows is related to the algae and fauna associated with the seagrasses. Within a species geographic range, high production rates are controlled by temperature, light, and nutrient availability. Adequate substrate and dessication are also important determinants of species distributions, as is disturbance, including storms, intense grazing, siltation, and excess nutrient loading (eutrophication). Most species are confined to sandy–muddy sediments, although a few species can grow over rock and in the intertidal zone.

Nitrogen (N) cycling processes in shallow coastal systems are influenced by seagrasses in several important ways. Seagrasses affect the timing and pathways of N processing through uptake and temporary retention in their tissue and the subsequent fate of this plant-bound N. In many (if not most) systems, N (and phosphorus) assimilation by seagrasses (and other primary producers) is of the same magnitude as watershed and atmospheric nutrient loading (Pedersen *et al.*, 2004). Thus, seagrasses and other marine primary producers are an important part of the estuarine filter and have a major impact on the fate and retention of nutrients as they transit through the system (McGlathery *et al.*, 2007). The possible fates of plant-bound N include mineralization and burial, grazing and subsequent excretion,

exudation, and advection/export of plant tissue. Seagrasses also influence physico-chemical conditions that affect the rates and pathways of bacterially and chemically mediated nutrient transformations such as mineralization, nitrification, N fixation, dissimilatory nitrate reduction to ammonium (DNRA), and sediment diagenesis. Changes in seagrass distribution and abundance in response to environmental change or restoration will likely have significant effects of the fate and transformations of N in nearshore coastal environments.

2. Nitrogen Inputs to Seagrass Ecosystems

Nitrogen inputs to seagrass ecosystems derive from both external sources of ''new'' N and internal sources of recycled N from sediment mineralization (and to a lesser extent from N excreted by consumers). The external sources of N to seagrasses include inputs from the watershed and airshed and bacterial N fixation within the system. Watershed nutrient inputs are enriched from fertilizer application, atmospheric deposition, and urban/domestic wastes, and are delivered to estuaries and coastal bays via surface and groundwater flow (see Seitzinger and Harrison, this volume). The production of inorganic fertilizer represents the single largest human alteration of the global N cycle, accounting for over half of the increase in bioavailable N (Galloway et al., 2003; Howarth et al., 2002; Vitousek et al., 1997). This is coincident with the explosive increase in coastal eutrophication from nutrient over-enrichment. The worldwide trend of increasing urbanization along the coastline, and shifts from forest to agricultural, suburban, and urban land use have accelerated delivery rates of nutrients and sediments to seagrass ecosystems (Nixon, 1997; Valiela et al., 1992). Atmospheric deposition of organic (DON) and inorganic N (NO_x) is enriched from the burning of fossil fuels, and is especially important in the N budget of shallow coastal bays and lagoons where seagrass abound and where the surface area to water volume ratio is high (Paerl, 1995). In some of these systems, the contribution of atmospheric N is of similar magnitude to that of groundwater N (Sigua and Tweedale, 2003). Nitrogen fixation accounts for 5–65% of seagrass N demand in diverse temperate and tropical systems (McGlathery et al., 1998; Welsh, 2000).

Internal recycling of N from sediment mineralization is a critically important N source for seagrasses, particularly in shallow coastal bays and lagoons that lack significant riverine inputs and where the sediment surface area is high relative to the volume of the water column (Chapter 8 by Bronk and Steinberg, this volume; Chapter 19 by Joye and Anderson, this volume; McGlathery et al., 2007). The nutrient demands of seagrasses appear to be met in large part by internal recycling of organic-bound nutrients in the sediment (Alcoverro et al., 2000; Hemminga et al., 1991; McGlathery et al., 2001a), although foliar uptake of ''external'' nutrients in the water column and root uptake of groundwater nutrients also may be important (Lepoint et al., 2002a; Stapel et al., 1996).

3. Nitrogen Controls on Production, Morphology, and Dynamics of Seagrasses

3.1. Evidence for nitrogen limitation

Nitrogen limitation is common in temperate coastal systems with siliclastic sediments (Howarth et al., 1988). However, benthic production in these systems is frequently limited by light availability rather than by nutrient availability (Dennison et al., 1987; Pedersen, 1995). The dichotomy of N limitation of primary production in temperate systems with siliclastic sediments and phosphorus (P) limitation in systems (typically tropical–subtropical) with carbonate sediments was first proposed by Short (1987), and has been attributed to (1) high rates of denitrification relative to N fixation (but see below) and (2) to the binding of P by carbonate sediments, which reduces P availability for plant uptake (Fourqurean et al., 1992; McGlathery et al., 1994; Morse et al., 1985; Short et al., 1990). However, there are many studies that show N limitation of seagrass or macroalgal growth in carbonate sediments (Erftemeijer et al., 1994; Ferdie and Fourqurean, 2004; McGlathery et al., 1992; Udy et al., 1999). The biogeochemical factors that may explain this variability include differences in sediment grain size (Erftemeijer et al., 1994), sorption–site saturation for P (McGlathery et al., 1994), and carbonate dissolution in the seagrass rhizosphere (Burdige and Zimmerman, 2002; Jensen et al., 1998; Long et al., 2008). There are also a few fertilization studies that indicate P limitation of seagrass growing in temperate systems or in sediments with low carbonate content (Harlin and Thorne-Miller, 1981; Murray et al., 1992; Perez et al., 1991). Clearly, species- and site-specific characteristics are more important than previously thought in determining N (versus P) limitation of seagrass.

For N-limited seagrass, an increase in ambient N concentrations, either in the sediment pore water or the water column, increases foliar or root uptake (Pedersen et al., 1997a,b; Perez-Lorens and Niell, 1995; Touchette and Burkholder, 2000). Higher N assimilation rates are indicated by increases in glutamine synthase or nitrate reductase activities (Pregnall et al., 1987; Touchette and Burkholder, 2000). Also, both the total tissue N content and specific N pools (nitrate, asparagines, glutamine) increase with higher N availability (e.g., Duarte, 1990; Erftemeijer et al., 1994; Terrados et al., 1999; Uddy and Dennison, 1997; Udy et al., 1999). The increase in tissue N content can be diluted by new growth if utilization is faster than uptake in high light environments (Peralta et al., 2002; Ruiz and Romero, 2001). If concentrations of NH_4^+ and NO_3^- exceed thresholds for upper tolerance limits, seagrass photosynthesis and growth decline (Burkholder et al., 1992, 1994; van Katwijk et al., 1997).

3.2. Nitrogen effects on seagrass architecture and production

Nitrogen enrichment changes specific morphologic characteristics as well as the allocation of plant biomass to aboveground and belowground tissues. Many studies report increases in leaf length and width, and shoot height and biomass in response to

N (and P) enrichment (e.g., Murray *et al.*, 1992; Terrados *et al.*, 1999; Udy and Dennison, 1997). Fertilization and nutrient gradient studies show that biomass allocation patterns change in predictable ways with increases in water column or sediment nutrient availability. The root–shoot ratio decreases with higher sediment nutrient availability, as plants allocate less biomass to root/rhizome tissue (Perez *et al.*, 1991; Powell *et al.*, 1989). When sediment nutrient concentrations are low, more biomass is allocated to belowground tissues to increase the surface area for nutrient uptake (Vogt *et al.*, 1993). There is insufficient information about how rhizome architecture changes with nutrient availability, including branching frequency and angle and internode length, although these characteristics are known to be as plastic in some species as leaf morphology.

Changes in biomass and productivity in response to nutrient enrichment are seen both at the individual and population levels. Responses of individual ramets include increases in leaf growth, length, and turnover (e.g., Lee and Dunton, 2000; Murray *et al.*, 1992; Udy and Dennison, 1997). At the population level, nutrient enrichment has been shown to increase both recruitment and shoot mortality (Perez *et al.*, 1991). Higher rates of mortality may be related to greater intra-specific competition for light, or to NH_4^+ or NO_3^- toxicity (Burkholder *et al.*, 1992; 1994; Hauxwell *et al.*, 2001; van Katwijk *et al.*, 1997; Perez *et al.*, 1994). The effects of N enrichment on allocation to flowering are not known, but may be important as this reproductive pathway requires substantial N resources.

3.3. Nitrogen effects on seagrass community structure and distribution

At the community level, N (and P) enrichment causes a shift in the dominance of primary producers, from seagrasses and perennial macroalgae to fast-growing macroalgae and phytoplankton (Duarte, 1995; Sand-Jensen and Borum, 1991; Valiela *et al.*, 1997). Increased nutrient loading related to anthropogenic activities appears to be responsible for 60% of the global seagrass loss (Short and Wyllie-Escheverria, 1996). Loss of seagrass is associated with shading from enhanced algal standing stocks (macroalgae, epiphytes, and/or phytoplankton; Hauxwell *et al.*, 2001, 2003; Neckles *et al.*, 1994; Nixon *et al.*, 2001; Short *et al.*, 1995; Twilley *et al.*, 1985). In addition, changes in the biogeochemical environment surrounding the seagrass roots and shoots, such as anoxia and increased sulfide and NH_4^+ concentrations, can lead to seagrass decline (Greve *et al.*, 2003; Hauxwell *et al.*, 2001). There is also evidence that in the more speciose tropical systems competition between seagrass species with N and P enrichment causes a shift in seagrass dominance before algal proliferation occurs (Fourqurean *et al.*, 1995; Udy and Dennison, 1997). Early indicators of eutrophication effects on seagrasses have been identified as increases in leaf elongation rates, shoot–root biomass ratios, and the ratio of leaf N to leaf mass, and a decrease in shoot density (Lee *et al.*, 2004; Nixon *et al.*, 2001).

In many systems, water column N concentrations set the maximum depth limit of seagrass by influencing phytoplankton biomass and light attenuation in the water column (Abal and Dennison, 1996; Nielsen *et al.*, 2002). Increased epiphyte biomass with higher N loading attenuates light at the scale of the seagrass blade (Brush and

Nixon, 2002; Howard and Short, 1986; Sand-Jensen, 1977), and can attenuate 10–90% of light potentially reaching the leaf surface. Also, bloom-forming macroalgae that form dense canopies up to 1 m thick over seagrass beds in high N waters can decrease light levels by >90% (Astill and Lavery, 2001; Krause-Jensen *et al.*, 1996; Peckol and Rivers, 1996), and particularly impact newly recruiting shoots that often lie entirely under, or within, the macroalgal mat (Hauxwell *et al.*, 2001). The reduction in seagrass photosynthesis that results from algal shading decreases oxygen translocation and release to the rhizosphere (Goodman *et al.*, 1995; but see Terrados *et al.*, 1999). This creates a positive feedback where the high concentrations of hydrogen sulfide that develop in the rhizosphere decrease nutrient uptake and plant energy status (Pregnall *et al.*, 1984), and result in further reductions in photosynthesis, growth, and leaf density, and sometimes, eventual mortality (Goodman *et al.*, 1995; Holmer and Bondgaard, 2001).

Benthic faunal communities are altered by changes in rates of primary production and a shift from seagrass to benthic algae with increased N loading (Diaz and Rosenberg, 1995; Grall and Chauvaud, 2002). During the early stages of nutrient enrichment, an increase in species richness, abundance, and biomass of macrofauna has been associated with an increase in food supply (Grall and Chauvaud, 2002). Also, several studies have shown that the structural complexity associated with increasing macroalgal biomass at low to moderate densities in seagrass beds reduces predation rates on mobile epifauna (e.g, crustaceans, fish) by providing more refuge (Isaksson *et al.*, 1994; Norkko, 1998; Pihl *et al.*, 1995; Raposa and Oviatt, 2000). As systems become more nutrient enriched and algal blooms occur, changes in oxygen and sulfide conditions cause the benthic infaunal community to shift from deep bioirrigating organisms to tube-building filter feeders which are less effective at mixing and irrigating the sediments (Henriksen *et al.*, 1980; Gray, 1989; Pearson and Rosenberg, 1978). Since these bioirrigating macrofauna stimulate coupled nitrification–denitrification in sediments by increasing the sediment surface area and oxygen penetration along burrow walls, and also the fluxes of water column nitrate into the sediments (Aller and Rude, 1988; Bronk and Steinberg, this volume; Joye and Anderson, this volume; Mayer *et al.*, 1995; Pelegri *et al.*, 1994), their loss will likely reduce the natural capacity of systems to buffer N loading. Ultimately, increased periods of sediment anoxia will result in a decrease in the abundance and diversity of benthic fauna and will reduce the role of seagrasses in supporting higher trophic levels (Deegan *et al.*, 2002; Gray, 1992, Norkko and Bonsdorff, 1996a,b).

4. Nitrogen Incorporation in Seagrass Biomass

Rates of N assimilation and retention vary with plant size, reflecting differences in relative surface area, and/or thickness (Sand-Jensen and Nielsen, 2004). In general, seagrasses have tissues that are lower in nutrients and higher in lignin, and have relatively lower photosynthetic potential and inherent growth rates, than benthic microalgae and ephemeral macroalgae (Duarte, 1995; Hein *et al.*, 1995). Seagrasses are able to meet their nutrient requirements in areas of relatively low

nutrient availability by reducing their dependence on nutrient sources through (1) exploiting tissue storage pools and (2) reclaiming nutrients from decaying tissues, and also by accessing sediment nutrient pools via their roots (Alcoverro *et al.*, 2000; Pedersen and Borum, 1993; Stapel and Hemminga, 1997).

4.1. Uptake

Seagrasses obtain nutrients both from sediment pore waters and from the overlying water column. Concentrations of inorganic N are typically one to several orders of magnitude higher in sediment pore waters than in the water column as a result of organic mineralization (Touchette and Burkholder, 2000) and nitrogen fixation in the sediment. Ammonium is the dominant source of inorganic N in the anoxic rhizosphere, with concentrations ranging from 1 to 200 M (Touchette and Burkholder, 2000). Leakage of oxygen from the lacunar system in the roots creates oxidized microzones where NH_4^+ is oxidized to NO_3^-; however, these microzones are limited to a few millimeters (Frederiksen and Glud, 2006) along the root tips, and bulk measurements of sediment pore water rarely detect NO_3^-. When NO_3^- is present in the pore water, it is more likely ''new'' N from groundwater inputs (McGlathery *et al.*, 2001a). In addition to inorganic forms of N, amino acids can contribute to seagrass nutrient demands, although there is relatively little work on DON uptake by seagrasses compared to marine algae (Bird *et al.*, 1998; Tyler *et al.*, 2003; Ward and Bronk, 2001).

The relative role of sediment versus water as a nutrient source to seagrasses depends on the concentrations of available nutrients for each source, the surface area of the leaves relative to the roots and their uptake capacities, and the thickness of the diffusive boundary layer surrounding the leaves and roots (Lepoint *et al.*, 2002a; Stapel *et al.*, 1996). These effects are important both within a single species where growth conditions vary, and between species with different morphologies. Earlier studies indicated that root uptake was the primary mechanism for meeting seagrass nutrient demands because of the high nutrient concentrations in sediment pore waters (e.g., Thursby and Harlin, 1982). There is considerable variation among species, however, and there is some evidence that root uptake can be limited by diffusion (Lee and Dunton, 1999; Stapel *et al.*, 1996). Several recent studies have shown that the contribution of leaf and root uptake is of approximately equal magnitude (Table 23.1; Lee and Dunton, 1999; Lepoint *et al.*, 2002a). Growth form also has an important influence on the relative importance of root vs. leaf uptake. Annual populations of the temperate seagrass, *Zostera marina*, may rely solely on leaf uptake, whereas perennial populations with greater root biomass have both significant root and leaf uptake (Hemminga *et al.*, 1994; Pedersen and Borum, 1993). As an extreme example, species such as *Phyllospadix torreyi* that grow on rocky substrate assimilate all nutrients through the leaves (Terrados and Williams, 1997). Because leaves can have relatively high nutrient uptake affinities, many seagrass species can assimilate N when water column concentrations are very low (Lee and Dunton, 1999; Pedersen *et al.*, 1997a,b). Water column nutrient enrichment may cause a shift from root to leaf uptake as the dominant process of nutrient acquisition. Nutrient uptake by seagrass leaves is influenced by the thickness of the diffusive

Table 23.1 Nitrogen uptake parameters for seagrass species based on Michaelis–Menten uptake kinetics

Seagrass species	Organ	Nutrient	V_{max} (μmol g DW^{-1}h^{-1})	K_m (μM)	$\alpha \cdot (V_{max}/K_m)$	Source
Temperate						
Z. marina	Leaf	NH_4^+	1.1–3.2			Short and McRoy (1984)
Z. marina	Leaf	NH_4^+	20.5	9.2	2.2	Thursby and Harlin (1982)
A. antarctica	Leaf	NH_4^+	5.9–43.1	9.5–74.3	0.6–0.8	Pedersen *et al.* (1997)
P. torreyi	Leaf	NH_4^+	96–204	9.3–34	6–10.3	Terrados and Williams (1997)
Z. marina	Leaf	NO_3^-		23		Iizumi and Hattori (1982)
P. torreyi	Leaf	NO_3^-	25–75	4.4–17	4.4–5.7	Terrados and Williams (1997)
Z. marina	Root	NH_4^+	0.9–2.9			Short and McRoy (1984)
Z. marina	Root	NH_4^+	211	104	0.5	Thursby and Harlin (1982)
Z. marina	Root	NH_4^+		30		Iizumi and Hattori (1982)
A. antarctica	Root	NH_4^+	1.1	4.7	0.2	Pedersen *et al.* (1997)
Tropical/subtropical						
Thalassia testudinum	Leaf	NH_4^+	8.3–16.4	7.6–15	0.6–2.8	Lee and Dunton (1999)
T. hemprechii	Leaf	NH_4^+	32–37	21	0.5–0.9	Stapel *et al.* (1996)
T. testudinum	Leaf	NO_3^-	3.7–6.5	2.2–38.5	0.2–1.7	Lee and Dunton (1999)
T. testudinum	Root	NH_4^+	8–73	34	0.03–0.3	Lee and Dunton (1999)

V_{max} is the maximum uptake rate, K_m is the half-saturaion constant, and α is the uptake efficiency at low nutrient levels (V_{max}/K_m).

boundary layer. Wave action may reduce the boundary layer thickness and enhance uptake by as much as an order of magnitude (Stevens and Hurd, 1997). Epiphytes also may reduce the boundary layer thickness under turbulent conditions by creating surface roughness, but they also compete with seagrasses for water column nutrients.

In a recent review of N and P metabolism in seagrasses, Touchette and Burkholder (2000) provide a detailed description of the mechanisms of NH_4^+ and NO_3^- uptake and assimilation. Nitrate is taken up via an active, inducible, protein-based transport system that is specific to NO_3^- and inhibited by anaerobic conditions. Ammonium uptake is apparently more complex, involving a low-affinity system based on passive movement of NH_4^+ through membrane channels, and a high-affinity system that requires the synthesis of proteins that transport NH_4^+ through membrane channels. Compared to marine algae, much less is known about organic N uptake. There is very limited evidence of species that are capable of both amino acid and urea uptake, and some that are not (Bird *et al.*, 1998; McRoy and Goering, 1974). Clearly, this is an area where more research is needed, especially for systems where concentrations of dissolved organic N exceed that of inorganic N in the water column (McGlathery *et al.*, 2001b).

Numerous estimates of Michaelis–Menten kinetic parameters for N uptake have been reported for both leaves and roots of various seagrass species, and show that uptake rates are both highly variable across species and between leaves and roots (Table 23.1). For most species, the uptake "affinity," which reflects the efficiency of nutrient uptake at low nutrient concentrations ($\alpha = V_{max}/K_m$), is generally higher for NH_4^+ than for NO_3^-. This indicates that many seagrasses are more efficient at NH_4^+ uptake, and this is consistent with the higher cost of NO_3^- reduction and assimilation. NH_4^+ uptake is also more efficient in leaves compared to roots (lower α for roots), which likely reflects the lower ambient concentrations in the water column than in the sediment porewaters. V_{max} reflects the uptake "capacity" (an indication of the number of active uptake sites; Touchette and Burkholder, 2000), and variability is so high that there appear to be no clear trends either between NO_3^- and NH_4^+ or between leaves and roots. A few studies have shown linear responses in both NH_4^+ and NO_3^- uptake for *Z. marina*, *Thalassia hemprichii*, and *Amphibolis antarctica*, rather than the substrate saturation of Michaelis–Menten kinetics (Paling and McComb, 1994; Roth and Pregnall, 1988; Stapel *et al.*, 1996). The reasons for these two kinds of responses in the same species is unknown, but might reflect an adaptation to a limiting and/or pulsed nutrient supply.

Once taken up into the plant cell, NH_4^+ is assimilated via the glutamine synthetase (GS)/glutamate synthase (GOGAT) pathway (see Touchette and Burkholder, 2000 for details). Ammonium typically does not accumulate in seagrass tissues, partly because of its toxicity to chloroplast function, and is rapidly assimilated into organic compounds. The same pattern has been shown for marine macroalgae (McGlathery *et al.*, 1996). Studies of GS activity in seagrasses (particularly *Z. marina* and *Posidonia oceanica*) indicate the importance of the leaves as well as the roots in NH_4^+ assimilation (Kraemer and Alberte, 1993; Kraemer *et al.*, 1997; Pregnall *et al.*, 1987). Temperature and light availability account for some of the seasonal differences in GS activity (Kraemer *et al.*, 1997), and ultimately, the supply of NH_4^+, carbon skeletons, and ATP from photosynthesis influence GS rates. Nitrate reduction to

NH_4^+ (via nitrite reduction) and subsequent assimilation into organic compounds is a more energy-expensive process. The activity of the enzyme nitrate reductase (NR) that catalyzes the initial reaction is induced by the presence of NO_3^- in the plant tissue and is influenced by light availability (see Touchette and Burkholder, 2000 for details). Nitrate reduction occurs both in the leaves and roots, but appears to be the highest in leaves where NO_3^- is typically stored in vacuoles and where energy and carbon skeletons are produced via photosynthesis. NR activity is usually directly related to carbohydrate availability. If carbohydrate availability is low, then NR response to enhanced NO_3^- availability also tends to be low (Touchette and Burkholder, 2001; Zimmerman *et al.*, 1987). Young *et al.* (2005) measured NR activity associated with epiphytes on seagrass leaves (*Posidonia sinuosa* and *A. anartica*) and showed that this was as important as NR in seagrasses as a sink for water column NO_3^-.

4.2. Turnover in plant biomass

Seagrasses have several nutrient conservation strategies that enhance nutrient use efficiency and reduce the demand for uptake of nutrients from the environment. Internal recycling, enhanced leaf longevity, and storage are important mechanisms that reflect a high efficiency for retaining nutrients within plant tissues, and are advantageous in nutrient-poor coastal waters. Nutrient conservation by resorption from senescing leaves and reallocation to young, growing leaves has been studied for several seagrass species (*Z. marina*, *P. oceanica*, *T. hemprichii*; Alcoverro *et al.*, 2000; Borum *et al.*, 1989; Invers *et al.*, 2002; Lepoint *et al.*, 2002b; Pedersen and Borum, 1992; Stapel *et al.*, 2001). It is common for leaf N concentration to decrease with increasing leaf age (Alcoverro *et al.*, 2000; Borum *et al.*, 1989; Kraemer and Mazzella, 1999). This reflects N resorption and recycling as well as leaching from ageing leaves. Invers *et al.* (2002) suggested that N is transported during resorption primarily as free amino acids. Resorption may be most important in temperate seagrasses in the spring-summer when the growth is high and when external nutrient concentrations are often depleted (Alcoverro *et al.*, 1997; Pedersen and Borum, 1993; Pedersen *et al.*, 1997a,b; Stapel and Hemminga, 1997).

　　The methods for calculating nutrient resorption efficiency have included either (1) a mass balance approach to determine the percent reduction of nutrients between mature green and senescent leaves, or (2) ^{15}N tracer studies to follow the reuse of labeled N in plant tissues. The mass balance approach is less precise in that it does not consider potential leaching losses. However, studies on the same species using the two methods agree reasonably well (Romero *et al.*, 2006), suggesting that leaching losses may be small. Estimates for nutrient resorption range from 10 to 50% of annual N requirements for a variety of species (Romero *et al.*, 2006). For example, Pedersen and Borum (1992) showed that internal recycling could contribute up to 12% of the annual N demand of *Z. marina* leaves, Stapel *et al.* (2001) determined N resorption accounted for 28% of the N demand of *T. hemprichii* in Indonesia, and Lepoint *et al.* (2002b) and Alcoverro *et al.* (2000) found that the annual contribution of recycled N for *P. oceanica* was 40%.

Leaf longevity is important for nutrient conservation, and the higher values for *P. oceanica* may be related to the long lifespan of the leaves (as high as 300 days). High leaf longevity increases the time for nutrient resorption and also reduces nutrient demand by decreasing the frequency of leaf formation. In an earlier review of the literature, Hemminga *et al.* (1999) calculated an average resorption efficiency of 20%, which is about half that of perennial terrestrial plants (Duarte and Hemminga, 2000).

Nitrogen can be stored in both rhizomes and leaves; this slows the turnover of N in plant tissues. Nitrogen can be stored on a short time scale of hours to days and on a longer (weekly to seasonal) time scale. Short-term storage usually involves uptake and reduction of NO_3^- when it becomes available as pulses, such as during storms or at night (Invers *et al.*, 2002; Romero *et al.*, 2006; Touchette and Burkholder, 2000). Ammonium would not be expected to accumulate in plant tissues due to its toxicity (McGlathery *et al.*, 1996). Seasonal storage occurs in response to imbalances in the supply and demand of N. Nitrogen accumulates in seagrass tissues during periods when availability is high and growth conditions (light and temperature) are suboptimal (Alcoverra *et al.*, 1997; Kraemer and Mazzella, 1999), and these are used to mobilize growth when conditions improve. Amino acids can be long-term storage pools in seagrasses (Invers *et al.*, 2002; Udy *et al.*, 1999), and soluble proteins also can be important (Murray and Larkum, 1991). Invers *et al.* (2002) found that free amino acids accounted for 50% of the N pools in *P. oceanica*, and were important for N storage in rhizomes over the winter.

5. Fate of Assimilated Nitrogen

The presence of seagrasses slows the overall rate of N cycling compared to algal–dominated systems (Duarte, 1995). Nitrogen that is assimilated by seagrasses is, for the most part, temporarily retained in the system on a time scale of weeks to months. This is longer than the retention time for either bloom-forming macroalgae (days–weeks) or microalgae (days). Seagrass-dominated systems are expected to have higher rates of burial than algal-dominated systems in part because of the inherently greater refractory content of seagrass tissue (and lower nutrient content), and in part because of the presence of belowground material (roots and rhizomes) that is less likely to be exported (Banta *et al.*, 2004; Buchsbaum *et al.*, 1991; Duarte and Cebrián, 1996; Enriquez *et al.*, 1993; Klap *et al.*, 2000).

Most of the N bound in seagrass tissue will ultimately become available through leakage from live tissue, remineralization through decomposition, and grazing and subsequent excretion (Cebrian, 2002; Duarte and Cebrián, 1996). This is shown conceptually in Figure 23.2. The timing of this retention and nutrient release can be important as it can slow the seaward transport of nutrients and reduce nutrient availability for other autotrophs during the growing season. For example, N that was temporarily retained in *Z. marina* leaf tissue in a Danish coastal bay was either nearly completely mineralized (95–98%) in the fall when phytoplankton blooms were less likely to occur, or were lost when the leaves were detached and transported from the system (Risgaard-Petersen and Ottosen, 2000). Some material may be removed from

the system by long-term burial or export as dissolved or particulate organic matter (Figure 23.2). Export of senescent leaf material may be an important loss of N from the system, and this exported material can fuel secondary production in adjacent intertidal and subtidal systems such as marshes, mudflats, and even deep basins (Melville and Connolly, 2005; Suchanek *et al.*, 1985).

5.1. Mineralization and burial

The major fate of seagrass aboveground biomass in modern seagrass systems is decomposition and transfer through the detrital food chain, either *in situ* or after being transported to adjacent regions. However, measured rates of decomposition vary, with studies reporting rates ranging from 15 to 95% of total plant production (Mateo *et al.*, 2006). This amount is greater than that for many terrestrial producers (Cebrian, 1999). Decay of leaf litter *in situ* may contribute as much as 10–46% of the annual N demands for leaf production of several species, including *A. antarctica*, *P. oceanica*, *Cymodocea nodosa* (Romero *et al.*, 2006, and references therein).

Tissue N may play an important role in decomposition and detrital processing of seagrass biomass. Several studies have shown a direct relationship between higher tissue N content in senescent and detrital material and faster decomposition rates (Harrison, 1989; Lopez *et al.*, 1998; Mateo and Romero, 1997; Perez *et al.*, 2001). This would be expected if N is limiting to decomposers. Lopez *et al.* (1998) did find that nutrient fertilization increased bacterial activity and reduced sediment organic matter content by >30% in N-limited *P. oceanica* meadows in the Mediterranean. However, there is some evidence from correlative work using data from studies of six species, including *T. hemprichii*, *T. testudinum*, *P. oceanica*, *Z. marina*, *Zostera noltii*, and *Syringodium filiforme* that detrital N content and decomposition rates were not strongly related (Enriquez *et al.*, 1993). Overall, the effect of tissue N content on decomposition rates of seagrasses appears to be more equivocal than what has been reported for salt marshes (Chapter 22 by Hopkinson and Giblin, this volume), and more research is needed to determine the influence of tissue N content on decomposition.

Some of the difficulty in generalizing among individual studies may be related to the type of material used (e.g. senescent, detrital, fresh), the method used, and the length of the incubation. Decomposition typically follows a 3-phase exponential pattern of decreasing weight loss: (1) a short, rapid phase of leaching of soluble compounds, (2) a slower phase of microbial decomposition and release of soluble compounds, and (3) the slowest phase of breakdown of refractory compounds. The time scale of the different phases of degradation may vary considerably among seagrass species. Different methods used for the same species also may support different conclusions. For example, Banta *et al.* (2004) found that over 86% of *Z. marina* root and rhizome detrital mass (as N) and 14% leaf detrital mass (as N) remained after nearly 2 yr of a litter bag experiment. However, Risgaard-Petersen and Ottosen (2000) modeled *Z. marina* decomposition and suggested that most of the N bound in sloughed plant material was released within one year. Studies have reported different time scales to reach the refractory phase, from several months (*Z. marina*; Banta *et al.*, 2004; Kenworthy and Thayer, 1984) to several years (*P. oceanica*; Mateo *et al.*, 1997; Romero *et al.*, 1994). This decay-resistant refractory

material has a low N content and high lignin content compared to fresh material (Klap *et al.*, 2000; Mateo and Romero, 1997).

Of the potential fates of seagrass biomass, burial is probably the least known. There are few published rates on N burial, and these come from a limited number of species that are know to accumulate large amounts of belowground material (Table 23.2). The presence of a refractory detrital pool is the main determinant of the potential for long-term burial of N in the sediments over the time scale of years to decades, even centuries (Banta *et al.*, 2004; Cebrian, 1999; Duarte and Cebrian, 1996). Refractory material is mostly derived from seagrass belowground biomass; however, allochthonous material imported from nearby terrestrial or intertidal systems may also be important. A few species (*Thallosodendron ciliatum*, *P. oceanica*) accumulate peat-like material similar to salt marshes, but this phenomenon is rare among seagrasses (Romero *et al.*, 2006). Romero *et al.* (1992) found that the buried material in a *P. oceanica* meadow lost 50–80% of its N content within the first 3–5 yr, and that a 10-yr-old detritus had essentially the same composition as a 1000-yr-old material. This buried material represented a long-term sink of 7% of the total plant N (Mateo *et al.*, 1997). Even in this extreme case of peat formation, burial represented a relatively small "loss" of N from the system. Losses of N in other non–peat seagrasses ecosystems would be expected to be even less significant. Nonetheless, some believe that burial of N accumulated in seagrass biomass may provide a natural buffering mechanism to increased nutrient loading during the early stages of eutrophication (de Wit *et al.*, 2001).

5.2. Grazing

Hebivores strongly influence the biomass, productivity, and plant community composition in many marine systems, although we know less about the impacts of grazing on seagrass ecosystems than on macroalgal assemblages (e.g., coral reefs, kelp forests, rocky intertidal). Herbivores consume 15–50%, on average, of seagrass production (Cebrian, 1999; Cebrian and Duarte, 1998), and this is considerably lower than for algal-based systems (Cebrian, 2004). The structure of herbivore communities associated with seagrass ecosystems has changed over time, and this likely has resulted in a reduction in the proportion of seagrass productivity that is directly grazed (Domning, 2001; Valentine and Heck, 1999; Williams and Heck, 2001). Historically, large herbivores including abundant turtles, sirenians (dugongs and manatees), and waterfowl impacted seagrass communities. Today, these large herbivores are reduced in number or are absent (e.g., Jackson *et al.*, 2001), and grazing is dominated by fish, urchins, and invertebrates that feed on seagrass blades and their epiphytes. There are few herbivores that consume seagrass roots and rhizomes directly (Valentine and Heck, 1999). Our current view of seagrasses as detritus-based systems is based on this modern situation, and may well have been different during historical times (Domning, 2001; Jackson *et al.*, 2001).

Leaf N content is an important determinant of food preference and the quantity of seagrass material consumed for some herbivores. Several field and laboratory studies have found a positive relationship between leaf N content and grazing by parrotfish, turtles and dugongs (e.g., Goecker *et al.*, 2005; McGlathery, 1995;

Table 23.2 Comparison of nitrogen (N) mineralization and burial rates in temperate and tropical/subtropical seagrass meadows

Seagrass species	Location	Mineralization (mmol N m^{-2} d^{-1})	Burial (mmol N m^{-2} d^{-1})	Reference
Temperate				
Z. marina	Izembeck Lagoon, Alaska	29.6		Iizumi et al. (1982)
Z. marina	Crane Cove, Alaska	33.9		Iizumi et al. (1982)
Z. marina	Great Harbor, Woods Hole, Massachusetts	16.1–80.4		Dennison et al. (1987)
P. oceanica	Fanals Point, Spain		0.1	Gacia et al. (2002)
P. oceanica	Mendes Islands, Gerano, Spain		0.22	Mateo and Romero (1997)
P. oceanica	Various Mediterranean sites		0.05–1.12[*]	Mateo et al. (1997) and Cebrian and Duarte (2001)
Tropical/subtropical				
T. hemprichii	Phuket Island, Thailand	0.83		Holmer et al. (2001)
H. beaudetti	Oyster Bay, Jamaica	16.4		Blackburn et al. (1994)
Zostera capricorni	Moreton Bay, Australia	12.9–35		Boon et al. (1986)
C. rotundata (Ehrenb. and Hempr. Aschers)	Phuket Island, Thailand	3.1		Holmer et al. (2001)
C. nodosa	Alfacs Bay, Spain		1.02	Perez et al. (2001)
C. nodosa	Alfacs Bay, Spain		0.35±0.12	Pedersen et al. (1997)

[*]Calculated from burial rates of 66.1–657.2 g DW m^{-2} yr^{-1} (Cebrian and Duarte, 2001) and rhizome/root N content of 0.35–0.86% DW (Mateo et al., 1997).

Zieman *et al.*, 1984). However, Valentine and Heck (2001) showed the opposite pattern in cage experiments where the urchin *Lytechinus variegates* consumed more seagrass in low-N treatments than in high-N treatments, presumably to compensate for the lower nutritional value of the food source. More work is needed in this area to determine the controls on food preference and mass consumed for these generalist herbivores. Some believe that herbivores cultivate the nutritional content of their food source by pruning away or removing older lower-N leaves and allowing the younger more N-rich leaves to dominate or another species to persist that is a preferred food source (low fiber, high N) (Bjorndal, 1980; Preen, 1995; Zieman *et al.*, 1984). Also, the epiphyte composition on the leaves (fleshy versus calcareous) will likely influence herbivore choice and consumption rates. Mesograzers (e.g., amphipods and gastropods) can control the abundance of algal epiphytes on seagrasses and can modulate the effects of increased nutrient loading (Hillebrand *et al.*, 2000; Lotze and Worm, 2002; Neckles *et al.*, 1993; Williams and Ruckelshaus, 1993). There are excellent recent reviews on grazing in seagrass ecosystems including Valentine and Duffy (2006) and Williams and Heck (2001).

Given the relatively low assimilation efficiency of most herbivores, little of the ingested leaf and epiphyte material will remain in consumer biomass. Most will be egested as feces or soluble compounds and will be remineralized. This suggests that where grazing is an important fate for seagrass material, most of the N bound in the plant material will be transferred to the decomposers. If the grazers are mobile, a portion of this N may be lost from the system (Preen, 1995).

5.3. Litter export

Plant material that is not decomposed *in situ*, permanently buried, or grazed may be lost from the system via mass transport. Seagrass leaves are transported primarily at the water surface due to their extensive aerenchyma tissue, compared to macroalgal material that moves either as bedload or as floating mats at the water surface. Export of belowground material is likely to occur only during storms (Bach *et al.*, 1996; Mateo *et al.*, 2006). Seagrass leaves may be transported longer distances as they settle to the bottom more slowly than macroalgae, and their surface movements are influenced by wind speed and direction as well as current speed. Water motion related to winds and currents determines the relative importance of export as a fate for seagrass biomass. Large-scale disturbance events such as storms may move large amounts of material to adjacent marsh and subtidal ecosystems and represents a nutrient loss from the seagrass ecosystem.

Transport of plant-bound nutrients is often not included in mass balance calculations of seagrass ecosystems in part because of the difficulty in doing representative sampling. Not including this mass transport can cause significant overestimates of nutrient retention in coastal bays. For example, Flindt *et al.* (2004) determined that for two European systems, Venice Lagoon, Italy and Roskilde Fjord, Denmark, the exclusion of mass transport of plant tissue would lead to an overestimate of N retention by 18–60%. Reports vary from negligible amounts to nearly 100% of aboveground biomass lost due to export (Mateo *et al.*, 2006). The nutritional (N) content of the seagrass may influence export, as higher decomposition rates of

high–N leaf litter may decrease the likelihood of export. Perez *et al.* (2001) estimated that export was 3-fold lower in a N-rich *C. nodosa* meadow than in a nearby N-poor meadow. In many systems, there is a seasonality of high leaf export that coincides with the timing of leaf shedding (Risgaard-Petersen and Ottosen, 2000).

5.4. Exudation

Compared to marine phytoplankton and macroalgae (e.g., Bronk and Steinberg, this volume; Tyler *et al.*, 2003; Ward and Bronk, 2001), we know little about DON leakage from seagrass tissues and the importance of this for N turnover and retention. A study by Jørgensen *et al.* (1981) suggested that leakage of amino acids from seagrass leaves and roots could contribute to standing stock DON pools; however, there has been little work to follow up on this idea. For marine algae, the composition of DON released from living tissue includes dissolved free and combined amino acids, dissolved primary amines, and urea, and release rates are related to light availability and nutrient status (Tyler *et al.*, 2003; Ward and Bronk, 2001). There have been studies of DOC leakage from seagrass leaves (Brylinsky, 1977; Moriarty *et al.*, 1986; Penhale and Smith, 1977), and one would expect this DOC release to be in simple organic compounds that also contain N. This is an area deserving of further investigation, and studies of DON exudation should include leakage from live tissues as well as leaching of soluble compounds during the initial states of decomposition.

6. SEAGRASS INFLUENCES ON BACTERIALLY MEDIATED NITROGEN CYCLING

Seagrasses have a major impact on sediment nutrient cycling by leakage of oxygen and dissolved organic matter (DOM) from the roots (Fig. 23.1). Both processes are largely photosynthetically driven. Oxygen produced by photosynthesis in the leaves is transported to the roots to support aerobic respiration via a well-developed lacunal system, a series of air channels comprising up to 60% of the total plant volume. This process occurs in the light; oxygen that is released to the sediment in the dark originates from the water column and enters the leaves by passive diffusion when the external oxygen concentrations exceed the internal concentrations (Borum *et al.*, 2006). Rates of passive oxygen influx to the leaves can be 10–30% of reported rates of photosynthetic oxygen production in *Z. marina* (Borum *et al.*, 2006). The oxygen that is not respired by the roots is released into the rhizosphere, creating oxidized zones in an otherwise reducing environment. Reported rates of oxygen release to the rhizosphere vary markedly (1–100% of total oxygen release to water column and sediments in light) for a range of marine and freshwater aquatic plants (Sand-Jensen *et al.*, 1982), and are relatively low for *Z. marina* (1–10%; Caffrey and Kemp, 1990; Sand-Jensen *et al.*, 1982). Fredericksen and Glud (2006) recently showed that oxygen release was restricted to the root tips
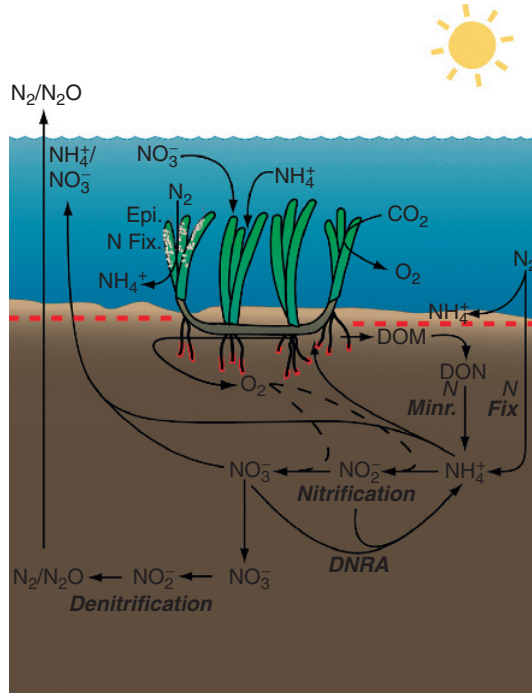
Figure 23.1 Seagrass influences on N cycling processes. The red line symbolizes the oxic–anoxic interface; oxic microzones around seagrass roots not shown in the figure are also important.

in *Z. marina* and only extended ∼8 mm along the root from the root tip. Oxic microzones of <100 μm extending from the root into the sediment have been shown to occur around roots in the light for *Z. marina* (Greve *et al.*, 2003), *Cymodocea rotundata* (Pedersen *et al.*, 1998), and *Halophila ovalis* (Connell *et al.*, 1999).

The DOM released from seagrass roots is typically in the form of simple organic carbon compounds that are the recent products of photosynthesis (Koepfler *et al.*, 1993; Ziegler and Benner, 1999), and these exudates influence bacterially-mediated N cycling processes. Bacterial productivity in the rhizosphere has been linked to these organic exudates. Moriarty *et al.* (1986) showed that 11% of recent photosynthate was released within 6 hurs into the rhizosphere of the tropical seagrass *Halodule wrightii*, and for *T. hemprichii* and *C. rotundata*, Holmer *et al.* (2001) showed that enough DOC was excreted by belowground tissues to support all anaerobic microbial activity in the rhizosphere. Seasonal and light-induced variation in N fixation and sulfate reduction rates have been associated with these root exudates (e.g., Holmer and Nielsen, 1997; Isaksen and Finster, 1996; McGlathery *et al.*, 1998; Welsh *et al.*, 1996). Also, there is typically a sub-surface peak in bacterial processes in the sediments where root biomass is highest (e.g., Blaabjerg *et al.*, 1998; McGlathery *et al.*, 1998; Welsh *et al.*, 1996).
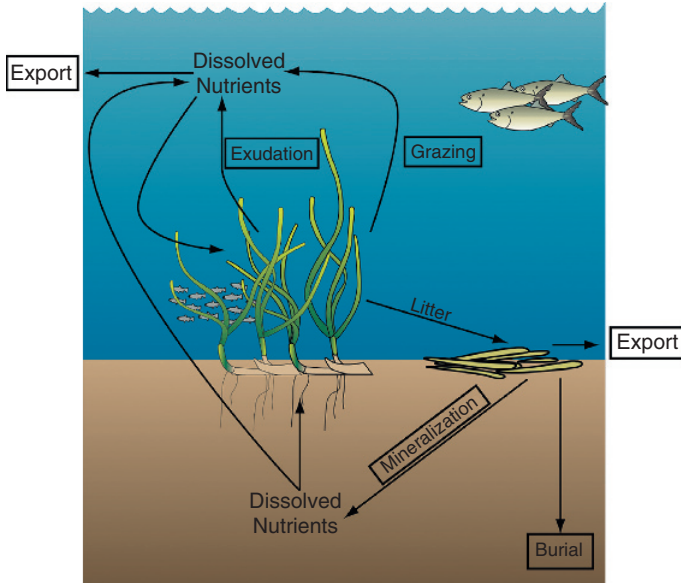
Figure 23.2 Conceptual model showing the possible fates of seagrass-bound nitrogen. Nitrogen temporarily immobilized in plant tissue can become available through exudation from live tissue, remineralization through decomposition, and grazing and subsequent excretion. Some material may be removed from the system through burial or export as dissolved or particulate organic matter.

6.1. Nitrogen fixation

Nitrogen fixation occurs associated with epiphytes on seagrass leaves, on the surface sediments, and deeper in the sediments in the seagrass rhizosphere (Fig. 23.1). Seagrass leaves provide a three-dimensional substrate in the photic zone that increases the surface area for autotrophic N fixers. The rates of N fixation of heterocystous cyano-bacterial epiphytes on seagrass leaves and on surface sediments tend to be high relative to those in the water column (e.g., Capone and Taylor, 1980; Capone *et al.*, 1992). This is true even in systems where a dense leaf canopy shades the sediment surface. It has been suggested that on an areal basis the activity of autotrophic N fixers is lower than that of heterotrophic bacteria N fixers (e.g., Capone and Taylor, 1980; O'Donohue *et al.*, 1991); however, this may not always be the case. The relative contribution of autotrophic vs. heterotrophic N fixation in seagrass beds likely depends on a number of factors, including the ratio of above- to belowground seagrass biomass, light and N availability, rates of sulfate reduction, and sediment redox conditions. More work is needed to understand how autotrophic and heterotrophic N fixation varies in seagrass meadows inhabiting different nutrient environments.

Heterotrophic N fixation in the sediments is enhanced by the presence of seagrasses relative to unvegetated sediments, both in temperate and tropical environments (e.g., Hansen *et al.*, 2000; McGlathery *et al.*, 1998; O'Donohue *et al.*, 1991; Welsh *et al.*, 1996). Reported rates vary tremendously between systems, although N fixation in general is higher in sediments of tropical seagrass meadows than temperate meadows (Table 23.3). The availability of organic substrate is a major factor

Table 23.3 Comparison of nitrogen (N) fixation rates in sediments of temperate and tropical/subtropical seagrass meadows

Seagrass species	Location	N Fixation (mmol N $m^{-2} d^{-1}$)	Reference
Temperate			
Z. marina	Vaucluse Shores, Virginia	0.36	Capone (1988)
Z. marina	Limfjord, Denmark	0.07–0.43	McGlathery et al. (1998)
Z. noltii	Arcachon Bay, France	0.10–0.41	Welsh et al. (2000)
Z. noltii	Arcachon Bay, France (winter)	0.007–0.014	Welsh et al. (1996)
Z. noltii	Arcachon Bay, France (summer)	0.14–0.52	Welsh et al. (1996)
Tropical/subtropical			
T. testudinum	Oyster Bay, Jamaica	1	Blackburn et al. (1994)
T. testudinum	Florida Bay, Florida	0.7–4	Kemp and Cornwell (2001)
T. testudinum	St. Lawrence, Barbados	1.93–10	Patriquin and Knowles (1972)
T. testudinum	Biscayne Bay, Florida	0.31	Capone and Taylor (1980)
T. testudinum	Barbados (June)	2.7	Capone and Taylor (1980)
T. testudinum	Bimini Harbor, Bahamas (summer)	0.36–0.38	Capone et al (1979)
T. testudinum	Bimini Harbor, Bahamas (summer)	0.86–0.99	Capone and Taylor (1980)
T. testudinum	Gulf of Carpentaria, Australia	1.1	Moriarty and O'Donohue (1993)
H. beaudetti	Oyster Bay, Jamaica	1	Blackburn et al. (1994)
T. hemprichii	Ishigaki Island, Japan	0.17–0.31	Miyajima et al. (2001)
Syringodium isoetifolium	Gulf of Carpentaria, Australia	1.1–3.4	Moriarty and O'Donohue (1993)
Enhalus acoroides	Gulf of Carpentaria, Australia	1.8	Moriarty and O'Donohue (1993)
Z. capricorni	Moreton Bay, Australia	0.7–2.9	O'Donohue et al. (1991)

controlling N fixation rates in seagrass sediments. Many studies have shown increases of N fixation by addition of organic compounds, and seasonal and diel variations that are consistent with the role of photosynthetic exudates stimulating N-fixing bacteria (e.g., Hansen *et al.*, 2000; McGlathery *et al.*, 1998; Welsh, 2000). Much of this activity (25–95% of total heterotrophic fixation) has been associated with sulfate

reducers (e.g., McGlathery *et al.*, 1998; Welsh *et al.*, 1996), which also are stimulated by root exudates (Blaabjerg *et al.*, 1998; Hansen *et al.*, 2000). Depth profiles of N fixation activity in *Z. marina* sediments showed a close association with root/rhizome biomass, with seasonal shifts in the magnitude and depth distribution of N fixation matching the shifts in belowground biomass (McGlathery *et al.*, 1998). N-fixing bacteria can colonize roots (Shieh *et al.*, 1989); rates of N fixation directly associated with roots and rhizomes vary from negligible (McGlathery *et al.*, 1998) to over 30% of heterotrophic fixation in the sediments (Nielsen *et al.*, 2001). The higher N fixation rates in seagrass-vegetated sediments in tropical/subtropical systems may in part be due to increased DOM release from the greater root/rhizome biomass of many tropical seagrass species. In tropical/subtropical seagrass meadows, sediment N fixation may provide more than 50% of plant N demand (Hanson *et al.*, 2000; O'Donohue *et al.*, 1991; Welsh, 2000), whereas in temperate systems the contribution to plant N demand is often lower (5–12%; McGlathery *et al.*, 1998; Welsh, 2000).

6.2. Denitrification

Many studies have estimated denitrification rates in seagrass-vegetated sediments, and a broad range of values have been reported (Table 23.4, see Devol, this volume for a review of the denitrification process). The variation appears to be related to the N availability in the sediments, as well as to species-specific characteristics (i.e. root oxygen release). A general pattern is emerging that under N-replete conditions oxygen release from plant roots stimulates coupled nitrification–denitrification, and under N-limiting conditions competition between plants and nitrifying bacteria decreases coupled nitrification–denitrification (see Ward, this volume for a review of the nitrification process). This same regulation of denitrification has also been suggested for sediments in which benthic microalgae are the dominant primary producers (An and Joye, 2001). For example, there is evidence for the temperate species, *Potomogeton perfoliatus* and *Z. marina*, that root oxygen release stimulates coupled nitrification–denitrification (e.g., Cornwell *et al.*, 1999; Flindt, 1994), and variations in rates are linked to the seagrass growth cycle and to variations in oxygen release (Caffrey and Kemp, 1992; Risgaard-Petersen *et al.*, 1998). In other studies, low rates of nitrification and competition between nitrifying bacteria and benthic microalgae in *Z. marina* and *Z. noltii* were believed to be the mechanisms responsible for the low rates of coupled nitrification–denitrification (Ottosen *et al.*, 1999; Risgaard-Petersen and Ottosen, 2000; Rysgaard *et al.*, 1996).

There are fewer reported measurements of denitrification in seagrass-vegetated carbonate sediments in tropical/subtropical environments than in temperate systems (Table 23.4). The available data suggest that denitrification rates are higher in tropical/subtropical seagrass meadows than in temperate seagrass meadows, with one exception (Table 23.4). More work is clearly needed to estimate rates in tropical and/or carbonate sediments. Higher denitrification rates may occur in carbonate sediments dominated by *T. testudinum* because this species allocates more biomass to roots and rhizomes than temperate species, and tends to be P-limited so that competition between bacteria and plants for N is unlikely to occur. Organic matter

Table 23.4 Comparison of denitrification rates in temperate and tropical/subtropical seagrass meadows

Seagrass species	Location	Denitrification (mmol N $m^{-2} d^{-1}$)	Reference
Temperate			
Z. marina	Vaucluse Shores, VA	1.7–5.0	Caffrey and Kemp (1990)
Z. marina	Limfjord, Denmark	0.08–0.4	Risgaard-Petersen et al. (1998)
Z. marina	Limfjord, Denmark	0.03	Ottosen et al. (1999)
Z. marina	Limfjord, Denmark	0.24	Risgaard-Petersen and Ottosen (2000)
Z. marina	Alaska	0.14–0.67	Iizumi et al. (1980)
Zostera novazelandica	New Zealand	0.09–0.36	Kasper (1983)
Z. noltii	Arcachon Bay, France	0.05–0.14	Welsh et al. (2000)
Z. noltii	Arcachon Bay and Etang du Prevost, France	0.33	Rysgaard et al. (1996)
Z. capricorni	Edmunds Bay, Australia	0.19	Eyre and Ferguson (2002)
Tropical/subtropical			
T. testudinum	Oyster Bay, Jamaica	2–4	Blackburn et al. (1994)
T. testudinum	Florida Bay, Florida	1.6–3	Kemp and Cornwell (2001)
T. hemprichii	Ishigaki Island, Japan	0.07–0.28	Miyajima et al. (2001)
H. beaudetti	Oyster Bay, Jamaica	2–4	Blackburn et al. (1994)

concentrations and bacterial oxygen demand also tend to be lower in tropical carbonates sediments, which may make oxygenation of the rhizosphere more efficient in these systems.

Studies on diel and seasonal patterns in denitrification activity in both temperate and tropical/subtropical seagrass meadows suggest that variations in coupled nitrification–denitrification are linked to variations in phytosynthetically-driven oxygen release and plant NH_4^+ uptake (Blackburn et al., 1994; Risgaard-Petersen and Ottosen, 2000; Risgaard-Petersen et al., 1998; Welsh et al., 2000). The relative effect of these two processes on denitrification should depend on whether N is limiting in the system. For example, under N-limiting conditions, NH_4^+ uptake and competition with denitrifiers would depress denitrification most when the plants

are most active photosynthetically during the day and in the summer. If N is not limiting, oxygen release by the seagrass and benthic microalgae in the sediments would stimulate coupled nitrification–denitrification the most in the light and during the summer. Both patterns have been reported. Blackburn et al. (1994) showed diel variation in porewater NH_4^+ and denitrification rates in tropical *Halodule beaudetti* (*wrightii*) meadows, with both being high at daybreak and declining during the day, presumably related to plant uptake of NH_4^+ and competition with nitrifiers. And Welsh et al. (2000) found higher denitrification rates in the dark and attributed this to shorter diffusion distance for NO_3^- to anoxic microzones in the rhizosphere.

Finally, a few studies have measured N fixation and denitrification simultaneously in seagrass meadows to determine if N gains by fixation can compensate for N losses via dentrification, and the results are equivocal (Tables 23.3 and 23.4). In temperate *Z. marina* meadows, one study indicated that denitrification roughly balanced N fixation (comparison in Risgaard-Petersen et. al., 1998, data reported in McGlathery et al., 1998), and another showed that N fixation rates exceeded denitrification rates on a seasonal basis (Welsh et al., 2000). Welsh et al. (2000) calculated that the net N_2 flux (N fixation–denitrification) averaged over the year was 5.4 μmol N m^{-2} h^{-1}. Of the three studies in tropical seagrass beds that have compared these rates, two indicated that gains by N fixation roughly equaled losses by denitrification (Kemp and Cornwell, 2001; Miyajima et al., 2001), and one reported denitrification rates that exceeded N fixation rates (Blackburn et al., 1994). More work is needed to determine what influences the balance between denitrification and N fixation in seagrass meadows and if this can be generalized among systems.

6.3. Mineralization

Nutrient cycling in seagrasses communities appears to be relatively conservative, with much of the nutrients required to support high rates of productivity met by recycling within the sediments in both temperate and tropical systems (Alcoverro et al., 2000; McGlathery et al., 2001b; Miyajima et al., 2001; Risgaard-Petersen and Ottosen, 2000). Mineralization rates appear to be higher in temperate than tropical seagrass sediments (Table 23.2); however, there is some overlap in the rates and there are few studies to compare. Seagrasses influence the production of N as NH_4^+ through mineralization by enhancing organic matter in the sediment. This occurs both through leaching and decay of senescent seagrass biomass and trapping of allochthonous particulate organic matter. Some studies have shown that as with salt marshes, much of the organic matter buried and decomposed in seagrass sediments is imported and that enhanced trapping of seston may be an efficient strategy for acquiring external nutrients (Boschker et al., 2000; Gacia et al., 2002; Pedersen et al., 1997a,b). Fine, organic-rich particles settle out as the water flow is slowed by the seagrass canopy, and the roots and rhizomes also help to stabilize the sediments and prevent resuspension (Gacia and Duarte, 2001; Kenworthy et al., 1982; Koch, 1999). The use of compound-specific stable isotopes has provided new insights into the origin of the organic matter that is decomposed in seagrass beds. Stable isotope ratios of branched polar lipid-derived fatty acids (PFLAs) of bacteria have been used to show that high bacterial production was supported by

benthic microalgae in some temperate systems (Boschker *et al.*, 2000), and by seagrass production (*T. testudinum*) in a tropical system (Jones *et al.*, 2003). Additionally, Barron *et al.* (2006) used ^{15}N-labelled algal material injected into *P. oceanica* sediments to show that seagrass meadows retained 4X more material than unvegetated sediments and that a portion of the material appeared in the seagrass biomass within a few days. The results of these studies indicate that not all microbial production in seagrass ecosystems is based on seagrass detritus as previously thought. More studies are needed to assess differences in mineralization between types of seagrass systems, which may relate to the importance of export and to seagrass community composition (availability of organic matter such as seagrass, microalgae, macroalgae, etc.).

Sulfate reduction is an important mineralization process in seagrass-vegetated sediments, and rates generally are higher than in nearby unvegetated sediments (Blaabjerg and Finster, 1998; Blaabjerg *et al.*, 1998; Holmer *et al.*, 2003). Increased input of allochthonous organic matter, organic exudates from living seagrass roots, and the presence of senescent seagrass material all likely stimulate sulfate reduction in vegetated sediments. A positive correlation between sulfate reduction rates and belowground biomass has been shown for *Z. marina* (Blaabjerg and Finster, 1998; Blaabjerg *et al.*, 1998); however, this was not the case for *P. oceanica* (Holmer *et al.*, 2003).

7. ARE SEAGRASS MEADOWS SOURCES OR SINKS OF NITROGEN?

Whether a seagrass meadow acts a net source or sink of N depends on the balance between processes (1) that contribute N to the system (N fixation, leaf uptake of water column N, root uptake of groundwater N, and import/sedimentation of allochthonous organic matter) and (2) those that contribute to a loss of N from the system (denitrification, leaching, leaf export, grazing and export). The source-sink role of seagrass ecosystems varies over diel, seasonal, and annual (and beyond) time scales.

Nutrient assimilation and temporary retention in plant biomass is an important process regulating the source-sink role of seagrass communities over all time scales. Seagrasses are highly productive (global average: 1012 g DW m^{-2} yr^{-1}; Duarte and Hemminga, 2000), and total annual N incorporation can exceed 2 mol N m^{-2} yr^{-1} (Pedersen *et al.*, 2004). For many systems, this assimilation rate is higher than the fluxes associated with microbial processes (e.g., N fixation, denitrification) by one to several orders of magnitude (Table 23.2–23.4), and is greater than external inputs of N in most systems (Pedersen *et al.*, 2004). Diel and seasonal variation in the source-sink role of seagrass beds depends in part on the growth requirements and N demand of seagrasses. For example, temperate *Z. marina* meadows have been shown to act as strong sinks for N from the beginning of the growing season in the spring to the time of maximum productivity in late summer; in the fall when decomposition rates were relatively high, but plant N demand low, the seagrass community switched to a source of nutrients (Risgaard-Petersen and Ottosen, 2000; Risgaard-Petersen *et al.*, 1998). Averaged over the year, N did not accumulate in above- or belowground

biomass in these seagrass meadows (Pedersen and Borum, 1993; Risgaard-Petersen and Ottosen, 2000). There are often diel patterns in N transfer driven by the seagrasses and algae associated with seagrass beds, with an efflux of nutrients during the dark and uptake from the sediment and water column in the light (Dudley *et al.*, 2001; Eyre and Ferguson, 2002; Larned, 2003; Miyajima *et al.*, 2001). Dudley *et al.* (2001) showed that epiphytes could remove more NH_4^+ and NO_3^- from the water column than the seagrass *Ruppia megacarpa* even though their biomass was only 25% of the seagrass biomass (Dudley *et al.*, 2001).

Several studies have attempted to either construct a mass balances N or follow the fate of seagrass biomass using ^{15}N as a tracer. Risgaard-Petersen *et al.* (1998) determined that over the growing season (April and August) for a *Z. marina* meadow plant uptake was the most important process influencing the role of the meadow, as an N sink, and that 82% of the assimilated N could be returned to the system via leaf shedding. Stapel *et al.* (2001) also found using isotopic tracers that much of the N in shed leaves (44%) in a *T. hemprichii* meadow in Indonesia was mineralized *in situ* and reassimilated by seagrasses. These studies and others (Hansen *et al.*, 2000; McGlathery *et al.*, 2001a) point to efficient sediment N cycling to meet plant demand. In contrast, Cebrian and Duarte (2001) followed the fate of *P. oceanica* leaf material in 16 meadows in the Mediterranean and found that grazing removed only a small percentage (13%), that 85% of the leaves were shed, and that most of these leaves (90%) were exported off the meadows.

When long-term nutrient accumulation occurs in seagrass meadows, it is either as increased biomass or as accumulation of slowly degrading detritus. Nitrogen accumulation in seagrass biomass most likely occurs during meadow development, as biomass accumulates. For example, Pedersen *et al.* (1997a,b) found that colonization and stand development of the N-limited seagrass *C. nodosa* in Mediterranean carbonate sediments resulted in a net accumulation of N (but not P) over a 5-yr period, with about half of the accumulated N accounted for by living and dead plant material. Nitrogen may also accumulate in the "matte" forming species, *P. oceanica*, for which a significant percentage of total seagrass production accumulates as refractory material in belowground detritus (dead roots and rhizomes) (Table 23.4; Mateo and Romero, 1997; Mateo *et al.*, 1997). There is some evidence that nutrient enrichment may cause a switch in mature meadows from being a sink to a source because the accumulation of nutrients in slowly degrading detritus is less likely since allocation to belowground biomass decreases and decay rates of leaves is higher (Perez *et al.*, 2001).

8. SUMMARY AND FUTURE DIRECTIONS

Seagrasses affect the rates and pathways of N cycling in lagoons and estuaries by (1) the temporary retention of N in their tissue and the subsequent fate of this tissue and (2) the release of DOC and oxygen from roots to the sediments that alters bacterial activities. For many systems, N assimilation rates are of the same magnitude as watershed and atmospheric N loading (Pedersen *et al.*, 2004), and thus seagrasses

are a critical part of the estuarine filter. Seagrasses slow the transport of N to the near-coastal zone, and appear to be net sinks of N on daily to seasonal time scales. This sink role is regulated by photosynthesis and nutrient uptake, with meadows acting as a sink during the day and during the active growing season. Whether seagrass meadows are a net sink on annual basis appears to depend on the developmental stage (young vs. old) and on nutrient conditions, with meadows in eutrophic environments possibly becoming a source of nutrients to the water column. At the plant level, we need to know more about exudation of DON from living seagrass tissue and its role in stimulating heterotrophic activity in the water column and sediments.

Bacterial activities associated with N fixation and mineralization are stimulated in seagrass-vegetated sediments relative to "bare" sediments with benthic microalgae. This does not appear to be the case for denitrification, and rates are often similar and low in vegetated and bare sediments. Although there is some evidence of enhanced coupled nitrification–denitrification in seagrass-vegetated sediments, seagrasses more often inhibit nitrification–denitrification due to low rates of nitrification in the oxic microzones surrounding the roots and to competition for ammonium with nitrifying bacteria. Methods still need to be improved to directly link plant metabolism with nitrification and denitrification rates in the rhizosphere over diurnal and seasonal time scales.

Over the last two decades we have developed a good understanding of N controls on the production, morphology and dynamics of seagrass ecosystems, and on the feedbacks of seagrass metabolism on N cycling processes. However, we lack sufficient empirical information to predict the changes in N cycling in seagrass-vegetated systems that will result from eutrophication. Responses of specific N processes (i.e., denitrification, mineralization, N fixation) to excess N loading will likely be nonlinear, as seagrasses are replaced by benthic algae and phytoplankton. There is also uncertainty about the role of consumer control in modulating the response of seagrass ecosystems to nutrient over-enrichment, and their influence on the resilience of these systems. Given the accelerating pace of seagrass degradation worldwide (Orth *et al.*, 2006), understanding the feedbacks of seagrasses on N cycling and the relative role of consumer interactions as systems become eutrophied is important for the conservation and management of these ecologically and economically important systems. These issues are also important for predicting the impacts of seagrass restoration on the transformations and fate of N on its trajectory from land to sea.

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Aspects of Marine Cyanobacterial Nitrogen Physiology and Connection to the Nitrogen Cycle

David J. Scanlan *and* Anton F. Post

Contents

1. Introduction

Nitrogen (N) is an essential nutrient for photosynthetic organisms for which the cellular demand is higher than that for phosphorus, iron, or trace metals. N sources that are commonly found in aquatic ecosystems include reduced and oxidized inorganic species, like ammonium and nitrate alongside organic N–compounds such as urea, amino acids etc. For decades N–availability has been considered the limiting factor for primary productivity by marine phytoplankton. This concept has recently been subjected to evaluation based on oceanographic findings showing that other nutrients, like phosphate and iron, limit primary production in large ocean provinces. Moreover, improved analytical methods have provided more detailed information on the spatial and temporal distribution of the various N–species. Furthermore, an increased understanding of the flow of matter via the microbial food web has resulted in an appreciation of high N–regeneration rates within the photic layer. Nevertheless, large oceanic regions, mostly in the (sub)tropics are characterized by N–deplete surface waters, from the surface down to below the 1% isolume. Such waters are more often than not populated with cyanobacteria as the

major phytoplankton group. These cyanobacteria may be either the microscopically small *Synechococcus, Prochlorococcus, Cyanothece*, and *Chrocosphaera* unicells or the large colonies of N_2-fixing *Trichodesmium* filaments. This chapter will largely focus on the N-metabolism, N-stress response and niche adaptation of these three major phytoplankton groups, as they impact significantly on the N-cycle in marine water bodies. Much emphasis will be put on the molecular ecology and phylogeny of these groups, which have seen a rapid development over the last decade. More rare planktonic species such as the filamentous N_2-fixing cyanobacteria *Richelia* and *Katagnymene* as well as those found in benthic ecosystems will not be considered here. Likewise we will not discuss the cyanobacteria found in atypical marine environments, like e.g., *Nodularia* populations in the Baltic Sea.

2. General Aspects of Cyanobacterial Nitrogen Metabolism

Cyanobacteria are key components of marine microbial communities contributing up to two-thirds of carbon dioxide fixed in oceanic systems. Although it is with C cycling that cyanobacteria are most notably associated, their contribution to the transformation of nitrogenous compounds is equally important, and indeed with roles in both regenerated and new N production and utilisation, they are also inextricably linked to the global N-cycle.

In nutrient-depleted surface layers of the ocean cyanobacteria often experience sudden pulses of N either by physical mechanisms such as up-wellings that provide nitrate to the euphotic zone or by biological mechanisms such as animal excretion that add regenerated N forms such as ammonia and urea. We thus consider here examples of N-metabolism and N-acquisition that encompasses the range of N substrates encountered by these organisms *in situ* (see Fig. 24.1) and including problems of aerobic N_2 fixation. For the latter we focus on the use of specialised regions, diazocytes, that provide a reduced oxygen microenvironment in *Trichodesmium* spp. as well as the temporal separation of nitrogen fixation and photosynthesis as found in *Cyanothece* and *Crocosphaera* spp. We will highlight where appropriate, and this is especially obvious in the unicellular picocyanobacterial genera *Prochlorococcus* and *Synechococcus*, the genotypic variation that can exist within a genus with respect to N resource utilisation and the concomitant N regulatory network, a feature that we can see no reason should be confined to these genera, but which we consider to be inextricably linked to the specific environmental niches occupied by particular genetic lineages. The importance of these picocyanobacteria to N-cycling should not be overlooked given their typical cell concentrations of between 10^6 and 10^8 cells l^{-1} and compared to *Trichodesmium* which is found at concentrations around 1 colony l^{-1} as a function of the integrated mixed-layer water column.

Of specific interest are issues that relate to the fine interplay between cellular processes and nitrogen scavenging from the environment. Studies of such interactions have shown much progress for some cyanobacteria (e.g., for *Trichodesmium*, see Section 2.1) but others still pose a challenge e.g., very little is known to date as to

1 - preferred nitrogen source
2 - alternative nitrogen sources
3 - N deprivation, internal stores e.g. phycobiliproteins
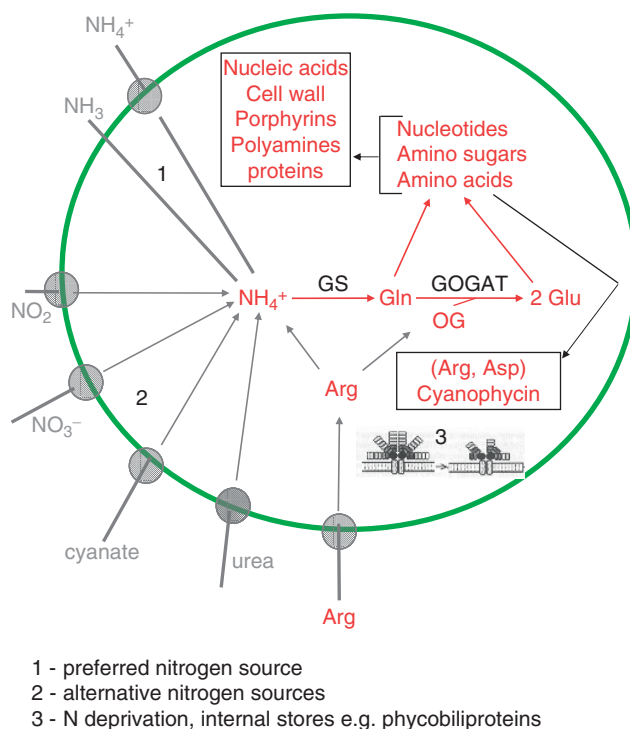
Figure 24.1 Cyanobacterial nitrogen incorporation as depicted at the level of (1) preferred N sources, (2) alternative N sources, and (3) the response to N starvation.

whether marine cyanobacteria have a cellular hierarchy with which they determine the presence of certain N compounds in the environment and establish the order for acquisition of these compounds. Based on purely theoretical considerations of energy requirements it would be logical to utilize ammonium preferentially, followed by nitrite, nitrate, and lastly the fixation of molecular nitrogen, provided that these sources are all available. A deeply mixed water column may contain such mixes and marine *Synechococcus* thrives in such environments with the capability of rapidly adapting to the differential utilization of the combined N sources (Lindell and Post, 1995, 2001; Lindell *et al.*, 1998, 1999). However, a ''pecking order'' for the use of these compounds (with the addition of organic N sources) has as yet not been determined with the exception of a preference for ammonium utilization over all other N sources, a property derived from the molecular regulation of N–stress responses (see Section 3). Once N has been acquired nitrogen assimilation by all (marine) cyanobacteria studied proceeds via the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway. GS is required for ammonium assimilation irrespective of the primary source of N. Elevated GS activity is characteristic of cells that are N limited or using N_2 as their N source although this is not true for all genera (e.g., *Prochlorococcus* see Section 2.3). Lastly, cellular processes need to be tuned to the presence and activity of nitrogenase. Nitrogenase, catalysing the reduction of atmospheric nitrogen to ammonium is irreversibly inhibited by exposure to molecular

oxygen, a major problem for oxygen-evolving photosynthetic organisms. As a result various adaptations have evolved that either involve (1) a spatial segregation of the process in specialised cells, heterocysts and diazocytes, in which photosystem II activity is strongly reduced or even lacking, or (2) a temporal separation, where N_2 fixation takes place in the dark.

2.1. Filamentous cyanobacterial N_2 fixers

In oligotrophic tropical and subtropical oceans planktonic marine cyanobacteria of the genus *Trichodesmium* are responsible for the bulk of nitrogen fixation providing more than half of the new nitrogen used for primary production (Capone *et al.*, 1997; Karl *et al.*, 1997). To put this into perspective, current estimates of global N_2 fixation are $\sim$240 Tg N year^{-1} with a marine contribution of 100–190 Tg N year^{-1}. Of this $\sim$100 Tg N year^{-1} are now considered contributed by *Trichodesmium* (Berman-Frank and Falkowski, 2003). The well documented surface accumulation of colonies (blooms) of this organism (Capone *et al.*, 1998) testifies to its importance as a major player in marine N_2 fixation dynamics and have led to much study of its ecology and biology (see e.g., Capone *et al.*, 1997; Carpenter *et al.*, 1992; Mulholland and Capone, 2000). We consider here then, only those more recent advances in its biology with specific respect to aspects of N metabolism that directly impinge on its wider role in the N cycle.

As a filamentous N_2 fixing cyanobacterium, *Trichodesmium* is unusual in being both non-heterocystous, but also in carrying out N_2-fixation maximally during the day (see Capone *et al.*, 1990, 1997; Karl *et al.*, 2002). This ''diel'' cycle of nitrogenase activity, found in both cultured (Chen *et al.*, 1996) and natural (Capone *et al.*, 1990; Zehr *et al.*, 1993) populations, is manifest as activity only during the light period and with maximal activities around midday. Such a cycle is consistent with protein data for nitrogenase with enzyme modification and inactivation during the late afternoon (Zehr *et al.*, 1993), prior to its degradation at night and with new nitrogenase being synthesized again each morning (Capone *et al.*, 1990; Wyman *et al.*, 1996). Similarly, in natural *Trichodesmium* populations, *nifH* (encoding one of the multi-subunit metalloproteins of nitrogenase) transcripts show a maximum between mid-morning and midday, decline during the afternoon and are largely undetectable at dusk. Such a cycle of gene expression has recently been shown, in cultures, to be due to a circadian rhythm, with cyclic nitrogenase gene expression persisting under constant conditions, the rhythms being trainable to daily light–dark (L–D) cycles by L–D cues; the rhythms are also temperature compensated (Chen *et al.*, 1998). Interestingly, *nifH* gene expression shows a pattern out of phase to the photosynthetic core polypeptide genes of photosystem II and I, *psbA* and *psaA*, respectively, the latter also being regulated in a circadian manner (Chen *et al.*, 1999). Circadian oscillation of gene expression seems then to underlie part of the mechanism allowing simultaneous photosynthesis and nitrogen fixation in *Trichodesmium* filaments. However, such transcriptional studies give data only on gene expression of ''bulk'' filaments and need to be put into the context that not all cells appear to be capable of N_2 fixation i.e., that spatial as well as temporal segregation of N_2 fixation and photosynthesis occurs within the photoperiod. Thus, immunological evidence has shown that

nitrogenase is localized to a subset of cells, now called diazocytes, clustered along regions of the filament in several *Trichodesmium* strains (El-Shehawy *et al.*, 2003; Fredriksson and Bergman, 1997; Janson *et al.*, 1994; Lin *et al.*, 1998). These diazocytes do not show the thickened cell wall typical of heterocysts, but appear to contain more cytochrome oxidase than surrounding cells (Bergman *et al.*, 1993), a denser thylakoid network dividing vacuole-like spaces into smaller units, less extensive gas vacuoles and smaller cyanophycin granules (Lin *et al.*, 1998). There is also an indication that these diazocytes may be shorter than adjacent cells (Janson *et al.*, 1994). However, this spatial segregation is not in itself sufficient to protect nitrogenase against oxygen evolution since active photosynthetic components (PSII and PSI complexes, RuBisCO and carboxysomes) are present in all cells. Instead, *Trichodesmium* has evolved the ability to rapidly switch on and off photosynthesis, within 10 or 15 min (Berman-Frank *et al.*, 2001a), with this modulation of oxygen consumption and production during the photoperiod being restricted to parts of the filament, often at their centres, a feature consistent with the presence of specialized cells for nitrogen fixation. Thus, a consensus mechanism is now emerging for this enigmatic simultaneous N_2 fixation and photosynthesis capacity in *Trichodesmium*—that the underlying circadian control of gene expression, and subsequent translational modification of nitrogenase which allows for high nitrogen fixation during the day is mirrored by a decline in photosynthetic production and increased light dependent oxygen consumption via the Mehler reaction which results in a net removal of oxygen, and particularly in specific cells clustered along a filament (Berman-Frank *et al.*, 2001a).

It seems then that nitrogen fixation in *Trichodesmium* appears to be a complicated interplay between both temporal and spatial controls of photosynthesis, respiration and nitrogen fixation. What is unclear though is how the circadian regulation of gene expression manifest in all cells is then overlaid with a more cell-specific regulatory network that allows for a window of opportunity for increased nitrogenase activity in specialized cells. The presence/absence or ''switching-on'' of a developmental cycle in some cells, and the role of global N regulators e.g., *ntcA* (see Section 3) will shed further light on this fascinating process.

The fact that nitrogen fixation does not occur in all cells of a *Trichodesmium* filament simultaneously implies some mechanism of N cycling between cells, and/or the concurrent uptake of other nitrogenous substrates, so as to maintain the intracellular N levels of individual cells. Indeed, *Trichodesmium* sp. strains NIBB1067 and IMS101 are capable of simultaneous N_2 fixation and utilization of combined N sources (Mulholland and Capone, 1999; Mulholland *et al.*, 1999). Furthermore, in these strains N_2 fixation accounts for ~15%, whereas the uptake of ammonium + glutamate accounts for around 85%, of total N uptake. Work with natural populations which demonstrates high rates of amino acid, DON, and ammonium release together with simultaneous uptake of amino acids (largely glutamate and glutamine) and ammonium suggests a cycle of release of fixed N by N_2 fixing cells and uptake by non N_2 fixing cells (Mulholland and Capone, 2000). While this release may fuel gross productivity of *Trichodesmium* spp. it likely also benefits the microbiota attached to *Trichodesmium* filaments, which may themselves be involved in the regeneration of dissolved inorganic and organic N.

2.2. Unicellular cyanobacterial N$_2$ fixers

Several unicellular cyanobacterial N$_2$ fixers have been described from the marine environment. All seem to share the common property of being aerobic diazotrophs and of being slightly larger (2.5–10 µm) in size to corresponding non N$_2$-fixing unicells (León *et al.*, 1986; Montoya *et al.*, 2004; Reddy *et al.*, 1993; Waterbury *et al.*, 1988; Zehr *et al.*, 2001). They have been described in the literature as belonging to the *Synechococcus*, *Synechocystis*, *Cyanothece*, and *Crocosphaera* genera, but there is some level of phylogenetic coherence among several of these cultured organisms (Mazard *et al.*, 2004; Turner *et al.*, 2001—see boxed area within Fig. 24.2) suggesting they should be unified into a single taxon. As well as free-living isolates, 16S rDNA sequences closely related to these organisms have been obtained from symbionts associated with the diatom *Climacodium frauenfeldianum* suggesting the potential of these types of organisms for nitrogen fixation within symbiotic relationships also (Carpenter and Janson, 2000; Falcón *et al.*, 2002, 2004a,b). Although the contribution of these unicellular cyanobacterial strains to global nitrogen fixation rates is not yet clear, indirect estimates from size-fractionated (10–0.2 µm) seawater samples (which include any nitrogen–fixing heterotrophs) suggests they can reach 2–100% of the rates of *Trichodesmium* at the same location (Falcón *et al.*, 2004a; Montoya *et al.*, 2004; Zehr *et al.*, 2001). Since there appear to be large differences in these rates between the Pacific and Atlantic Oceans (Falcón *et al.*, 2004a), and indeed even over small spatial scales within the same ocean basin (Montoya *et al.*, 2004) it would appear that, depending on location, these are major diazotrophs in oligotrophic marine ecosystems. The spatial heterogeneity in fixation rates and abundance of these coccoid cyanobacterial diazotrophs might reflect differences in aeolian Fe input or Fe:P ratios (Falcón *et al.*, 2004a) or may be a result of changes in water column temperature (Mazard *et al.*, 2004). Certainly, temperature appears important in dictating the distribution of heterocystous versus non-heterocystous cyanobacteria in oceanic systems (Staal *et al.*, 2003). Thus, differences in the temperature dependence of oxygen flux, respiration, and N$_2$ fixation activity were shown to be critical in explaining how *Trichodesmium* performs better than heterocystous species at higher temperatures, and it may be that some of these factors are important for unicellular nitrogen–fixing species too.

The N physiology of these marine unicellular N$_2$ fixers has been restricted to only a few strains (Falcón *et al.*, 2004b; León *et al.*, 1986; Mitsui *et al.*, 1986, 1987; Waterbury *et al.*, 1988) and with most data from a single isolate, *Cyanothece* sp. ATCC 51142 (= strain BH68; e.g., see Colón-López and Sherman, 1997; Li *et al.*, 2001; Reddy *et al.*, 1993; Schneegurt *et al.*, 1994; Sherman *et al.*, 1998). A consistent feature is the full temporal separation of photosynthesis and N$_2$ fixation, a process which is likely controlled by an endogenous circadian rhythm (see Sherman *et al.*, 1998). N$_2$ fixation, respiration and photosynthesis all show peaks with a periodicity of 24 h, with photosynthesis 12 h out of phase relative to N$_2$ fixation. In *Cyanothece* sp. ATCC 51142 such rhythms can occur under continuous light (LL), light–dark (L–D) or continuous dark (DD) conditions, the latter via heterotrophic growth on glycerol. Under L–D growth N$_2$ fixation is restricted to dark periods. The establishment and maintenance of these rhythms appears complex but includes tight regulation of expression of the *nifHDK* operon, with net
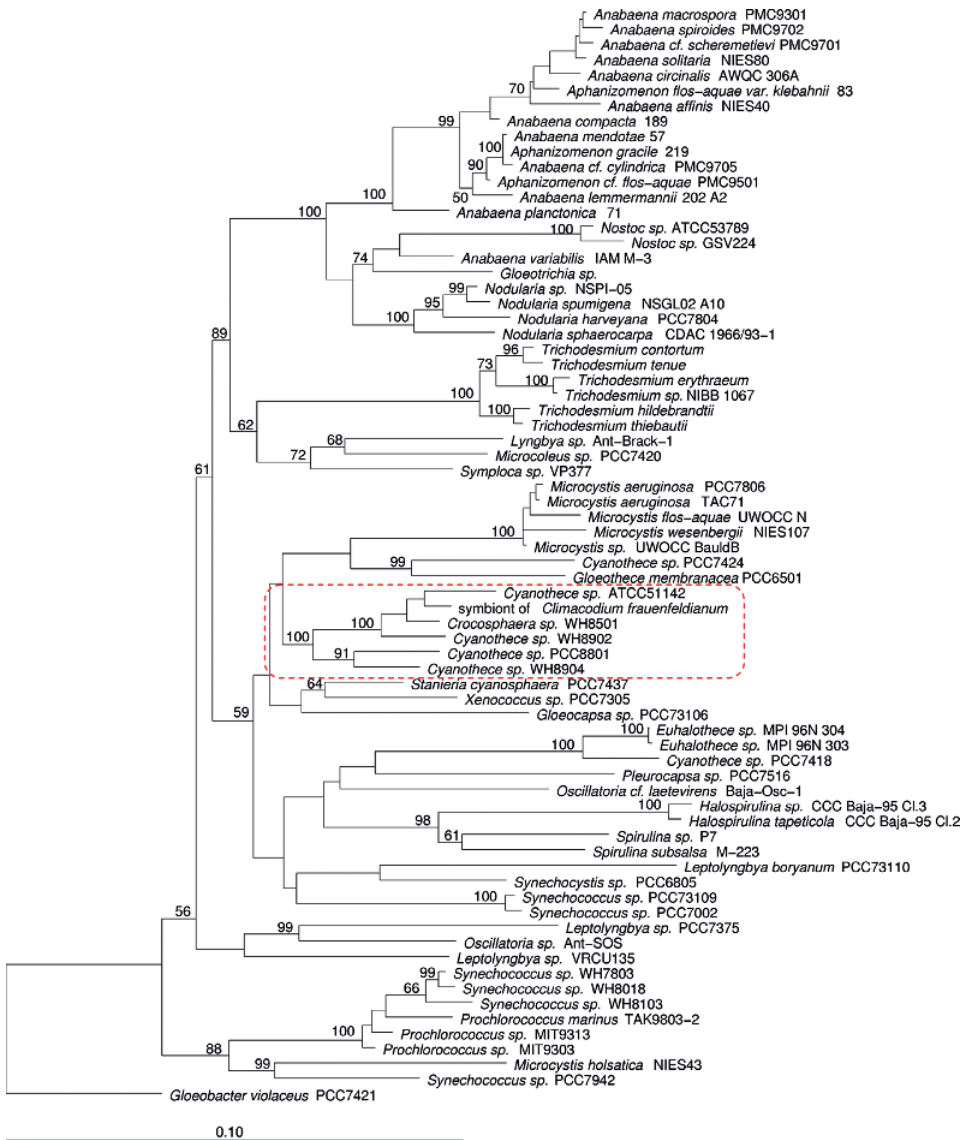
Figure 24.2 Phylogenetic tree showing the relationships of marine and freshwater cyanobacteria, including diazotrophs and non-diazotrophs, inferred from 16S rRNA gene sequences. The tree was constructed by the neighbour joining method with Jukes-Cantor correction. The region highlighted encompasses a lineage that only includes unicellular nitrogen-fixing isolates. The scale bar represents 0.1 nucleotide substitutions per position.

accumulation of *nifHDK* transcripts only during a small percentage of the diurnal cycle irrespective of culture growth conditions (i.e., L–D, LL, or DD). L–D growth conditions do demonstrate, however, the most highly synchronised regulatory behaviour of the *nifHDK* operon, with transcription confined to the first four hours of the dark period during which a peak of transcription occurs and

followed by a rapid disappearance (Colón-López and Sherman, 1997). This peak of transcriptional activity coincides with the peak of nitrogenase activity. This relatively simple pattern of nitrogenase activity contrasts with a complicated pattern of regulation of photosynthetic activity (reviewed by Sherman *et al.*, 1998). Photosynthetic changes appear to favour PSII and noncyclic electron flow during the light period, whilst PSI and cyclic flow (ATP synthesis) are favoured early in the dark period. Interestingly, these temporal patterns of photo-synthesis and N_2 fixation are mirrored by circadian oscillation of C and N storage products. Thus, glycogen granules formed during the light period remain at high levels 4 h into the dark period before rapidly disappearing (presumably as a result of respiratory utilisation—see Falcón *et al.*, 2004b). Conversely, cyanophycin gran-ules only begin to appear 4 h into the dark period, accumulate during the later stages of the dark period and gradually disappear during the light period as the cyanophycin is utilised. Cyanophycin polymerisation/depolymerisation appears then to be an important mechanism for storage and utilisation of nitrogen in *Cyanothece* sp. ATCC 51142 at least, and contrasts with what appears to be the more typical degradation of phycobilisomes as a N store in unicellular non-N_2 fixers (Li *et al.*, 2001).

2.3. Unicellular non-N$_2$-fixers

The vast equatorial open ocean gyres are typified by oligotrophic conditions low in available N, but it is in these waters that picocyanobacteria <2 μm dominate. This picocyanobacterial component encompasses the genera *Synechococcus* (Scanlan, 2003; Waterbury *et al.*, 1986) and *Prochlorococcus*, the latter genus comprising probably the most abundant photosynthetic organism on Earth (Partensky *et al.*, 1999). The high numerical abundance of these unicellular cyanobacteria make them potentially important players in N recycling, and as contributors to new and regenerated production. Predicting the precise role that these organisms play in N cycling requires knowledge not just of the physical (e.g., stratified vs mixed) and chemical (e.g., nutrient concentrations that equate to oligotrophic, mesotrophic, or eutrophic conditions) nature of the water column though. It also requires an understanding of the genetic composition of the *in situ* population, and knowledge of the N resource capacity of these populations (see Fig. 24.3). Indeed, *Synechococcus* and *Prochlorococcus* populations can occupy a variety of environmental niches that are not entirely consistent with what is known with respect to N resource utilisation. Thus, in stratified oligotrophic areas *Synechococcus* generally establishes a population maxima in surface waters, where nitrate concentrations are low or undetectable, though nearly all cultured strains, but with a few exceptions (Fuller *et al.*, 2003; Moore *et al.*, 2002), can utilise nitrate for growth. Conversely, *Prochlorococcus* usually possesses a sub-surface maxima and may be the dominant phytoplankter at the base of the euphotic zone close to the nitracline. However, surprisingly all *Prochlorococcus* strains so far studied lack the ability to grow on nitrate (García-Fernández *et al.*, 2004; Moore *et al.*, 2002).
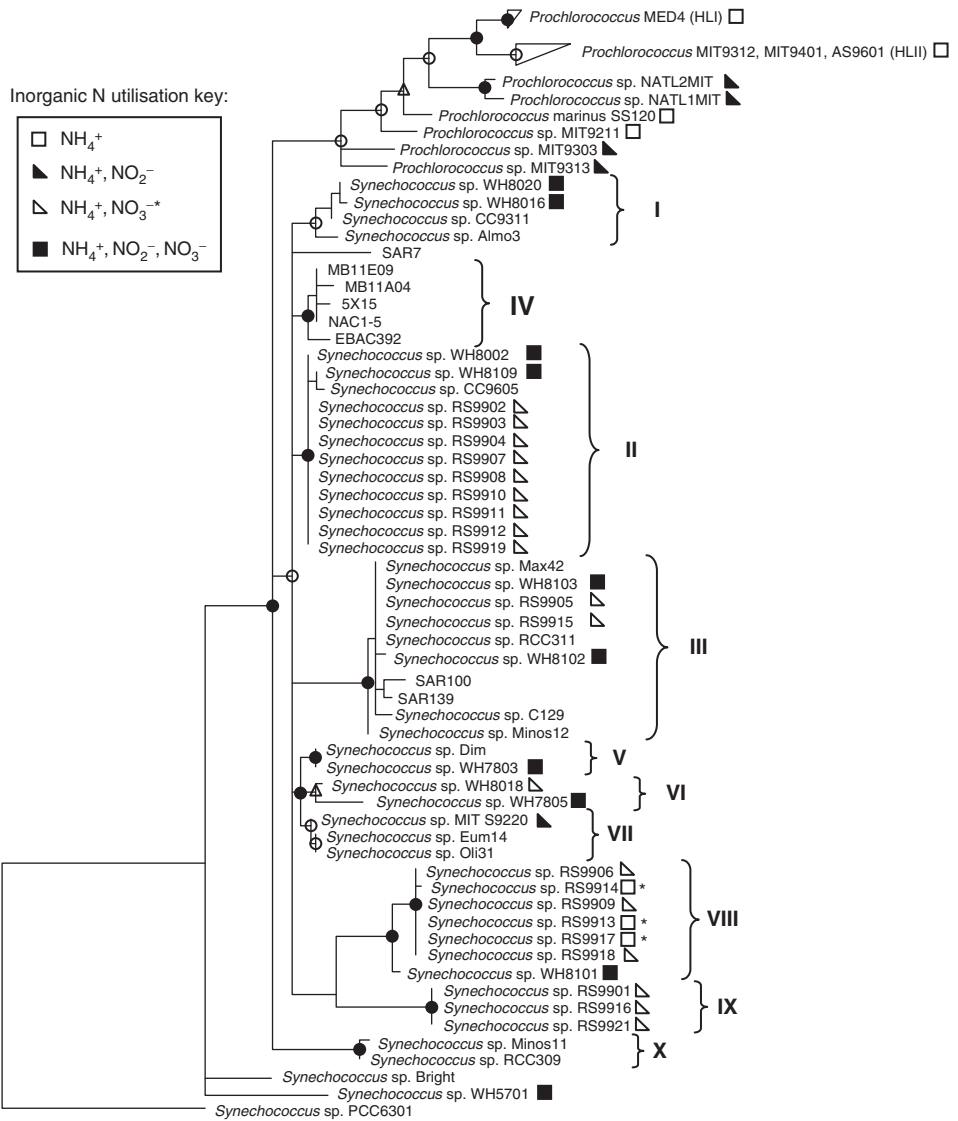
Figure 24.3 Evolutionary relationships of the 16S rRNA gene in *Prochlorococcus* and *Synechococcus* isolates (based on Fuller *et al.*, 2003). Symbols to the right of each strain show N resource utilisation capacity i.e., the ability of each strain to utilise nitrate, nitrite, or ammonium as sole N source. *Nitrite not tested. The scale indicates 0.1 substitutions per position.

For *Synechococcus* this apparent contradiction can be reconciled with the need of members of that genus to fuel phycobilisome synthesis. The capacity to utilise all available N sources, including episodic pulses of up-welled nitrate, would then be critical for construction of such a N-rich light–harvesting macromolecular structure. A low cell density at depth would then be consistent with the incident light levels at depth not providing sufficient energy to reduce nitrite to ammonium (Moore *et al.*, 2002). Overlying obvious differences in N resource utilisation amongst different *Synechococcus* strains, are more subtle differences in N physiology (see Scanlan, 2003) that are also likely an adaptation to the specific environmental niche occupied by individual genotypes.

For *Prochlorococcus* the lack of nitrate utilisation amongst isolates is mirrored by utilisation of ammonium and nitrite N sources which is partitioned amongst closely related, but distinct ecotypes, a feature correlating well to the environmental niche where these resources are available and the known niche of these ecotypes (Moore *et al.*, 2002; Fig. 24.4). Indeed, the lack of utilisation of nitrite, nitrate and urea in specific strains is correlated with actual gene loss e.g., of the nitrite and nitrate reductase structural genes or the nitrate transporter (Rocap *et al.*, 2003; see Fig. 24.5 and Section 5). Interestingly, natural populations of *Prochlorococcus* can take up significant amounts of dissolved organic nitrogen in the form of amino acids (Zubkov *et al.*, 2003). Thus, at an oligotrophic site in the Arabian Sea *Prochlorococcus* was responsible for 33% of the methionine turnover by all bacterioplankton. This data suggests significant competition for reduced N compounds in the form of
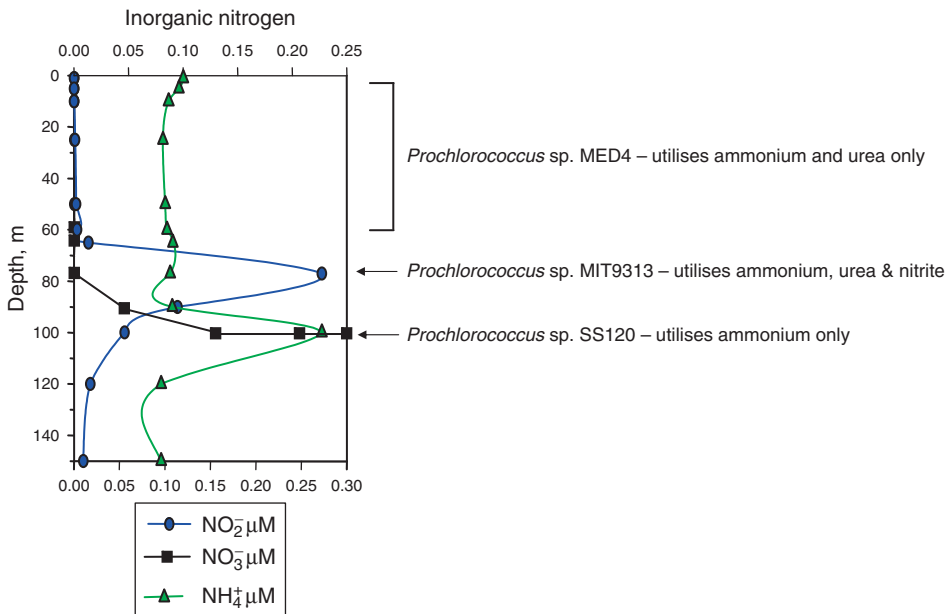
Figure 24.4 Schematic diagram of potential N substrate profiles in a stratified water column, mapped against the known 'partitioned' distribution of specific *Prochlorococcus* genotypes/ecotypes from molecular ecological studies (e.g., see Moore *et al.*, 1998; Steglich *et al.*, 2003; West and Scanlan 1999).
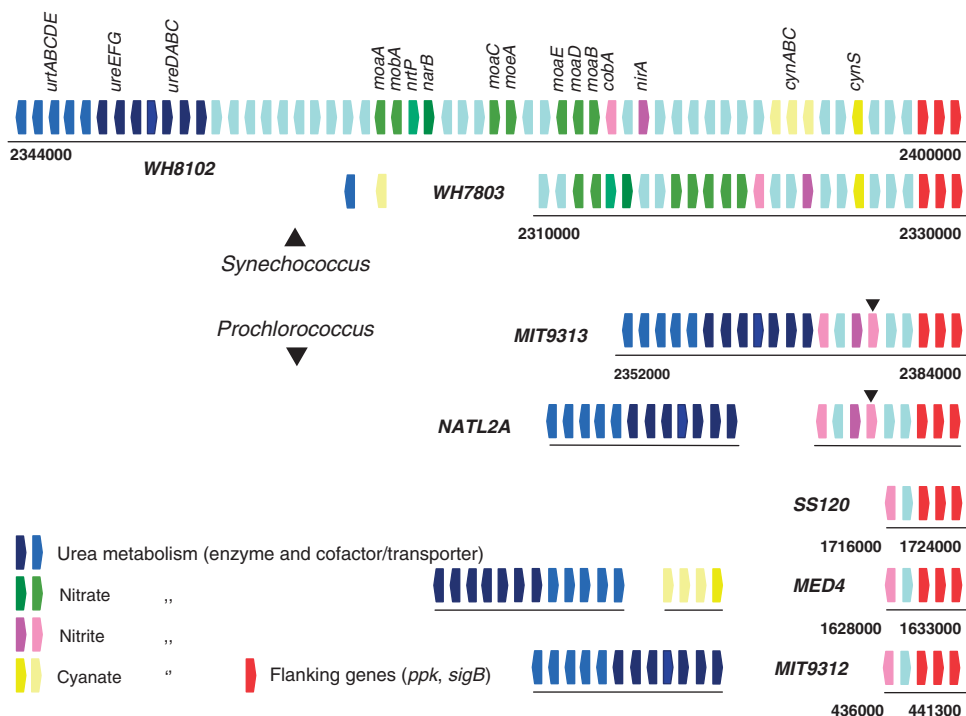
Figure 24.5 Evolution of N acquisition capacity in marine cyanobacteria. *Synechococcus* sp. WH8102 has a wide capacity for N utilisation including nitrate, nitrite, ammonium, and urea. *Prochlorococcus* sp. MIT9313 can utilise ammonium, urea, and nitrite, MED4 ammonium and urea whilst *Prochlorococcus* sp. SS120 only uses ammonium as a N source—consistent with genomic information (Dufresne *et al.*, 2003; Palenik *et al.*, 2003; Rocap *et al.*, 2003). Additional information on the *Synechococcus* sp. WH7803, *Prochlorococcus* sp. MIT9312 and NATL2A genomes are courtesy of Dufresne and Partensky; Steglich and Chisholm resp.

amino acids exists between these autotrophs and heterotrophs in natural populations, particularly in oligotrophic areas. High rates of *Prochlorococcus* methionine uptake were also observed along a transect across the Southern Atlantic gyre, and with high red fluorescing *Prochlorococcus* populations showing a fourfold higher cellular uptake rate than low red fluorescing populations (Zubkov *et al.*, 2004). Thus, within-genus differences also appear important in this aspect of the N nutrition of these organisms.

3. REGULATION OF NITROGEN METABOLISM

Much of the N-metabolism of marine cyanobacteria observes the same basic scheme as that known for widely studied freshwater species (see Flores and Herrero 1994; Herrero *et al.*, 2001 for reviews). Nitrogen acquisition and metabolism in marine cyanobacteria are finely tuned to photosynthetic activity, the availability of additional nutrients, and other environmental factors. Most, if not all, of N-metabolism is funnelled through the GS–GOGAT pathway which—on balance—links ammonium

to oxoglutarate to yield glutamate. This glutamate forms the building block for the synthesis of other amino acids and essential cell components. Alternative N–sources like dinitrogen, nitrate, nitrite, urea, and cyanate are converted into ammonium following their uptake and they subsequently enter cell metabolism via the same route. However, compounds like amino acids and oligopeptides should also be considered as N-sources (see Section 2.3). Although very little is known about their utilization, genome sequences of *Prochlorococcus*, *Synechococcus*, and *Trichodesmium* carry genes with good similarity to those known to encode amino acid and oligopeptide transporters.

The acquisition and metabolism of N is encoded by a vast array of genes, often deposited in operons, the transcription of which is controlled by a single transcriptional activator *ntcA*, a 24 kD DNA binding protein. The *ntcA* sequence is highly conserved among cyanobacteria and it acts specifically in N stress responses (Lindell and Post, 2001). *ntcA* has been identified in all marine cyanobacteria, including both culture isolates as well as in natural samples, whilst its expression has been characterized in *Synechococcus* sp. WH7803 (Lindell and Post, 2001; Lindell *et al.*, 1998), *Synechococcus* sp. WH8103 (Bird and Wyman, 2003), *Prochlorococcus* sp. MED4 (Lindell *et al.*, 2002), *Cyanothece* sp. BK68K (Bradley and Reddy, 1997) and *Trichodesmium* sp. IMS101 (Wang and Post, unpublished results). Interestingly, *ntcA* expression patterns differ considerably, both between organisms but also during different growth conditions within the same organism. Thus, *Synechococcus* sp. WH7803 expresses *ntcA* at three distinct levels, minimal expression when ammonium is sufficient, intermediate expression when ambient ammonium is <1 µM, and maximal expression when cells are N-deprived (Lindell and Post, 2001).

In *Prochlorococcus* sp. PCC9511, a surface-adapted strain (Rippka *et al.*, 2000), *amt1*, encoding a high affinity ammonium transporter, is expressed at high levels both in the presence of ammonium as well as during all but the severe stages of N-deprivation. This constitutive *amt1* expression, and the absence of a typical *ntcA* binding site upstream of *amt1*, has led to the proposal that *amt1* is not regulated by *ntcA* in this *Prochlorococcus* strain (Lindell *et al.*, 2002). Whether constitutive *amt1* expression occurs in *Synechococcus* sp. WH7803 and other marine *Synechococcus* strains awaits further investigation, but interestingly there is a recent report of constitutive expression of *nirA*, encoding nitrite reductase, in the marine *Synechococcus* sp. strain WH 8103 (clade III), i.e., with little effect of ammonium on *nirA* transcription (Bird and Wyman, 2003). Such regulation contrasts with the enhanced expression of *nirA* and *amt1* in the absence of ammonium in freshwater *Synechococcus* strains (see for example Vazquez-Bermudez *et al.*, 2002) and highlights the regulatory constraints placed on these organisms in the N-poor marine ecosystem, a feature though that may well differ subtly between coastal and open-ocean strains.

4. ASSESSMENT OF *IN SITU* NITROGEN-STATUS

One of the outstanding challenges in biological oceanography is the determination of the limiting step in marine primary productivity. Both abiotic (nutrients, light, temperature etc.) and biotic factors (grazing, viruses, allelochemicals) are of

significance, but it is generally believed that productivity is ultimately controlled by nutrient supply. Determining the limiting step in marine productivity is not only important for basin-scale carbon and nutrient flux estimates, but also affects our understanding of ecosystem structure and functioning. Over the last decade it has become increasingly clear that nutrient limitation changes dynamically over space and time, e.g., distinct provinces of the Pacific Ocean are controlled by either N (see e.g., Tyrrell and Law, 1997), P (Karl, 1999), or Fe supply (Coale *et al.*, 1996). A comparable situation occurs at different sites in the Atlantic Ocean (Graziano *et al.*, 1996; Mills *et al.*, 2004). Moreover, invasion by diazotrophic cyanobacteria may drive an N-controlled phytoplankton community into one limited by P-supply. Furthermore, the main taxonomic groups within the phytoplankton community may be controlled by different nutrients, e.g., diatom blooms are controlled by silicate as opposed to P-controlled blooms of diazotrophic cyanobacteria. Nevertheless, it is fair to state that nitrogen is likely the main nutrient that limits phytoplankton productivity as N-requirements of each phytoplankton group are 1 to 2 orders of magnitude greater than those for P and Fe, respectively. As cyanobacteria are reportedly the most abundant phytoplankton fraction in nutrient-deplete oligotrophic waters they routinely meet and adapt to limited N-supplies.

As mentioned above, the *ntcA* gene is commonly found in cyanobacteria and it is the sole gene controlling N-stress responses in cyanobacteria, non nitrogen-fixing and nitrogen-fixing species alike. In the marine unicellular cyanobacteria *Synechococcus* and *Prochlorococcus*, transcription of the *ntcA* gene is up-regulated when ammonium is lacking (Lindell and Post, 2001; Lindell *et al.*, 1998). The threshold ammonium concentration below which *ntcA* expression is up-regulated was determined to be <1 μM in *Synechococcus* sp. WH7803 cultures (Lindell and Post, 2001). *ntcA* is thus expressed over an ecologically relevant concentration range. Moreover, *ntcA* expression was shown to be specific to N-stress responses and to derive from a single gene copy (Lindell and Post, 2001). It is also specific for marine cyanobacteria (Post, 2005). The enhanced expression is required for both nitrite and nitrate utilization in *Synechococcus* sp. WH7803 (Lindell *et al.*, 1998, 1999) and *ntcA* expression may thus have the potential for differentiating between regenerated and new primary production by cyanobacterial phytoplankton. In order to use *ntcA* probing as a means to assess the N-status of marine cyanobacteria, a protocol was developed which studies *ntcA* expression of natural populations relative to their basal and maximal transcript levels (Lindell and Post, 2001). The latter were obtained from the rapid response of *ntcA* expression to additions of 1 mM ammonium and 100 μM methionine-sulfoximine (MSX)—a GS inhibitor—respectively (Lindell and Post, 2001). Based on this protocol it was shown that *Synechococcus* populations in the northern Gulf of Aqaba were not N-deprived over the 1999 annual cycle (Post, 2005). A consecutive study during 2000 established that such *Synechococcus* populations express *ntcA* at an intermediate level shortly after a deep mixing event which injected micromolar concentrations of nitrate into the photic zone (Lindell *et al.*, 2005). *Synechococcus* populations were found to be ammonium sufficient in all other periods even though ammonium concentrations did not exceed 60 nM (Lindell *et al.*, 2005). It would appear then that *ntcA* expression by *Synechococcus* in nature responds to the ammonium flux rather than to ammonium concentration, the latter

being the case for cultures. Although *ntcA* probing allows for unequivocal assessment of the N-status of marine cyanobacteria, the applicability of the protocol may not be universal. On the one hand, not all cyanobacteria express *ntcA* at distinct levels consistent with their N-nutrition. For example, the diazotrophic cyanobacterium *Trichodesmium* sp. IMS101 (see Section 2.1) does not down-regulate *ntcA* transcript to basal levels in the presence of ammonium (Wang and Post, unpublished results). On the other hand, assessment of maximal levels of *ntcA* expression requires the uptake of added MSX via an amino acid transporter. Absence of such a transport system in e.g., *Prochlorococcus* spp. requires that different GS inhibitors, like glufosinate (Aflalo *et al.*, 1999), are used for N-status assessment in such organisms.

5. Genomics

A recent development in the biology of cyanobacteria concerns the sequencing of whole genomes. The first cyanobacterial genome to be sequenced was that of *Synechocystis* sp. PCC6803, a model organism in photosynthesis studies (Kaneko *et al.*, 1996). The sequencing of marine cyanobacterial genomes followed on the heels of this breakthrough. The interest in these genomes was triggered by the significant contribution of marine cyanobacteria to the "biological pump," i.e., the exploration of the genetic potential required to withdraw CO_2 from the surface ocean (and thus atmosphere) and deposit particulate organic matter in the deep ocean. Currently, several marine cyanobacterial genomes are at various stages of sequencing, annotation, and publication, whilst others have either been published or are at an advanced stage. Fully annotated genome sequences are available for *Prochlorococcus* sp. strains MED4, MIT9313, and SS120 (Dufresne *et al.*, 2003; Rocap *et al.*, 2003) along with that of *Synechococcus* sp. strain WH8102 (Palenik *et al.*, 2003). Further genome sequences are in advanced stages of annotation. *Prochlorococcus* sp. strains MIT9312 and NATL2A along with *Synechococcus* sp. strains WH7803, RCC307, and CC9311 are expected to provide much complementary information regarding the evolution of this group of unicellular picocyanobacteria, their physiological traits and their genetic potential to address environmental conditions encountered in distinct ocean niches. In addition, a number of other *Synechococcus* genomes have been selected for sequencing (http://www.moore.org/microgenome/strain-list.aspx). As well as those of *Prochlorococcus* and *Synechococcus*, genomes of the nitrogen-fixing marine cyanobacteria *Cyanothece* sp. *Trichodesmium erythraeum* strain IMS101 and *Crocosphaera watsonii* are currently being analyzed (http://www.jgi.doe.gov). Together these genomes are expected to yield a wealth of information on nitrogen acquisition capabilities, nitrogen metabolism, and nitrogen stress responses in these species.

Based on the genomes available at the time this chapter was written, we can make the following statements. Both *Prochlorococcus* and *Synechococcus* have relatively small genomes ranging in size from roughly 1.6 to 2.5×10^6 nucleotides (Dufresne *et al.*, 2003; Palenik *et al.*, 2003; Rocap *et al.*, 2003). They appear to share a common gene pool of some 1300 genes, with the remainder of the genes mostly encoding

functions that define adaptation to their respective (micro) niches. The genes required for basic nitrogen metabolism are scattered over the genome of *Synechococcus* sp. WH8102, the species thought to be most closely related to the ancestor of the unicellular marine *Synechococcus–Prochlorococcus* clade. The ammonium transporter *amt*, *glnA*, and *glts* encoding GS–GOGAT, *glnB* encoding the regulatory protein P_{II} and the N-stress response gene *ntcA* are all found at different locations on the genome (Palenik *et al.*, 2003). On the other hand, genes required for the uptake of alternative nitrogen sources, e.g., nitrite, nitrate, urea, cyanate are found as a large cluster of tightly grouped genes (Fig. 24.5).

The 11 genes required for the urea transporter and the urease enzyme are organized in three adjacent clusters. Likewise the ∼17 gene cluster for nitrate utilization includes the nitrate permease gene *nrtP*, the nitrate reductase gene *narB* and genes involved in molybdopterin synthesis, supplying the nitrate reductase cofactor. Finally, the four genes required for cyanate uptake and hydrolysis are located in this genome region. Whereas nitrite and nitrate are widely available in the environment, and marine *Synechococcus* can utilize either of these as its sole source of nitrogen, cyanate is a poorly known compound both in terms of cyanobacterial nitrogen metabolism and as a nitrogen source in the marine environment.

This large cluster of genes can be identified in the other marine *Synechococcus* and *Prochlorococcus* genomes currently available by use of the flanking genes *ppk–sigB* (polyphosphate kinase, sigma factor B) on one side and the *pyrG* (glutamine amidotransferase) gene on the other. Comparison of this genome section among the various closely related strains indicates a dynamic plasticity in its make-up (Fig. 24.5), possibly relating to nitrogen sources available in different ocean niches (Fig. 24.4). *Synechococcus* sp. WH7803, known for its inability to grow on urea, lacks the genes for urea utilization. All the *Prochlorococcus* strains known are incapable of nitrate utilization (Moore *et al.*, 2002) and the genes encoding this function are all missing supposedly due to a single deletion event (Rocap *et al.*, 2003). The low-light adapted *Prochlorococcus* strains MIT9313 and NATL2A retained the genes for nitrite utilization and indeed grow on nitrite as the sole nitrogen source (Moore *et al.*, 2002; Rocap *et al.*, 2003). This phenotype is consistent with the depth distribution of low-light adapted *Prochlorococcus* which centers around depths where the primary nitrite maximum is found. As cyanate has not been identified as a nitrogen source in surface waters, it is as yet unclear what the significance is of the presence of the cyanate utilization genes. However, key genes in this genome region, required for the utilization of the various nitrogen sources, are characterized by the presence of an *ntcA* binding site in the promoter region suggesting they are controlled by *ntcA*. Likewise the nitrogen metabolism genes *glnA* and *glnB* have putative *ntcA*-binding sequences in their promoter regions and are likely controlled by *ntcA*. The situation is less obvious for the utilization of other nitrogen sources, like amino acids, oligopeptides etc. for which open reading frames with high similarity to genes encoding transporters for these compounds are found on the genomes. Such open reading frames more often than not lack putative *ntcA* binding sites and thus their function in the nitrogen stress response remains as yet unresolved.

Interestingly, the nitrogen stress regulon of the unicellular marine picocyanobacteria appears much simpler than that of their freshwater counterparts such as

Synechocystis sp. PCC6803 and *Synechococcus* sp. PCC7942. These freshwater strains can carry secondary transcriptional activators like *ntcB*, encoding a *lysR*-type transcriptional activator required for optimal utilization of nitrate (Aichi and Omata, 1997; Aichi *et al.*, 2001), *gifA*, *gifB*, encoding inhibitory factors for GS activity (Garcia-Dominguez *et al.*, 2000), and *nblA* required for the timely degradation of the phycobilisome under environmental stress including nitrogen deprivation (Collier and Grossman 1994; Grossman *et al*, 1994; Luque *et al.*, 2001; Schwarz and Grossman, 1998) all of which appear absent in marine strains. The lack of the *ntcB* gene in the marine *Synechococcus* genomes so far analysed is perhaps surprising given the often massive surface blooms of these organisms in waters freshly enriched with nitrate. In addition, these genomes lack genes required for the synthesis and degradation of cyanophycin, an N-storage polypeptide commonly found in other cyanobacteria and including some marine phycoerythrin-containing *Synechococcus* (Wingard *et al.*, 2002) suggesting marine *Synechococcus* is mostly incapable of exploiting ambient nitrogen levels beyond its cellular requirements.

   Although the genomes of the nitrogen fixing *Cyanothece* sp. *Trichodesmium eythraeum* strain IMS101 and *C. watsonii* are at an advanced stage of analysis, no complete annotation file has yet been made public. However, these genomes are much larger than those of *Synechococcus* and *Prochlorococcus* ranging in size from ~5.5 to 7.8 × 10^6 nucleotides. This is slightly surprising as e.g., *Cyanothece* and *Crocosphaera* are unicellular and with a cell diameter of <8 μm are of the same size class as *Synechococcus*. The larger genome sizes likely reflects the inclusion of the extensive family of *nif* genes as well as genes required for the spatial segregation of the carbon and nitrogen fixation processes along the filaments of *Trichodesmium* (Berman-Frank *et al.*, 2001a; Janson *et al.*, 1994; Lin *et al.*, 1998) or the temporal segregation of these processes in the unicells (Bradley and Reddy, 1997; Reddy *et al.*, 1993). Although they are often considered as obligate nitrogen fixers, the genomes of these cyanobacteria are likely to have further common features given that they can utilize a wide variety of nitrogen sources, very much like their non nitrogen-fixing counterparts.

6. CYANOBACTERIA AND THE NITROGEN-CYCLE

   The marine cyanobacteria are mainly found in the (sub)tropical ocean and sometimes extend into waters at more temperate latitudes. They thus thrive in waters which are either "permanently" stratified or ones that are subject to seasonal stratification. Such waters are preconditioned to become nitrogen-deplete, so that selective pressure favours nitrogen fixing cyanobacteria that inject new nitrogen into the photic zone. They also favour the abundance of picocyanobacteria with their extremely low sinking rates and their surface-to-volume ratios that supports life near the diffusion limit for dissolved nutrients. It is interesting to note that cyanobacterial species are mostly found in the strongly illuminated surface layers. *Trichodesmium*, *Synechococcus* and *Prochlorococcus* have all been described as populating surface layers in dense communities. Of these, *Trichodesmium* is considered as an

obligate nitrogen fixer and thus to be engaged in new primary production only. Likewise, *Synechococcus* may be characterized as contributing to new primary production. It is most abundant in recently mixed water columns and up-welling areas, water bodies that are enriched in nitrate. Natural populations have been shown to adapt to utilize this nitrogen source (Lindell *et al.*, in press; Post, 2005). Low light adapted *Prochlorococcus* are found at depths that coincide with the primary nitrite maximum and such types can utilize nitrite but not nitrate (Moore *et al.*, 2002; Rocap *et al.*, 2003). They may thus also contribute to new primary production, although it is estimated to be quantitatively less significant. Low light adapted *Prochlorococcus* populations are less abundant than those of high light adapted *Prochlorococcus* and light may become a limiting factor as these populations experience ambient light of <5% of surface intensities. Species that are involved in regenerated production—traditionally considered the main contribution of cyanobacteria—belong both to *Synechococcus*, which maintains a sizable population in nitrogen deplete surface water over extended periods, as well as high light adapted *Prochlorococcus*. These latter organisms, which contribute significantly to regenerated production, rely solely on urea and ammonium and they are most abundant in layers deplete of oxidized nitrogen sources like nitrate. Much less is known on the spatial and temporal distribution of the diazotrophs *Cyanothece* and *Crocosphaera* though Fe levels and water column temperature appear important determinants (Falcón *et al.*, 2004a; Mazard *et al.*, 2004; see Section 2.2).

Cellular N-quota and thus N-requirements of phytoplankton in general, and their cyanobacterial component in particular, exceed those of other inorganic nutrients. Hence one would intuitively expect nitrogen to be the factor limiting marine phytoplankton biomass and its productivity. However, cyanobacteria also have the capacity to upset the canonical Redfield ratio, the "natural" balance relating N:P both as dissolved nutrients and plankton mass. Thus, *Trichodesmium* forms a source of nitrogen input in the surface ocean, but it constitutes a sink for phosphate. Hence, the very process of N_2-fixation may inject significant amounts of new nitrogen into the photic layer. This nitrogen can become immediately available via ammonium excretion or follow a more convoluted route via the marine food web. Whereas little is known about all other N_2-fixers, *Trichodesmium* is known for its potential for ammonium release (see Section 2.1). Grazing pressure on *Trichodesmium* is considered not extensive, with the copepod *Macrosetella* as the main grazer, although *Trichodesmium* specific fatty acids have been identified in other marine zooplankters including larger tunicates (Post *et al.*, 2002). Water bodies known to carry *Trichodesmium* populations have been shown to exhibit properties consistent with P-stress (Sanudo-Wilhelmy *et al.*, 2001). Thus, in the nutrient deplete waters of the Gulf of Aqaba, one may find occasional blooms of *Trichodesmium* (Post *et al.*, 2002). During such periods phytoplankton typically shows enhanced alkaline phosphatase activities (Li *et al.*, 1998) and the cyanobacterial P-stress protein PstS is highly expressed during this period (Fuller *et al.*, 2005). *Trichodesmium* populations are themselves P-stressed with high levels of alkaline phosphatase activity (Dyhrman *et al.*, 2002; Stihl *et al.*, 2001) and enhanced PstS (Scanlan, unpublished results). Alternatively, one should consider that *Trichodesmium* populations may become Fe-limited. Both a low Fe supply in certain ocean provinces and the high

Fe-requirements of *Trichodesmium* as compared to other phytoplankters (Berman-Frank *et al.*, 2001b) contribute to this notion. Whether Fe limits N_2-fixation in free-living cyanobacteria like *Crocosphaera*, *Cyanothece*, and possibly even in endosymbionts like *Richelia* needs to be established. Certainly, the two former cyanobacteria do have the capacity to respond to Fe-limiting conditions (Webb *et al.*, 2001).

7. FUTURE PERSPECTIVES

Over the last two decades developments in flow cytometry, molecular biology, microbial genomics, N-isotope studies etc have propelled cyanobacteria into a role as key players in the field of biological oceanography. On the other hand such studies have raised many more questions that need to be addressed. Firstly, genome information indicates that N-stress responses may differ substantially with different cyanobacteria carrying different gene pools for the acquisition of alternative N compounds. Some of these N compounds have not been recognized as ''common'' N sources in the marine environment. The fact that many cyanobacteria have genes with a high degree of similarity to amino acid, cyanate, nitrite, oligopeptide transporters and to enzymes required for the incorporation of these N compounds into the cell's metabolism attest to the fact that these compounds may have some significance. Especially interesting in this respect is the fact that diazotrophic cyanobacteria considered to be obligate N_2-fixers have the capacity to address a wide suite of inorganic and organic N species.

Other, unicellular, N_2-fixers have only recently been shown to be significant contributors to the marine phytoplankton communities (Falcón *et al.*, 2004a; Montoya *et al.*, 2004; Zehr *et al.*, 2001). Many aspects of their spatial and temporal distribution as well as the environmental factors determining the niche they occupy, are still unresolved.

Lastly, recent studies have shown an unexpected genetic diversity among, the morphologically uniform, marine *Synechococcus* with at least 10 distinct clades identified (Fuller *et al.*, 2003; Rocap *et al.*, 2003). It needs to be established whether such *Synechococcus* clades represent different ecotypes adapted to different niches in the ocean environment. Moreover, little is known whether certain clades represent stable populations over seasonal cycles and/or multi-annual time periods.

In the near future we anticipate further progress in ocean acidification as a result of increased atmospheric CO_2 concentrations (Caldeira and Wickett, 2003) with sea surface pH potentially reaching as low as 7.8, a decrease of ~0.5 pH units since the middle of the 20th century. More extensive periods of stratification and a spreading of oxygen-minimum zones in the world's oceans are also expected. Each of these processes is likely to impact on the oceanic N-cycle and the role cyanobacteria play within these systems. Specifically, these climate induced changes are likely to have significant effects on the composition of marine cyanobacterial communities and hence on the N dynamics they carry out.

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VIRUSES, BACTERIA, AND THE MICROBIAL LOOP

Ian Hewson *and* Jed A. Fuhrman

Contents

1. INTRODUCTION

Study of the role of microorganisms in the marine environment has led to the concept of amicrobial loop in the oceans, which cycles nutrients through organisms smaller than 10 um. Our concept of the marine microbial loop has undergone

considerable change since formalization over 20 years ago because newly recognized metabolic capabilities, types of organisms, revised rates of metabolic processes as well as a greater understanding of the genetic diversity of microorganisms have been observed. Here we review developments in our understanding of the microbial loop as it relates generally to all marine microorganisms as well as to the marine N cycle, focusing specifically upon prokaryotes (bacteria and archaea) and the role of viruses in the emerging microbial loop paradigm.

The microbial loop is a critical component of the marine nitrogen cycle in which viruses, prokaryotes, and unicellular eukaryotes play distinct roles. Nitrogen transformations e.g., nitrogen fixation, ammonification, nitrification, ammonium oxidation, and denitrification are mediated by prokaryotes, and the formation of autochthonous dissolved organic nitrogen is a direct result of either intracellular component leakage into surrounding waters by grazing, apoptosis or viral lysis, or by direct excretion from phytoplankton. Thus, the microbial loop plays a key role in both the generation and uptake of both organic and inorganic nitrogen in the ocean. Understanding of the dynamics of marine microbial communities has undergone substantial revision in the past three decades since initial descriptions of the microbial food web in the 1970s (Pomeroy, 1974) and formalization of the microbial loop by (Azam *et al.*, 1983) (Fig. 25.1). Classical ideas that bacteria were responsible solely for remineralisation of zooplankton and fish wastes (Steele, 1974) and unable to derive nutrition from the pool of dissolved organic C (Krogh, 1934; Sverdrup *et al.*, 1942), were then challenged by studies rejecting ideas of tightly-closed grazing food chains with little flow of organic matter to microheterotrophs (le B. Williams, 1981), and later by studies demonstrating that 10–50% of primary production and about 40% of reduced inorganic N is consumed directly by prokaryotic nanoplankton (Andrews *et al.*, 1971; Fuhrman and Azam, 1980, 1982; Hagstrom *et al.*, 1979; le B. Williams, 1981; Pomeroy, 1974; Sieburth *et al.*, 1977; Suttle *et al.*, 1990a) (Fig. 25.2). The definition of ''bacteria'' in original models has been revised to include not only bacteria (''heterotrophic'' bacteria including photoheterotrophic genera) and cyanobacteria, but also an entirely new domain, the Archaea. Until 1992, Archaea were originally believed to occur only in ''extreme'' environments, but since have been found in mesopelagic (100–500 m), pelagic (Fuhrman *et al.*, 1992) and later in surface coastal (DeLong *et al.*, 1992) waters, but also in large abundance in the deep ocean (Fuhrman and Ouverney, 1998; Karner *et al.*, 2001), where they may play a significant role as dominant nitrifiers (Ward Chapter 5, this volume). Observation of a ubiquitous, abundant, and diverse viral assemblage in the marine environment further challenged understanding of the microbial carbon transfer (Bergh *et al.*, 1989; Proctor and Fuhrman, 1990; Wommack and Colwell, 2000). Even the lifestyles of the protozoan component of microbial communities has undergone revision to include not only heterotrophic and autotrophic, but also mixotrophic eukaryotes (Caron, 2000).

The purpose of this chapter is to emphasise the role of the microbial loop in the oceanic nitrogen cycle. It begins by briefly introducing classical concepts of microbial loop function, then shifts to the increase in our knowledge that has occurred in the past three decades. It then introduces the components of the microbial loop that
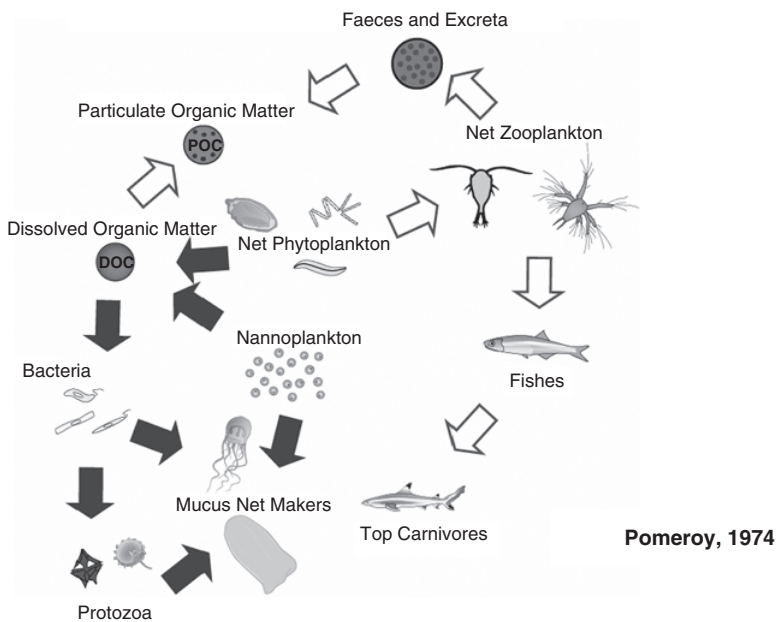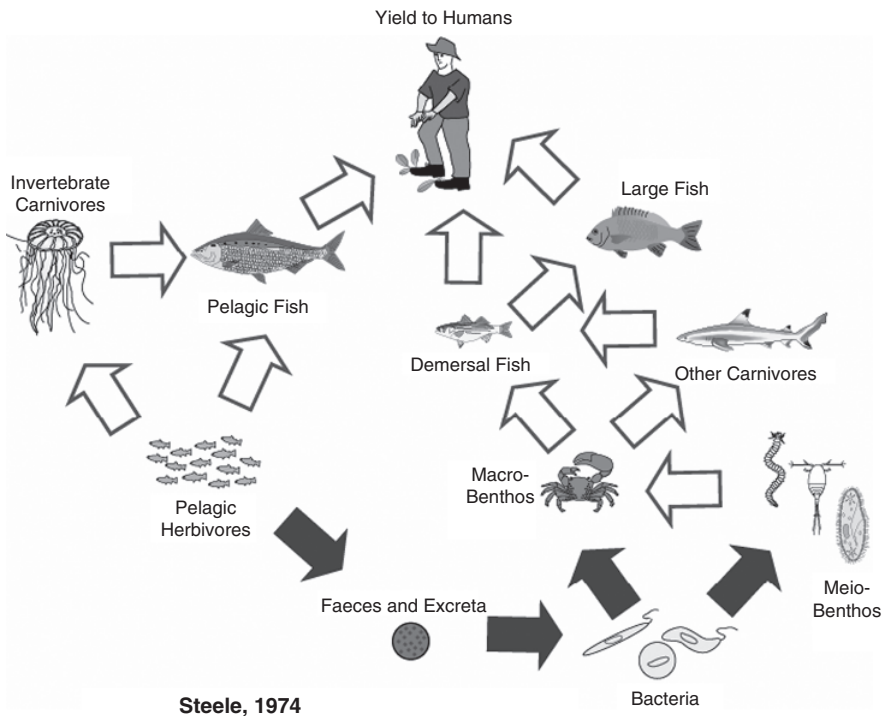
Figure 25.1 Two classical marine food webs adapted from Steele (1974) and Pomeroy (1974), where microbial loop cycling is indicated by red arrows. "Net" phytoplankton and zooplankton were defined as those collected in 20 μm plankton nets; "nannoplankton" were those cells passing through plankton nets. One significant step in the Pomeroy (1974) model was the inclusion of dissolved organic matter (DOM) which is remineralised by bacteria and thereby made available to higher trophic levels.
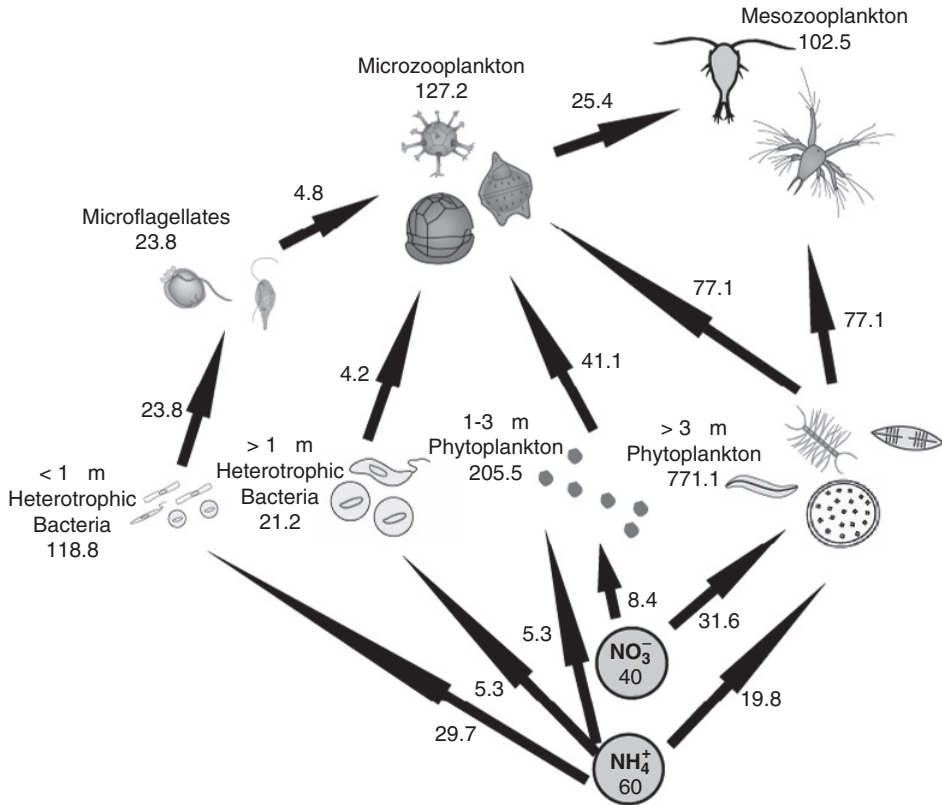
Figure 25.2 Contemporary microbial loop with nitrogen fluxes under homogenous N supply, redrawn from Suttle *et al.* (1990b). The numbers next to each arrow indicate the relative partitioning of inorganic N into different functional groups of bacteria, protists and metazoa, and the numbers beneath groups of organisms indicates the biomass of C in each size class. Under pulsed N supply, a greater proportion of NO_3^- and NO_4^+ is transferred to larger organisms than to smaller organisms.

exist in contemporary conceptualizations and models, including viruses as the new members of the microbial loop paradigm.

1.1. Historical context for the microbial loop

Observations of submicron-sized living particles in aquatic environments can be attributed originally to Antonie van Leeuwenhoek (van Leewenhoek, 1677) who examined lake water samples through a microscope, and observed protozoa, metazoa, and bacteria. Early and continuing attempts to isolate bacteria from the marine environment (Gran, 1901; Russel, 1892, 1893) were later found to be inconsistent with reports of large abundances of bacteria-like organisms in seawater, and were interpreted to mean that the majority of bacteria in the marine environment were not active (i.e., "the great plate count anomaly" (Jannasch and Jones, 1959; Staley

and Konopka, 1985)). Accurate counts of bacteria were not obtained until the advent of epifluorescence microscopy and DNA stains in the late 1970s (Ferguson and Rublee, 1976; Hobbie et al., 1977; Watson et al., 1977), and measurements of their activity have since confirmed that most bacteria show at least some measureable activity (Fuhrman and Azam, 1982; Tabor and Neihof, 1982b).

Initial concepts of the role of microbes in the marine ecosystem suggested that they were mostly decomposers of macro-organism faeces in sediments (Steele, 1974), before the observation of high bacterial abundance in the oceans. But a forward thinking microbiologist (Pomeroy, 1974) recognised bacteria as reminer-alising dissolved organic matter (DOM) and feeding higher trophic levels. A few years earlier, higher levels of chlorophyll–a associated with ''nanophytoplankton'' (i.e., passing through phytoplankton nets) than to ''net'' phytoplankton (i.e., trapped within a net) were found, which suggested that photosynthesis may be dominated by smaller phototrophs (Malone, 1971). With the discovery of unicellular cyanobac-teria (which account for most chlorophyll–a in the ocean) (Chisholm et al., 1988; Johnson and Sieburth, 1979; Waterbury et al., 1979) it became clear that bacteria played far more complicated roles in marine ecosystems than originally thought, and caused a re-think of the role of prokaryotes in material and energy cycling in the ocean. Subsequent models of the marine food webs contain shunts of DOM from zooplankton and higher organisms (secondary production) and phytoplankton exu-dation (primary production) into bacteria (Azam et al., 1983).

Until the late 1980s it was thought that the bacteria in the ocean comprised only a few different species due to low morphological diversity observed in culture (Giovannoni et al., 1990). A greater understanding of the diversity of bacteria was gained when advances in molecular biology allowed universal, evolutionarily-conserved regions (particularly 16S ribosomal RNA) within the bacterial genome to be sequenced (Pace et al., 1986). This indicated a wide richness of marine bacteria, many of which were not culturable by classical enrichment techniques (Giovannoni and Rappe, 2000; Jannasch and Jones, 1959). Further developments in molecular techniques allowed discrimination of bacteria involved in different aspects of nutrient cycling based upon functional gene sequences (Zehr et al., 2000).

More recently, sequencing of large segments of microbial genomes from nature has elucidated new energy-assimilating pathways, for example that involving the retinal–containing pigment bacteriorhodopsin (Beja et al., 2000). With discoveries of phylogenetically diverse previously unrecognised prokaryotic ecophysiological pro-cesses (Beja et al., 2000; Rocap et al., 2003; Venter et al., 2004; DeLong et al., 2006; Rusch et al., 2007), and a greater understanding of the size-spectra and metabolic plasticity of protists (Caron, 2000; Sherr and Sherr, 2000), the conceptual microbial loop has become vastly more complicated than originally conceived.

1.2. Components of the microbial loop

In its simplest form, the microbial loop comprises autotrophs, heterotrophs and an abiotic pool of DOM. These organisms are <100 μm and in basic models comprised bacteria, flagellates, ciliates, zooplankton, phytoplankton, and cyanobacteria (Azam

et al., 1983). The microbial loop described in recent years has been expanded to include viruses, heterotrophic bacteria, pico- and nano-cyanobacteria, anoxygenic aerobic/anaerobic phototrophic bacteria, heterotrophic flagellates, heterotrophic diatoms, and microzooplankton (a catchall term which refers to all hetero- and mixotrophic protists which phagocytise particles and are <200 μm in length). Bacteria in the early microbial loop models included all cells <1 μm and hence encompassed Bacteria and Archaea, the latter of which can comprise up to 60% of bacterioplankton in deep sea environments (DeLong *et al.*, 1999; Fuhrman and Ouverney, 1998; Karner *et al.*, 2001). The composition of the DOM pool has also received considerable study in the past 20 years. DOM is comprised of a spectrum of compounds of variable refractability ranging from low molecular weight (i.e., <2 kDa) monomers, lipids, fatty acids, complex condensates, and amino acids, to high molecular weight (>100 kDa) aliphatic algaenans, proteins, nucleic acids, and polymers (Gelin *et al.*, 1999; Ogawa and Tanoue, 2003). Of these compounds, those with high molecular weight are taken up more rapidly and consequently are younger, while low molecular weight compounds are less utilised by bacterioplankton and therefore turn over slowly (Amon and Benner, 1996; Loh *et al.*, 2004). DOM may form complex aquatic gels (Azam, 1998) which result in ''hotspots'' of microbial activity and possibly diversity (Long and Azam, 2001; Seymour *et al.*, 2004). The net result of research in the past two decades is a more complete understanding that the seemingly homogeneous environment of surface waters is in fact heterogeneous on the micrometer scale, and encompasses a wide variety of metabolic pathways and capabilities.

1.3. Viruses as a member of the microbial loop

Isolation of bacteriophage directly from coastal and pelagic seawater (Moebus, 1980) and observation of high viral abundances in marine plankton (Bergh *et al.*, 1989; Proctor and Fuhrman, 1990) and sediments (Danovaro and Serresi, 2000; Hewson *et al.*, 2001a,b,c; Paul *et al.*, 1993), raised interest in their biogeochemical and ecological roles in the marine environment (Fuhrman, 1999; Wommack and Colwell, 2000). While some reviews of bacterivory indicated close coupling between bacterial production and loss via grazing in some environments (Strom, 2000), others suggested that grazing was insufficient to account for all production (Hamels *et al.*, 2001; McManus and Fuhrman, 1988). Data showing high rates of viral mortality in both the water column (Proctor and Fuhrman, 1990) and sediments (Hewson and Fuhrman, 2003) have shown that viruses must be included in contemporary models of the microbial loop. In some cases, viruses may be the dominant form of bacterial mortality and consequent organic N release (Bratbak *et al.*, 1993; Hewson and Fuhrman, 2003).

   This review focuses on four key research areas directly related to the microbial loop which are critical to the oceanic N cycle and for which the picture is slowly evolving. First, the opening of the black box of marine bacterial taxonomy and physiology is reviewed, which provides context for later sections addressing bacterial ecology and physiology. The distribution of secondary production in the oceans is then reviewed since a global- and ecosystem-scale biogeochemical view of bacterial

activities in seawater is warranted in the context of the marine N cycle. A review of viral lysis as a newly recognised shunt of N through the microbial loop follows, since this source of mortality has important consequences for the cycling of organic N in marine plankton. This work will also review key differences between pelagic and sediment microbial food webs, since sediments are sites of important N cycling processes. Finally, it concludes with a discussion of several promising future directions for research on the role of the microbial loop in the oceanic N cycle, as well as a new view of the microbial loop.

2. BACTERIAL DIVERSITY AND PHYSIOLOGY

Early studies of marine bacteria via culture-based methods indicated a low abundance in seawater (Sverdrup *et al.*, 1942; ZoBell, 1941), and that their assemblages were not diverse (Waksman, 1934). Reliable enumeration techniques developed in the late 1970s indicated that typical bacterial abundances in marine surface waters exceeded colony-forming unit (cfu) estimates by over two orders of magnitude (Hobbie *et al.*, 1977), with abundances of typically 10^9 cells l^{-1} in surface waters. Employment of microautoradiographical and molecular techniques has since indicated that bacterioplankton and sediment bacteria have a spectrum of activities (Fuhrman and Azam, 1982; Tabor and Neihof, 1982b; Ouverney and Fuhrman, 1999a,b). The percentage of "actively growing" bacteria (i.e., those growing at a rate that makes them detectable above thresholds of these techniques) can range from below 20% to greater than 90% (Karner and Fuhrman, 1997; Luna *et al.*, 2004; Smith and del Giorgio, 2003). The advent of flow cytometry has allowed observation of cellular DNA content and has indicated that bacterioplankton contain a range of DNA concentrations, which has been argued to be an indicator of physiological state (Button and Robertson, 1989; Gasol *et al.*, 1999). Rates of bacterial production measured by "bulk" techniques (i.e., radioactive thymidine or leucine incorporation (Fuhrman and Azam, 1982; Simon and Azam, 1989)) represent averages among the entire prokaryotic community. Reportedly "live" and "dead" bacteria in marine systems can be observed by use of vital stains (i.e., those reporting metabolite formation within cells or associated with cell wall permeability), however, these may be somewhat misleading since there are likely cells within assemblages that fall below the detection threshold of such stains (which are described incorrectly as "dead") (Sherr *et al.*, 1999) or which can rise above detection thresholds when favorable conditions occur (Choi *et al.*, 1999).

2.1. Broad phylogenetic affiliation of bacteria

The term "bacteria" in the ocean is commonly used to describe prokaryotes (Archaea and Bacteria). In the last 20 years, there has been an explosion in the understanding of the phylogeny of bacteria that is based primarily upon culture-independent sequencing of the universally conserved genes (i.e., those on which there is strong evolutionary selection) involved in ribosome function (Giovannoni and Rappe, 2000; Olsen *et al.*,

1986; Pace *et al.*, 1986). Early molecular studies utilised length heterogeneity in electrophoresis of extracted 5S rRNA (Fox and Woese, 1975; Stahl *et al.*, 1981) from mixed, low-diversity lake communities to examine assemblage structure (Hofle, 1988). A second approach, in which mixed community DNA was sheared and ligated into lambda phage DNA was proposed, but not widely adopted, probably due to the difficulty in obtaining universally conserved fragments in lambda libraries (Schmidt *et al.*, 1991). Later studies sequenced part of the 16S rRNA gene amplified with PCR primers designed from cyanobacterial sequences (Giovannoni *et al.*, 1990), and by amplification using universal primers (Fuhrman *et al.*, 1992, 1993) from DNA extracted from natural mixed communities. 16S rRNA is larger than 5S rRNA (*ca.* 1492 bp *cf.* 126 bp). The 16S rRNA gene contains both conserved and variable regions and provides greater phylogenetic resolution than 5S rRNA. Further studies examining the intergenic spacer region (lying between the 16S and 23S rRNA genes) have indicated even more phylogenetic microdiversity among closely related taxa (Brown and Fuhrman, 2005, Garcia–Martinez *et al.*, 1999; Garcia–Martinez and Rodriguez–Valera, 2000; Orcutt *et al.*, 2002). Very recently, whole metagenome (i.e., the combination of all genomes of all organisms present at a single location) shotgun sequencing has been applied to marine bacterioplankton communities and has indirectly provided further appreciation of the depth of microdiversity in communities (Rusch *et al.,* 2007; Venter *et al.,* 2004). Interesting, in the first such marine metagenomics study, the genomes of the most common marine taxa (SAR–11) could not be closed (i.e., assembled computationally into a continuous chromosome) due to high microdiversity (Giovannoni, 2004).

PCR amplification biases are a concern in studies examining bacterial diversity. Biases investigated include uneven operon copy number (Crosby and Criddle, 2003), bias in template annealing temperatures (Suzuki and Giovannoni, 1996; Suzuki *et al.*, 1998) and differential extractability of bacterial cells (Polz and Cavanaugh, 1998; Polz *et al.*, 1999). However there has been at least one report of few net PCR biases, with proportional amplification of genes in mixed-culture conditions (Leuders and Friedrich, 2003). Most marine bacteria are oligotrophic and thus are slow growing, hence they probably contain only single or a small number of operon copies (Klappenbach *et al.*, 2000). It is interesting as well to note that clone libraries constructed using PCR based techniques (Fuhrman *et al.*, 1993; Giovannoni *et al.*, 1990) have very similar proportions of phylogenetic groups to those estimated with PCR-independent approaches (e.g., metagenomics and lambda phage libraries) (Schmidt *et al.*, 1991; Venter *et al.*, 2004).

Prokaryotic communities in the marine environment are comprised primarily of eleven broad groups of organisms, many of which have relatively unknown physiology due to their low culturability on enriched media (Giovannoni and Rappe, 2000). For example, the family Proteobacteria includes a cluster of phylotypes, described as the SAR–11 group (in the α-Proteobacteria), named for their original sequence recovery in the Sargasso Sea (Giovannoni *et al.*, 1990). These are abundant (making up ∼30% of total counts (Morris *et al.*, 2002)) and ubiquitous in the world's oceans (Morris *et al.*, 2002), yet are only recently culturable and therefore have almost completely unknown physiology (Rappé *et al.*, 2002; Simu and Hagstrom, 2004). Aside from the α-Proteobacteria (SAR–11, SAR–116, *Roseobacter*), marine plankton contains diverse and abundant γ-Proteobacteria

and cyanobacteria. Cytophaga–Flavobacteria–Bacteroides (CFB) group bacteria, Actinobacteria, Planctomycetales, and Cren- and Euryarchaeota round out the total but typically each make up a relatively small proportion of clone libraries in most pelagic waters (Giovannoni and Rappe, 2000). In marine sediments, the δ- and ε- Proteobacteria comprise a greater proportion of communities than in the water column, along with CFB and Actinobacteria (Cifuentes et al., 2000; Madrid et al., 2001; Todorov et al., 2000). β-Proteobacteria are relatively uncommon in marine clone libraries, but typically dominate freshwater systems (Fuhrman and Ouverney, 1998; Fuhrman et al., 1993; Giovannoni and Rappe, 2000; Methe et al., 1998; Venter et al., 2004). Whole-metagenome shotgun sequencing and bacterial artificial chromosome (BAC) library studies provide a future direction to overcome some culture limitations (De Long, 2002; Schmidt et al., 1991; Venter et al., 2004; Rusch et al., 2007, DeLong et al., 2006) and may elucidate unexpected physiological capabilities that can be investigated in studies of field samples or pure cultures (Fuhrman, 2003). Among nitrogen cycling bacterial guilds (bacteria here referring to both Bacteria and Archaea) there is a spectrum of diversity associated with each function.

2.2. Common features of bacterial physiology

Bacteria and Archaea, as prokaryotes, are the simplest and oldest life-forms on earth, having evolved early in the Achaean era 3.4–3.8 Ga (Schopf, 1993). Prokaryotic DNA is not contained within a nucleus and prokaryotic cells contain no membrane-bound organelles. All bacteria contain a cell wall composed of membrane and peptidoglycan. Bacteria contain generally smaller genomes than eukaryotes, however they have a higher percentage of biomass in nucleic acids than most protists. Marine heterotrophic bacterioplankton contain on average 2 fg DNA cell^{-1} (Fuhrman and Azam, 1982). The average C biomass of heterotrophic and autotrophic bacteria ranges from 12 to 280 fg C cell^{-1} but most studies of heterotrophic bacteria converge on 10–20 fg C cell^{-1} (Carlson et al., 1999; Caron et al., 1995; Christian and Karl, 1994; Ducklow, 2000; Fagerbakke et al., 1996; Fukuda et al., 1998; Heldal et al., 2003; Lee and Fuhrman, 1987). The C content of cells depends primarily upon growth rate (Heldal et al., 1996) and on the size of cells (Troussellier et al., 1997). Volumetric conversion factors based upon cultures range from 65 to 290 fg C μm^{-1} (Ducklow et al., 1992; Fagerbakke et al., 1996; Heldal et al., 2003; Troussellier et al., 1997), however in natural bacterioplankton samples they range from 32 to 400 fg C μm^{-3} (Fagerbakke et al., 1996; Lee and Fuhrman, 1987; Simon and Azam, 1989; Vrede et al., 2002). Marine bacteria (autotrophs and heterotrophs) range in diameter from 0.2 to 3 μm, however most heterotrophs are small (0.2–1 μm) encompassing volumes of $<$0.004–0.524 μm^3 (Caron et al., 1995; Carlson et al., 1999; Christian and Karl, 1994; Lee and Fuhrman, 1987).

Bacteria may live aerobic, anaerobic or microaerophilic lifestyles, and do not necessarily utilise O_2 as a terminal electron acceptor; many can use a suite of organic and inorganic compounds. Bacteria may have autotrophic (cyanobacteria and nitrifying bacteria), heterotrophic (supposedly most planktonic bacteria) or chemoautotrophic (utilising energy from reduced inorganic compounds) physiologies. Additionally, some prokaryotes have been observed to contain plastic metabolism and mixotrophic physiologies, however it is unclear whether these provide

significant energy for metabolism, or if they may have unknown ecological advantages (Beja *et al.*, 2000; de la Torre *et al.*, 2003; Schwalbach *et al.*, submitted for publication). Recent metagenomic studies of bacterioplankton has suggested that mixotrophy may be very common (Venter *et al.*, 2004; Rusch *et al.*, 2007). Observation of variable physiology and its implications for biogeochemical cycling complicates our view of the microbial loop, and in part requires opening the bacterial "black box" in biogeochemical models including unicellular prokaryotes.

2.3. Carbon:nitrogen ratios in bacteria

The atom ratio of C:N in marine bacterial biomass depends upon the type of bacteria, as well as their growth rate (Heldal *et al.*, 1996). The first descriptions of elemental ratios in marine plankton can be attributed to Vinogradov (Vinogradov, 1935) who described C:N:P ratios in large phytoplankton (diatoms and "Peridinians" i.e., dinoflagellates) of 100:18:3 (Sverdrup *et al.*, 1942). Later descriptions of plankton >20 μm, predominantly phytoplankton, found C:N:P ratios of 106:16:1, referred to as the Redfield ratio (Redfield, 1958). Difficulty in separating elemental analysis signatures of bacteria from detritus inhibited estimates of their elemental ratios until the advent of X-ray microanalysis which allowed the composition of single cells to be determined. These studies have revealed a range of ratios for both cultured and natural marine bacteria (Fagerbakke *et al.*, 1996; Heldal *et al.*, 2003; Vrede *et al.*, 2002). The source of variation has remained unclear, however environmental conditions and the physiology of bacteria may explain some of the variability (Table 25.1).

Table 25.1 Size and elemental composition of example eubacterial taxa based upon X-ray microanalysis. The natural bacterioplankton includes values from the oligotrophic subtropical gyres to highly productive coastal embayments

Physiology	Taxa	C content (fg cell^{-1})	Cell Vol. (μm^3)	Atom C:N	Ref.
Heterotroph	*Vibrio natriegens* culture	62–350	0.93–3.5	3.8	Fagerbakke *et al.*, (1996)
Phototroph	*Prochlorococcus* culture	17–34	0.08–0.22	8.8–9.9	Heldal *et al.*, (2003)
	Synechococcus culture	120–250	0.62–1.60	8.2–10.0	
Anaerobe	*Haloanaerobium praevalens*	137–756	5.01–20.30	2.9–3.3	Oren *et al.*, (1997)
Natural bacterioplankton		6–48	0.11–0.41	2.0–8.3	Fagerbakke *et al.*, (1996), Goldman *et al.*, (1987), Fukuda *et al.*, (1998)

The ratio of C:N decreases with increasing growth rate, presumably due to greater synthesis of N–rich nucleic acids and protein (Norland *et al.*, 1995). This is consistent with reports of high C:N ratios in the oligotrophic subtropical gyres, and lower ratios in productive coastal embayments (Fukuda *et al.*, 1998). The higher C:N ratios in photosynthetic bacterioplankton than in heterotrophs reportedly reflects their inclusion of carbon-rich thylakoid membranes and other photosynthetic apparati (Heldal *et al.*, 2003).

The ratio of N:P is frequently used to determine growth limitation in marine ecosystems when the water column concentrations of inorganic N and P species is known. Heterotrophic bacterial N:P ratios are between 3.5 and 9.0 (Goldman *et al.*, 1987; Fagerbakke *et al.*, 1996), while cultures of *Prochlorococcus* sp. and *Synechococcus* sp. are higher at 8.0–43.0 depending upon strain and growth conditions (Heldal *et al.*, 2003). Interestingly, the N:P ratios in heterotrophic bacteria are substantially less than in larger phytoplankton, which typically follow Redfield stoichiometry (16:1 (Redfield, 1958)). This could be due to larger nucleic acid:protein ratios and lack of chloroplasts in heterotrophic bacteria when compared to eukaryotic phytoplankton (Fagerbakke *et al.*, 1996; Heldal *et al.*, 2003).

3. DISTRIBUTION OF BACTERIAL SECONDARY PRODUCTION IN THE MARINE ENVIRONMENT

Bacterial secondary production, the synthesis of new bacterial cellular components from organic molecules taken up from the surrounding environment, has long been of interest to marine biogeochemists since it provides estimates for elemental flow through the submicron size fraction. Until the advent of molecular techniques, it was assumed by some marine scientists that most bacteria, including those observed by recently-developed epifluorescent techniques, were inactive or moribund because estimates of colony-forming units in enrichment culture from seawater samples fell well short of observed cell abundances (Hobbie *et al.*, 1977; ZoBell, 1941). However, development of enrichment culture-independent techniques has indicated a spectrum of bacterial activities, ranging from undetectably to highly active cells (Cottrell and Kirchman, 2004; Fuhrman and Azam, 1982; Gasol *et al.*, 1999; Karner and Fuhrman, 1997; Ouverney and Fuhrman, 1999a,b; Tabor and Neihof, 1982b). In this section, we review methods of measurement currently used to assess bacterioplankton production, as well as summarise current knowledge about secondary production rates in a range of environments.

3.1. Methods of measurement

The biogeochemical activities of microbes in the oceans was of interest since initial reports of elemental cycles in the oceans (Redfield, 1958). Initial attempts to determine rates of heterotrophic synthesis focused upon dark respiration rates of

bulk water samples, however these were confounded by the presence of algae (Sorokin, 1964). Demand for bacterioplankton-specific growth rate estimates resulted in several methods of estimating turnover of planktonic bacterial communities. The frequency of dividing bacterial cells, as observed by epifluorescence microscopy, provided among the first, though not widely adopted, estimates of bacterial growth rate in marine environments, and was based upon the assumption that the relative number of visibly dividing cells was proportional to growth rate (Hagstrom et al., 1979). Incorporation of labeled amino acid substrates into DNA or protein provided another way of estimating activity, since the incorporation rate is putatively proportional to bacterial growth rate, and has been used in virtually all studies of bacterioplankton production since 1982. Incorporation of ^{3}H-thymidine directly into DNA was the first widely used approach in studies of bacterioplankton growth and production rates in the marine environment (Fuhrman and Azam, 1982), and more recently ^{3}H-leucine has been used to determine the rate of protein synthesis and growth or production in marine bacteria (Kirchman et al., 1985; Simon and Azam, 1989). As these are ''bulk'' analyses, they represent averages of bacterial activity across the entire community based upon conversion factors of cells per amount of labeled compound incorporated (Fuhrman and Azam, 1982). There have since been several estimates of these conversion factors which in most studies converge on $1.1–1.8 \times 10^{18}$ cells mol^{-1} of total thymidine and $100–150 \times 10^{18}$ cells mol^{-1} leucine (Coveney and Wetzel, 1988; Ducklow et al., 1992; Fuhrman and Azam, 1982; Kirchman, 1992; Kirchman and Ducklow, 1993; Kirchman et al., 1982; Pulido-Villena and Reche, 2003; Simon and Azam, 1989), with similar estimates of bacterial production using both approaches (Fuhrman and Azam, 1982; Fuhrman and Noble, 1995; Kirchman, 1992; Simon and Azam, 1989), which have been extensively reviewed elsewhere (Ducklow, 2000).

3.2. Distribution of bacterial secondary production

Bacterial secondary production is dependent upon supply of dissolved organic carbon (Carlson and Ducklow, 1996) or inorganic nutrients (Kirchman, 1994; Kirchman and Rich, 1997) and is controlled in part by temperature (Shiah and Ducklow, 1994). It is not surprising therefore that the most productive bacterioplankton communities occur in subtropical and temperate estuaries, where warm temperatures and high allochthonous DOC inputs sustain high bacterial growth rates (Table 25.2). However, temporal changes in bacterioplankton production are tightly correlated to primary productivity such as in the Ross Sea and Southern Ocean, which are highly seasonal (Ducklow et al., 2001) and where consistently low temperature is not an overwhelming controlling factor. Other ''hotspots'' of bacterioplankton activity occur in oxic/anoxic boundaries and hydrothermal vents. Oxic/anoxic boundaries occur in only a few locations in the water column (e.g., Black Sea, Cariaco Trench, Arabian Sea, Eastern Tropical Pacific), but are biogeochemically critical regions for water column denitrification and processes such as anammox (Dalsgaard et al., 2003; Devol, 2003), and also occur within virtually all sediments. Within hydrothermal vent fluid, most frequently found in the bathyl ocean,

Table 25.2 Examples of bacterioplankton production and growth rates in surface waters of various environments ranging in temperature and nutrient concentrations. Production rates were converted using published data and conversion factors of 2×10^{18} cells mol^{-1} TdR (Fuhrman and Azam, 1982), 1.5×10^{17} cells mol^{-1} leu (Simon and Azam, 1989), and 20 fg C cell^{-1} (Lee and Fuhrman, 1987; Troussellier et al., 1997)

Habitat	Location	Bact. abund. ($\times 10^8$ cells l^{-1})	Bact. prod. ($\times 10^5$ cells l^{-1} day^{-1})	Ref.
Arctic	Laptev Sea	1.8–20.0[a]	0.2–64.0	Saliot et al., (1996)
Antarctic	Ross Sea (summer)	30–50[b,c]	31.8–84.8	Ducklow et al., 2001
	Ross Sea (spring)	2.0[a]	2.4–3.5	Ducklow et al., 2001
	Southern Ocean	3.0–3.6[a]	300–3400	Church et al., (2000)
Oligotrophic	Equatorial Pacific	6.0–8.0[c]	12.5–18.3	Ducklow et al., (1995)
	East China Sea	0.76–13.5[d]	6–53	Shiah et al., (2003)
Temperate	North Atlantic	7.46–26.7[a]	46.7–146	Ducklow et al., 2002
	North Atlantic	2.02–2.30[a]	556–2220	Teria et al., (2003)
Estuaries	Senegal River	27.0–810.0[a]	440–9480	Troussellier et al., (2004)
	Lena River	2.0–20.0[a]	14.8–156.0	Saliot et al., (1996)
	Chesapeake Bay	21.5–101.0[a]	846–3150	Shiah and Ducklow, (1995)

[a] ^{3}H-TdR.
[b] Estimated for upper 50 m of the water column.
[c] Measured by both ^{3}H-TdR and ^{3}H-leu
[d] Assuming euphotic zone depth of 20 m.

prokaryotic abundances are enhanced 2–3-fold over surrounding bathypelagic waters (Juniper et al., 1998) and importantly, prokaryotes dominate food web processes in this habitat type.

Deep (meso-, bathy-, and abyssopelagic) bacterioplankton communities turn over more slowly than epipelagic communities, dramatically demonstrated during accidental sinkings of the submersible *Alvin* and cruise liner *Titanic*. In these cases, strong preservation of food materials (Jannasch et al., 1970), and structural materials (Holden, 1985), respectively, suggested that bacteria in deep water environments are slow to metabolise. Recent study of bathypelagic assemblages in the Northwest Pacific showed turnover of bacterial C every 1–30 years, compared to bacteria in surface waters which turned over every 10–20 days (Nagata et al., 2000). Similar

deep-water results have been demonstrated in the North Atlantic Ocean (Hansell and Ducklow, 2003). Deep water bacterioplankton communities rely upon a patchy rain of POC from overlying waters for growth requirements (Cho and Azam, 1988; Hansell and Ducklow, 2003).

4. Viral Infection and Mortality

Viral activities in the marine environment have been observed for decades, primarily by isolation of lytic bacteriophage (Arloing and Sempe, 1926; Asheshova, 1926; Fejgin, 1926; Kakimoto and Nagatomi, 1971; Kriss and Rukina, 1947; Kriss et al., 1967; Spencer, 1955, 1960) and by observation of virus particles internalized within phytoplankton cells (Pienaar, 1976). However viruses were not considered important in marine ecosystems because of their low observed abundance by plaque titer (Moebus, 1980), which was partially due to low culturability of host bacteria (Giovannoni et al., 1990). The first attempt to directly observe the assemblage of viruses in marine plankton noted $<10^4$ particles ml^{-1}, and apparently greatly under-estimated their abundance because most viruses passed through the 0.2 μm filters used in that study (Torella and Morita, 1979). Further development of staining methods and protocols for concentrating viruses from seawater onto electron microscope grids enabled researchers to view an astonishing abundance of viruses in seawater samples from both open-ocean locations (Proctor and Fuhrman, 1990; Proctor et al., 1988) and coastal (Bergh et al., 1989; Borsheim et al., 1990) waters. It became clear that not only were viruses abundant in marine ecosystems, but they may cause significant mortality of marine microorganisms (Proctor and Fuhrman, 1990, 1992; Suttle et al., 1990a,b) and therefore may play significant roles in biogeochemical cycles. Key aspects of marine viral ecology are reviewed here, however readers are also pointed to recent reviews (Fuhrman, 1999; Fuhrman and Suttle, 1993; Weinbauer, 2004; Wommack and Colwell, 2000) for more comprehensive information.

4.1. Virus abundance in marine waters and sediments

The abundance of viruses in the marine environment has been investigated using multiple approaches, initially by plaque-titer on specific hosts (Spencer, 1960), by electron microscopy (Bergh et al., 1989; Borsheim et al., 1990; Proctor and Fuhrman, 1990; Proctor et al., 1988; Torella and Morita, 1979), nucleic acid staining (e.g., DAPI (Hara et al., 1991), YoPro (Xenopoulos and Bird, 1997), SYBR Green I (Noble and Fuhrman, 1998), and SYBR Gold (Chen et al., 2001)) and epifluorescence microscopy, and by flow-cytometry (Chen et al., 2001; Marie et al., 1999). There have been numerous studies inter-calibrating different staining and microscopic techniques (Hennes and Suttle, 1995; Noble and Fuhrman, 1998; Proctor, 1997; Suttle, 1993; Weinbauer and Suttle, 1997). Virus-sized particle concentrations range from 10^5 to 10^8 VLP ml^{-1} in surface waters (reviewed in Wommack and Colwell, 2000)

however are typically 1–2 orders of magnitude less abundant in sub-euphotic zone (Hara *et al.*, 1991, 1996; Noble and Fuhrman, 1998) and hydrothermal vent plume waters (Juniper *et al.*, 1998). In estuaries, virus abundance can exceed 10^8 VLP ml^{-1} (Hewson *et al.*, 2001b; Wommack *et al.*, 1999a), representing an intermediate of abundance in completely fresh (10^8–10^9 VLP ml^{-1}) (Leff *et al.*, 1999; Maranger and Bird, 1995) and completely marine waters (10^6–10^7 VLP ml^{-1}) (Bergh *et al.*, 1989; Proctor *et al.*, 1988; Wommack and Colwell, 2000). The ratio of virus to bacteria abundance in marine waters ranges from 1 to 100, however is typically 10–30 in most pelagic and deep environments (Fuhrman, 2000; Weinbauer, 2004; Wommack and Colwell, 2000). These ratios are similar to the number of viruses produced per marine bacterium upon lysis (the ''burst size'') which ranges from 5 to 200, but is more commonly reported within the range of 10–50 (Wommack and Colwell, 2000). It is important to note that TEM and epifluorescence counts of viruses may underestimate viral abundances due to fast viral decay within preserved samples (Wen *et al.*, 2004), which may in turn affect the ratios of viruses to bacteria, since preserved bacteria decay less rapidly (Gundersen *et al.*, 1996).

By contrast, the observation of high abundance of viruses in sediments has received considerably less attention than the water column, possibly due to the difficulty of separating viruses from other particulate matter (Fig. 25.3). Initial reports of very high virus abundance in benthos, up to 10^9–10^{10} VLP cm^{-3} (Maranger and Bird, 1996; Paul *et al.*, 1993) suggested that viruses could be important components in sediment microbial food webs, however their origin was unknown. Empirical studies in a range of environments including high suspended matter–containing harbour waters demonstrated that the arrival of adsorbed viruses on sinking particles could only account for a tiny fraction of observed abundance, and that most viruses are produced autochthonously (Hewson and Fuhrman, 2003). The ratio of viruses to bacteria in marine sediments is highly variable depending on study location. Surface sediments in estuarine and coastal sediments have been
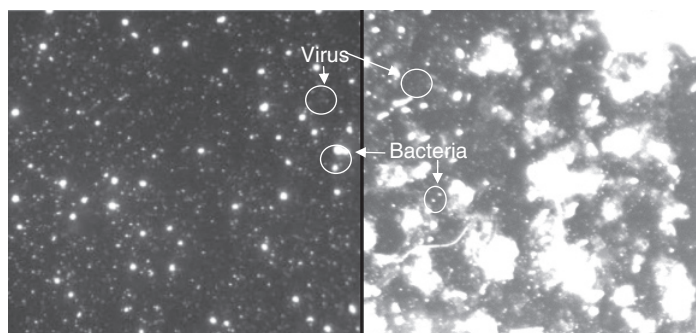
Figure 25.3 Viruses, bacteria, and other microorganisms in plankton (left) from Southern California and from sediments (right) from Florida Bay. Note bacterial morphological diversity is greater in sediments than in the water column but viruses and bacteria are harder to distinguish from detritus.

reported to contain a wide range of virus to bacteria ratios (0.2–71). Some studies have found that the virus-to-bacteria ratios in sediments are significantly higher than in overlying waters (Hewson and Fuhrman, 2003; Hewson et al., 2001a,b,c), while others have found lower benthic virus:bacteria ratios than in overlying waters (Danovaro and Serresi, 2000; Danovaro et al., 2001, 2002), but most studies converge on ratios of 1–7 in sandy marine environments (Danovaro et al., 2001; Drake et al., 1998; Hewson and Fuhrman, 2003; Hewson et al., 2001a,b,c, 2003; Mei and Danovaro, 2004; Middelboe et al., 2003). Similarly, bathyl and abyssal sediments also contain a range of virus-to-bacteria ratios (0.1–5; most reports 0.8–2 (Danovaro and Serresi, 2000; Danovaro et al., 2002; Hewson and Fuhrman, 2003)). The great variability in virus-to-bacterium ratios in marine sediments suggests that local geochemical features (i.e., sediment type) or biological fluxes (e.g., sedimentation) and processes (e.g., bacterial production) may have a strong impact upon the persistence of viruses in different regions.

The geochemical composition of viruses in marine environments is most similar to bacteria hosts, however there are important differences. Viruses are composed of protein and nucleic acids (DNA or RNA) in an approximate 1:1 ratio (Ackermann and DuBow, 1987). Viruses contain C, N, and P in the calculated ratio 10:4.5:1 (Hewson and Fuhrman, 2003). Since viruses are extremely small (size range ca. 15–300 nm but typical diameter of 40–50 nm (Steward et al., 1996b; Suttle et al., 1991)) and contain only small amounts of nucleic acids (15–300 kbp (Fuhrman et al., 2002; Wommack et al., 1999a,b)), they are not believed to comprise a large fraction of DOM in the ocean (Fuhrman, 1999). Using a bacterial conversion factor of 350 fg C μm^{-3} (Lee and Fuhrman, 1987), and average diameter of virus of 50 nm, each virus particle probably only contains 0.2 fg C, hence with an average abundance of 10^9 VLP l^{-1} (Wommack and Colwell, 2000) they only comprise approximately 15 nM DOC and 6 nM DON out of typical DOC concentrations of 40–60 μM (le and Williams, 2000) and DON concentrations of 3–20 μM (Capone, 2000). Although they are not a large component of DOC themselves, their effects on bacterial communities by killing bacteria and thereby releasing their dissolved and particulate intracellular components is potentially a significant component of marine elemental cycling.

4.2. Rates of viral mortality and nitrogen release

Viruses have three potential lifestyles, two of which have been observed in the marine environment (Fuhrman and Suttle, 1993): (1) Lytic viruses cause cell lysis shortly after successful infection, typically about one generation after infection (Proctor et al., 1993); (2) Lysogeny results when temperate phage successfully infect cells, but the viral DNA becomes incorporated into the genome as a ''prophage'' and does not enter the lytic cycle until stimulated to do so by either internal or external factors (discussed in later section) (Jiang and Paul, 1994, 1996; Weinbauer and Suttle, 1996). The third lifestyle, chronic infection, is like lysogeny except live cells extrude viruses over many generations and virus release is sub-lethal (Fuhrman and Suttle, 1993).

Lytic viral mortality of bacteria in marine systems has been studied with 3 basic approaches; (1) by examining the percentage of visually infected bacterial cells,

(2) by examining rates of viral decay, or (3) by examining rates of viral production. The percentage of visually infected bacteria is determined by staining bacteria with a heavy metal stain (typically uranyl sulfate) (Proctor and Fuhrman, 1990, 1991; Proctor et al., 1993; Weinbauer and Suttle, 1996), then observation of virus particles within cells by transmission electron microscopy. Using assumptions about the relative percentage of time during which infected bacteria can be recognized (by the presence of electron–dense shading within bacterial cells), it is possible to calculate the percentage of infected cells (Proctor and Fuhrman, 1990). Since viral abundance in pelagic marine waters is presumably stable over long time scales, viral decay must approximate production and can therefore be used to estimate it. Viral decay is measured primarily by three approaches. By treating samples with compounds that kill bacteria, but do not effect enzyme activities (e.g., KCN), production of viruses is stopped; The rate of decrease in virus abundance over time is equivalent to the production rate (Heldal and Bratbak, 1991; Suttle and Chen, 1992). In the second approach, in which a subset of virioplankton is stained with the nucleic acid fluorochrome SYBR Green I, the disappearance of fluorescently labeled virus (FLV) can be used to estimate viral decay in an approach similar to isotope dilution (Noble and Fuhrman, 2000). A third approach to assess viral decay in natural waters is to add a large titer of cultured bacteriophage to seawater, then to determine abundances of these over time (Wommack et al., 1996). To directly estimate the production of viruses, two approaches are used. The first method is to determine the accumulation rate of radiolabeled (with ^{3}H or ^{33}P), DNAse resistant particles into the 0.02–0.2 μm size fraction (Steward et al., 1992, 1996a,b). More recently, production has been determined by a dilution technique where virioplankton is mostly removed from bacteria by dia- or tangential–flow filtration and virus abundance increase monitored by microscopy (Hewson et al., 2001b; Wilhelm and Suttle, 1999, 2000; Wilhelm et al., 2002). In production estimates, mortality is calculated with a conversion factor which includes estimates of burst size to estimate the number of bacteria that need to be lysed to maintain the observed production rate.

Reports of lytic viral mortality in surface waters of the ocean average near 20–25% (range 4–74%) of mortality (reviewed in Weinbauer, 2004; Wommack and Colwell, 2000), which in some environments is similar to the proportion of mortality attributed to protists (Fuhrman and Noble, 1995). The large range in viral mortality is due mainly to the range of environmental conditions in which virus infection has been measured, but also due to variability in estimates of host burst size, which can effect mortality rate calculations by over an order of magnitude (Wommack and Colwell, 2000). Viral lysis results in the release of intracellular compounds and, of course, viruses into surrounding waters. Assuming a generous burst size for a small marine bacterium of approximately 50 VLP cell^{-1}, then most cellular C and N is released into surrounding waters upon lysis. Assuming viral lysis results in 2–20% of bacterioplankton mortality per day (Proctor and Fuhrman, 1990), viral lysis could release 4–40 nM N day^{-1} into DOM (calculated). It is interesting to note that lytic virus infection may have diel variability (Winter et al., 2004a,b), with most initial virus infection occurring at night and lysis at noon or afternoon.

Cyanophage of filamentous cyanobacteria (Hewson et al., 2001a, 2004; Ohki, 1999; Ohki and Fujita, 1996) and marine representatives of the unicellular

phytoplankton genera *Prochlorococcus* and *Synechococcus* (Sullivan *et al.*, 2003; Suttle, 2000; Suttle and Chan, 1993, 1994; Waterbury and Valois, 1993; Wilson *et al.*, 1993) have been isolated and observed for some time. Both are of particular interest due to their role in primary production and global carbon sequestration. Lytic cyanophage are believed to cause only modest mortality of *Synechococcus* ($5–10\%$ d^{-1}) in oligotrophic waters, less in temperate waters (Fuhrman, 1999; Suttle and Chan, 1994; Waterbury and Valois, 1993) and most culturable strains of this genus have been reported to be resistant to co-occurring cyanophage (Sullivan *et al.*, 2003; Waterbury and Valois, 1993). This suggests minimal impact of viruses upon total abundance of *Synechococcus*. Recent study has demonstrated that cyanophage infecting *Prochlorococcus* have a range in host specificities, with some cyanophage infecting only a single strain of cyanobacterium, while others infect both *Prochlorococcus* (both high- and low-light adapted strains) and *Synechococcus* (Sullivan *et al.*, 2003). Lysogeny in marine *Synechococcus* may be an important source of mortality (McDaniel *et al.*, 2002; Williamson *et al.*, 2002), but the stimuli causing prophage to enter the lytic cycle remain unclear.

Viral pathogens of eukaryotic phytoplankton have been observed for some time (Pienaar, 1976), and now almost every group of phytoplankton in marine waters has been demonstrated to be affected by viral lysis (Brussaard, 2004; Brussaard *et al.*, 1999; Nagasaki *et al.*, 1994, 2004; O'Kelly *et al.*, 2003; Suttle *et al.*, 1990a,b, 1991; Tarutani *et al.*, 2000; VanEtten *et al.*, 1991). Despite this, there is little information on the rate of viral mortality that can be attributed to "phycoviruses" under ambient conditions, because it is difficult to distinguish eukaryotic viruses from bacteriophages in indirect observation techniques. Direct observation of cells of *Micromonas pusilla* indicate that 2–10% of cells lyse per day under ambient conditions (Cottrell and Suttle, 1995), while observation of *Emiliana huxleyii*, *Ostreococcus taurii*, and *Aureococcus anophagefferens* indicate that viral mortality upon bloom decline could be up to 80–100% (Bratbak *et al.*, 1993; Milligan and Cosper, 1994; O'Kelly *et al.*, 2003). Clearly, viruses have significant impacts upon eukaryotic phytoplankton, which may cause substantial N release upon lysis.

To date, the only study attempting to examine viral mortality of metazoan zooplankton found no strong effects of concentrated viral communities upon copepod physiology or fecundity (Drake and Dobbs, 2005), but viruses of prawns (Decapoda) have been known for some time (Bonami *et al.*, 1990; Tsai *et al.*, 2000). Recent discovery of a diverse array of RNA Picorna virus-like sequences in surface waters of the Georgia Strait and north of Oahu suggests that zooplankton viruses may proliferate in marine waters (Culley *et al.*, 2003; Culley and Steward, 2007). Picorna-like viruses, which are within the same family as those infecting prawns could conceivably also infect dominant microcrustacean zooplankton in the ocean (e.g., Copepods, Amphipods). Clearly this area remains a potentially significant research direction for future studies.

4.3. Lysogeny and horizontal gene transfer

Lysogeny, the process by which temperate viral DNA becomes integrated into host genomes could be a major source of mortality for marine microorganisms if induction of lysogens is significant. However, the stimuli causing induction are not well understood. There have been detailed studies of natural factors impacting lysogens in

single-species cultures. Bioegeochemically, it is difficult to assess the potential impact of lysogens on nutrient cycling, since methods of detection (e.g., addition of strong mutagens or UV irradiation to induce lytic activity) are unrealistic of natural marine stimuli (Jiang and Paul, 1994; Weinbauer and Suttle, 1996). Addition of mitomycin C, which is the only inducing agent among cultures of prokaryotes consistently causing increases in virus abundance in incubations (Wommack and Colwell, 2000), has revealed a variable percentage of lysogens, from 0 to 27% (most studies fall within the range of 3–10%) of all bacterioplankton in oceanic surface waters (Jiang and Paul, 1994, 1996; Ortman *et al.*, 2002; Weinbauer and Suttle, 1996, 1999; Weinbauer *et al.*, 2002, 2003), 0–79% (most studies 2–38%) in estuarine waters (Cochran and Paul, 1998; Jiang and Paul, 1996; McDaniel *et al.*, 2002; Weinbauer *et al.*, 2003) and 16–84% in sub-euphotic waters (Weinbauer *et al.*, 2003). The abundance of lysogens has been demonstrated to have a seasonal cycle, with greatest occurrence in months of lowest bacterial and phytoplankton production, suggesting that this lifestyle was selected for when host densities were low and least active (Cochran and Paul, 1998; McDaniel *et al.*, 2002; Williamson *et al.*, 2002).

Another aspect of lysogeny of interest to biogeochemists is the role of viruses in gene transfer (i.e., transduction). Lysogeny is believed to cause significant exchange of host materials and introduce new genes into hosts (Chiura, 1997; Jiang and Paul, 1998; Paul *et al.*, 2002). For example, lysogenic conversion of *Vibrio cholerae* and possibly the cyanobacterium *Microcystis aeruginosa* cause hosts to produce toxins (Vance, 1977; Waldor and Mekalanos, 1996). Recent observations of genes encoding for phosphate metabolism (*pho*) and for the production of proteins essential for photosynthetic apparatus (D1 proteins) have been observed in phage infecting *Roseobacter* SI01 (Ritalic,*et al.* 2000) and *Prochlorococcus* (Lindell *et al.*, 2004), respectively (reviewed in Paul *et al.*, 2002). Observation of environmental viral metagenomes has indicated that the a large numbers of viral DNA open reading frames (ORFs) that were affiliated with phage encode for unknown functions (Breitbart *et al.*, 2002, 2004), however roughly half of ORFs were similar to Bacterial, Archaeal, and eukaryotic sequences in GenBank (National Center for Biotechnology Information's sequence database). While to date no sequences similar to key nitrogen cycling genes have been recovered, it is likely that within the unknown fraction of viral ORFs there are genes that are involved in nitrogen processing.

5. Marine Sediments Compared to the Water Column

Marine sediments are an important compartment for N cycling. Shelf sediments in particular are sites of active denitrification (Codispoti *et al.*, 2001), and estuarine, coastal, and coral reef sediments can be important sites of nitrogen fixation (Burns *et al.*, 2002; Capone, 1983; Capone *et al.*, 1992). Despite extensive study of biogeochemical processes in sediments, the study of the microbial loop in this compartment has had considerably less attention, possibly due to difficulty in studying sediment microorganisms. There are two primary differences between water column and sediment microbial processes. The first of these is the presence

of an abiotic matrix of sediment material which limits diffusion and onto which charged particles and solutes may absorb. The second primary difference is that sediments are the sink for both autochthonous and allochthonous organic matter from overlying waters (especially within estuaries), and thus, unlike the water column, are extremely rich environments. In this section, we review current knowledge of the sediment microbial loop, and compare rates of loop processes with the water column.

5.1. Bacterial production in sediments

Sediments are often extremely nutrient rich environments, hence it is not surprising that bacterial abundances there are typically an order of magnitude more than in overlying waters per unit volume of porewater (Bird et al., 2001; Danovaro and Serresi, 2000; Drake et al., 1998; Hewson et al., 2001a,b,c; McDaniel and Capone, 1985), nor is it surprising that benthic microalgal biomass exceeds phytoplankton biomass in shallow-water benthic environments (e.g., coral reefs) by over an order of magnitude (Heil et al., 2004; MacIntyre et al., 1996). The method used to measure bacterial production in sediments is similar to that in the water column, that is, by incorporation of radiolabeled thymidine or leucine (Fuhrman and Azam, 1982; Simon and Azam, 1989), then extraction of sediment macromolecules and determination of radioactivity (van Duyl and Kop, 1994).

Benthic bacterial production rates cover a wide range of activity from 0.1 to 600 mg C m^{-2} day^{-1}, representing approximate growth rates of 0.1–5.1 day^{-1} (Alongi, 1994; Dixon and Turley, 2001; Epstein, 1997; Findlay, 1995; Hamels et al., 2001; Hietanen et al., 1999; Hondeveld et al., 1995; Mei and Danovaro, 2004; Nodder et al., 2003; Poremba and Hoppe, 1995; Sander and Kalff, 1993; Sinsabaugh and vanDuyl et al., 1997; Turley and Dixon, 2002; Vanduyl et al., 1993). Bacterial production is related to sediment grain size, with coarser sediments being less productive than silty or muddy sediments (Hamels et al., 2001). The supply of organic matter from overlying waters is also related to sediment bacterial production, with significant correlations between suspended particulate organic carbon and production rates in deep sea sediments (Hietanen et al., 1999; Nodder et al., 2003; Suits and Arthur, 2000; Turley and Dixon, 2002), however lag times between inputs and stimulation of bacterial production have been noted under algal blooms (vanDuyl et al., 1997). Through multiple correlation analysis, temperature and sediment chlorophyll-a levels have also been demonstrated to explain variability in sediment bacterial production rates (Sander and Kalff, 1993). Since sediments also contain large quantities of dissolved and particulate organic N (Guldberg et al., 2002; Middelboe et al., 2003), bacterial hydrolysis of these compounds probably plays a large role in their decomposition in marine systems.

5.2. Grazing, bacterivory, and viral lysis in marine sediments

Bacterial production in planktonic marine environments is believed to be tightly controlled by a combination of bacterivory and viral lysis (Proctor and Fuhrman, 1990; Sherr and Sherr, 1994). While it is tempting to apply these loss factors to

models of benthic marine food webs (Epstein, 1997), several studies have demonstrated that protozoan grazing in sediments is insufficient to account for benthic bacterial production (reviewed in Hamels *et al.*, 2001; Kemp, 1990). However, meiofaunal grazing has been demonstrated to remove significant proportions of daily bacterial and benthic microalgal production (Epstein, 1997; Montagna, 1995), and grazing by protozoa has been observed to remove roughly half of bacterial production per day in intertidal sand flats (Epstein, 1997; Hamels *et al.*, 2001; Hondeveld *et al.*, 1995). Grazing by larger fauna, such as deposit feeding Holothurians has been demonstrated to significantly increase sediment bacterial productivity (Moriarty *et al.*, 1985).

In shallow water environments, benthic microalgae make up a large component of sediment autotrophic production (Heil *et al.*, 2004; Kendrick *et al.*, 1998; Thornton *et al.*, 2002). Their high biomass overlying anoxic sediments has profound impacts upon denitrification efficiency in benthic compartments, since photosynthesis deepens the oxic layer depth, while depleting NO_3^- (Dong *et al.*, 2000). Removal of benthic microalgae has been demonstrated to result from meiofaunal and macrofaunal grazing (Epstein, 1997; Moriarty *et al.*, 1985), although attempts to simulate enhanced viral lysis of benthic microalgae *in situ* resulted in only modest effects on bacterial assemblage composition and protistan photosynthetic rate (Hewson *et al.*, 2001c).

Viral lysis in sediments has received only recent attention as a source of mortality of bacteria (Hewson and Fuhrman, 2002, 2003; Mei and Danovaro, 2004; Middelboe *et al.*, 2003) and has been shown to kill up to 14% of bacterial biomass per hour in highly productive sediments, accounting for 9–57% of total sediment bacterial mortality (Mei and Danovaro, 2004). Lysogeny investigated by mitomycin C addition has been reported to account for only 0.1–1.8% of all bacteria (Mei and Danovaro, 2004) across a range of sediment types. This is despite reports of a high number of ORFs from sediment metagenomes belonging to the *Siphophage* family, many members of which are temperate (i.e., causing lysogeny (Breitbart *et al.*, 2004)). The limited data suggest that unlike in the water column, lysogeny may be of only limited importance in this compartment. An alternative explanation is that it is difficult to induce sediment lysogens with mitomycin C.

A combination of bacterivory and lytic viral activity appears to account for roughly similar amounts of bacterial biomass loss in marine sediments, which is comparable to the dynamics in overlying waters (Strom, 2000). However, rates of bacterivory and viral lysis are strongly influenced by sediment type and organic matter loading (Hamels *et al.*, 2001; Hewson and Fuhrman, 2003), which is ultimately linked to local geography and productivity of overlying waters.

6. FUTURE DIRECTIONS OF MICROBIAL LOOP RESEARCH

This chapter has reviewed several key aspects of the marine microbial loop that have become clearer in the past 20 years. Many of the developments in our understanding have come about thanks to novel molecular techniques and advances in microscopy. While conceptualised models of the microbial loop have changed dramatically since first conceived (Ducklow, 1983; le and Williams, 1981; Pomeroy,

1974) and formalised (Azam *et al.*, 1983), to include new pathways and processes, there is still a large "black box" of information yet to be fully understood. In the following section we briefly preview four promising areas of future research on microbial loop processes, however we note that there are undoubtedly many others to come.

6.1. Metagenomics and metatranscriptomics

The desire to discover novel genes in the marine environment has given rise to whole genome shotgun sequencing of viral and prokaryotic communities, whereby entire, mixed community genomic DNA is fractured into millions of small fragments, then sequenced and attempts made to reconstruct individual genomes. This has already begun to provide interesting information on the organisms intrinsic to the microbial loop (Breitbart *et al.*, 2002, 2004; Venter *et al.*, 2004; Rusch *et al.*, 2007 and DeLong *et al.*, 2006). This approach is an extension of early work (Olsen *et al.*, 1986; Pace *et al.*, 1986; Schmidt *et al.*, 1991) which sheared whole community DNA, then used bacteriophage λ, containing small pieces of this sheared extract, as a library. The technique was the precursor for the approach known today as BAC library analysis (De Long, 2002, DeLong *et al.*, 2006), and was the first entire-metagenome attempt to understand diversity in marine bacterioplankton. The sequencing of entire environmental metagenomes is related to complete sequencing of cultivated marine viral (Rohwer *et al.*, 2000), bacterial (Heidelberg *et al.*, 2000), cyanobacterial (Palenik *et al.*, 2003; Rocap *et al.*, 2003), and eukaryotic genomes (Armbrust *et al.*, 2004). Annotation of single genomes and metagenomes has provided information on unexpected and previously unknown metabolic pathways (Fuhrman, 2003), such as phosphonate utilisation in *Synechococcus* (Palenik *et al.*, 2003). One of the most exciting discoveries made using BAC or metagenomic approaches has been observation of genes encoding for bacterial rhodopsins, a suite of retinal-containing pigments which serve a variety of potential functions (Fuhrman *et al.*, 2008), including the generation of energy from sun energy (i.e., phototrophy) (Beja *et al.*, 2000), or regulatory (i.e., sensory) functions (Wang *et al.*, 2003). Discovery of these genes, and observation of a small percentage of bacteria bearing the photosynthetic pigment bacteriochlorophyll-*a* (Kolber *et al.*, 2001) have suggested that phototrophy may be more widespread and not as restricted to chlorophyll-*a* than previously thought. Flow cytometric sorting, whole genome amplification, and pyrosequencing technologies promise to provide complete genome sequences from uncultivated microorganisms important in the N cycle. Metatranscriptomics, the study of community gene expression, also promises to highlight dominant metabolic processes which may be under-represented in genomic surveys (Poretsky et al., 2005). The recognition of genes from cultured organisms within environmental metagenomes will potentially elucidate new N–cycling pathways which have not been considered in previous models of biogeochemical cycling, however recognition of the function of such genes is only possible if metagenomic studies are coupled with culture studies.

6.2. Culturing previously unculturable microorganisms

Study of metabolic capabilities of numerically dominant components of marine ecosystems have been hampered by low culturability of these taxa. Early work showed that bacteria could be serially diluted and grown in filtered seawater in lab

(Ammerman *et al.*, 1984; Fuhrman and Azam, 1980). Recent extensions of this culturing theory have enabled labs to develop nontraditional approaches to isolating marine microorganisms, for example extinction—dilution (Button *et al.*, 1993; Connon and Giovannoni, 2002; Rappé *et al.*, 2002), and isolation of single cells away from colonies on enriched media (Simu and Hagstrom, 2004). These methods have allowed culture of the ubiquitous and highly abundant SAR-11 clade of marine bacterioplankton (Rappé *et al.*, 2002; Simu and Hagstrom, 2004), however it should be noted that these cultures are not easy to grow or study. The isolation of such organisms, and studies of cultured organism genomics, will open doors on both physiological studies and interactions with other marine microbes in the microbial loop. The axiom that >90% of bacterioplankton taxa are not culturable may be partially a consequence of dependence upon enrichment for isolation (Giovannoni and Rappe, 2000), and ultimately understanding of dominant prokaryotes in the environment may progress when novel techniques such as those applied to SAR-11 cultures are applied on a massive scale.

6.3. Viral and autocatalysed mortality of key nitrogen-processing organisms

Virus infection of bacteria involved in nitrogen processing has been observed for decades (Adolph and Haselkorn, 1973; Khudyakov, 1977; Mendzhul *et al.*, 1991; Safferman *et al.*, 1972), however it is only recently that the implicated in releasing fixed nitrogen (Hewson *et al.*, 2004; Ohki, 1999; Ohki and Fujita, 1996; Suttle, 2000). Programmed cell death, a form of autocatalysed mortality, has also received little study in marine microbial ecology, probably due to difficulty in distinguishing this process from induction of lysogens (Bidle and Falkowski, 2004), and is believed to be restricted to colonial prokaryotes or eukaryotes. Recent observation of viral and autocatalytic lysis of *Trichodesmium* cultures (Berman-Frank *et al.*, 2004; Ohki, 1999) and large estimates of viral mortality under *in situ* conditions (Hewson *et al.*, 2004), suggest that viruses play underappreciated roles in controlling abundance and N fluxes from important N-cycling microorganisms. Observation of high rates of viral lysis within sediments (reviewed in Mei and Danovaro, 2004), for example, raises interesting questions about the role of viral lysis in regulating processes mostly carried out in the benthic domain (e.g., denitrification, nitrification). Moreover, viral activities have been hypothesized to be important in determining the taxonomic and clonal composition of marine bacterial assemblages (Thingstad, 2000; Thingstad and Lignell, 1997), by killing the winner of resource competition and thereby maintaining diversity. Experimental approaches to addressing this interaction have provided important results on the effects of viruses on total bacterial and eukaryotic assemblage composition and diversity (Hewson *et al.*, 2001c, 2003; Schwalbach *et al.*, 2004; Winter *et al.*, 2004b), but there have been no reports to date of virus effects on composition of nitrogen cycling guilds of bacteria. Since apoptotic cell death is unlikely to occur in unicellular bacterioplankton or sediment bacteria, it is unlikely that this process affects greatly nitrogen cycling in the microbial loop.

6.4. Nitrogen fixing heterotrophic bacteria

Nitrogen fixation was until recently believed to be dominated by large, filamentous cyanobacteria, such as *Trichodesmium* and *Kategynemenae* (reviewed in Capone *et al.*, 1997). It was not until DNA and RNA-based PCR techniques were applied to the <20 μm size fraction in oligotrophic seawater that nitrogenase activity by unicellular cyanobacteria was detected (Zehr *et al.*, 1998, 2001). Since initial descriptions, these cyanobacteria have been found to be highly active and have nitrogenase activity rates similar to larger diazotrophs per unit sea surface area (Falcon *et al.*, 2004; Zehr *et al.*, 2001; Montoya *et al.,* 2004). In addition to autotrophic cyanobacteria, heterotroph nitrogenase sequences have also been recovered as genes and gene transcripts from bacterioplankton in both Atlantic and Pacific oceans (Falcon *et al.*, 2002; Zehr *et al.*, 1998, 2001; Church *et al.,* 2005a,b), suggesting that heterotrophic bacteria may also contribute to nitrogen fixation in open ocean pelagic environments (Fuhrman and Capone, 2001; Zehr *et al.*, 2001). Extensive study of the distribution of nitrogenase genes in the Chesapeake Bay has demonstrated that heterotrophic diazotrophs have variable distribution throughout the estuary and with season (Short *et al.*, 2004) and at least 2 phylotypes actively express their nitrogenase genes (Short and Zehr, 2007). Future studies using microarray technology promise to elucidate the diversity of non-cyanobacterial diazotrophs in open-ocean conditions (Taroncher-Oldenburg *et al.*, 2003; Hewson *et al.,* 2007). Combined with quantitative molecular tools (e.g., quantitative PCR) (Short *et al.*, 2004) and stable isotope incorporation (Montoya *et al.*, 2004; Zehr *et al.*, 2001) they should elucidate the role of this previously underappreciated source of N in the microbial loop.

7. CONCLUSION: A NEW MICROBIAL VIEW—THE GENE LOOP

Our understanding of the microbial loop has changed dramatically since formalisation (Azam *et al.*, 1983; Ducklow, 1983). Elucidation of new metabolic pathways, incorporation of viruses (Fuhrman, 1999; Fuhrman and Suttle, 1993; Fuhrman *et al.*, 1992), and a further unfolding of the "black boxes" of both biotic components (i.e., bacteria, viruses, and protists) through molecular tools, and abiotic components (i.e., DOM composition) through advances in chemistry, have made our view of the microbial loop more complex. It has been argued that since genes freely exchange between prokaryotes in the marine environment by transduction and viral infection, the bacteria in the ocean represent a supergenome where diversity among communities has evolved as a consequence of random recombination (Sonea and Panisset, 1983). Recent observation of high levels of microdiversity (Acinas *et al.*, 2004; Brown and Fuhrman, 2005) has demonstrated that evolution of bacterial taxa is not random, but rather individuals within communities undergo selection to enable them to exist within tightly-defined niches (Giovannoni, 2004; Kimura, 1968). Ultimately, the types of organisms existing within communities hypothetically depend upon the physical conditions present, which in turn is a consequence of biological activity. Incorporation of all metabolic pathways into current conceptualisations of the microbial loop results in extremely complicated webs of interactions. This complexity is exacerbated by discovery of metabolic plasticity in a large number of microorganisms (Caron, 2000),

and observations of microdiversity in some closely related bacteria occupying similar, but not exactly the same niche (e.g., ecotypes) (Garcia-Martinez and Rodriguez-Valera, 2000; Rocap *et al.*, 2002). Since the lines between metabolisms, and between different interactions becomes blurred, we must move towards a more continuous idea of the microbial loop in affecting biogeochemistry and ecology in the marine environment.

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Nitrogen Consumption and Metabolism in Marine Zooplankton

Deborah K. Steinberg *and* Grace K. Saba

Contents

1. Introduction

Zooplankton play a pivotal, multi-faceted role in the ocean nitrogen cycle. They consume organic nitrogen and thus exert control on primary production, as well as sustain the growth of phytoplankton via N excretion, thus enhancing primary production (Miller and Landry, 1984). Release of dissolved organic nitrogen (DON) by sloppy feeding or excretion by zooplankton can also support heterotrophic bacterial growth and may be the principal pathway of dissolved organic matter from phytoplankton to bacteria (Carlson, 2002; Jumars *et al.*, 1989). Zooplankton transport particulate organic nitrogen (PON) to depth via production of sinking fecal pellets (Altabet and Small, 1990; Small *et al.*, 1989). Diel vertically migrating zooplankton contribute to vertical export of N by

consuming organic particles in the surface waters at night and metabolizing the ingested food as ammonia, DON, or via production of fecal pellets below the mixed layer during the day (Longhurst and Harrison, 1988; Schnetzer and Steinberg, 2002; Steinberg *et al.*, 2002).

   This chapter will focus on the important role that zooplankton play in nitrogen cycling by ''following the path of the food'' from ingestion to all aspects and stages of metabolism (Fig. 26.1). We begin with a discussion of N consumption, including the nitrogenous composition of available food types, rates of N consumption, and effects of food quality. We then discuss the various aspects of N metabolism, emphasizing the importance to biogeochemical cycling and coupling with other major elements (carbon, phosphorus). The contribution of zooplankton N regeneration to the N budget and requirements for phytoplankton growth will be covered in Bronk and Steinberg (in this volume). Recent methods for measuring zooplankton consumption and metabolism can be found in the *International Council for the Exploration of the Sea (ICES) Zooplankton Methodology Manual* (Harris *et al.*, 2000), thus we do not cover methodology herein. In addition, the analytical procedures for
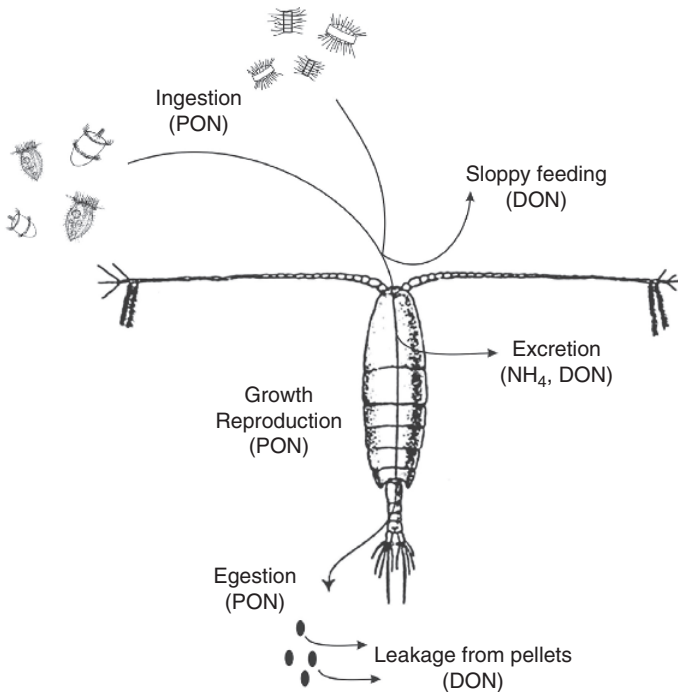
Figure 26.1 Pathways of nitrogen consumption and metabolism by zooplankton discussed in this chapter. Prey (other zooplankton such as the ciliates shown here, or phytoplankton) PON of differing quality and quantity are ingested by zooplankton. Some of the DON in the prey is lost to the surrounding water due to sloppy feeding, while the rest is ingested. A portion of the PON that is ingested is assimilated, or taken up into the organism's tissues, and the rest is egested as fecal pellets which may subsequently leach DON. Of the N that is assimilated, a portion is excreted as ammonia (NH_4) or DON, and the rest goes into the animal's growth and reproduction (= secondary production) (Adapted from Møller *et al.*, 2003).

measuring the products of N metabolism (i.e., various forms of particulate and dissolved N) are described in McCarthy and Bronk (in this volume). There are several helpful prior reviews on N nutrition (Roman, 1983) and metabolism (Bidigare, 1983; Caron and Goldman, 1990; LeBorgne, 1986; Regnault, 1987) which this chapter builds upon. As most previous reviews have focused on crustacean mesozooplankton (>200 μm), we have made an effort to include information on both gelatinous zooplankton and microzooplankton (<200 μm, most of which are protozoans) herein.

2. Consumption of Nitrogen

2.1. Biochemical (nitrogenous) composition of food for zooplankton

2.1.1. Phytoplankton

A significant proportion of ocean primary production is grazed by zooplankton. A recent synthesis of microzooplankton grazing rate estimates indicate microzooplankton graze an average of 60–75% of the daily primary production across a wide range of open-ocean to coastal environments (Calbet and Landry, 2004; Landry and Calbet, 2004), and mesozooplankton are responsible for removing 10–40% of the daily primary production (Calbet, 2001). Phytoplankton N content consists mostly of protein (65–85% of cell N) and amino acids (6–12% of cell N); however, nucleic acids, pigments, and inorganic N also contribute to phytoplankton N content (Geider and La Roche, 2002; Lohrenz and Taylor, 1987; Lourenco et al., 1998). Phytoplankton have a heavy N requirement due to their photosynthetic apparatus protein complexes such as the enzyme ribulose 1,5-biphosphate carboxylase (RUBISCO), and reaction center II protein D1 (Falkowski et al., 1989; Geider et al., 1993). The elemental composition of phytoplankton reflects the changes in these protein macromolecules relative to high carbon carbohydrates and lipids such as those found in the cell wall (Beardall et al., 2001; Goldman et al., 1979). While the Redfield ratio is consistent for non-nutrient limited marine phytoplankton at high growth rates (Geider and La Roche, 2002), phytoplankton C:N, N:P, and C:P ratios can deviate greatly from Redfield due to differences in nutritional and growth conditions. Thus, differences in the N content and chemical composition of phytoplankton depends on the species, as well as a number of factors including growth rate, nutrient limitation, cell size, temperature, and irradiance levels (Hecky et al., 1993; Perry, 1976).

Differences in the nitrogen content between phytoplankton taxa can be due to differential growth rate (Goldman et al., 1979), as well as cell size, shape, structure (e.g., presence of a vacuole), and composition (e.g., amino acid requirements) (Beardall et al., 2001; DeYoe and Suttle, 1994; Quigg et al., 2003). For example, Moal et al. (1987) found that in exponential growth phase, diatoms with vacuoles had the lowest levels of protein, protein:chlorophyll a, and total N content (24–141 fg protein μm^{-3}, prot:chl a = 30–172, 5–26 fg N μm^{-3}, respectively) compared to dinoflagellates absent of vacuoles (43–434 fg protein μm^{-3}, prot:chl a = 222–740, 7–45 fg N μm^{-3}, respectively). Nutrient limitation and the resulting

decrease in growth rate (Fernandez *et al.*, 1996; Sterner and Hessen, 1994) can affect phytoplankton N content via a decrease in internal pools of amino acids, inorganic N storage, and protein—including photosynthetic proteins RUBISCO and D1, and an increase in carbohydrate and lipid storage, thus increasing the C: N (Beardall *et al.*, 2001; Dortch, 1982; Dortch *et al.*, 1985; Falkowski *et al.*, 1989; Geider *et al.*, 1993; Goldman *et al.*, 1979; Harrison *et al.*, 1990; Moal *et al.*, 1987). Moal *et al.* (1987) found that C, N, and protein density in many species of diatoms, dinoflagellates, cryptophytes, and haptophytes can decrease as much as 50% from exponential to stationary phase. The N content of a common brown tide alga, *Aureococcus anophagefferens*, off Texas, USA, varies with different inorganic N sources (DeYoe and Suttle, 1994). *A. anophagefferens* grown on ammonium (NH_4), their preferred N source, had higher N (1.3 pg cell^{-1}), chlorophyll *a* content, and a C:N near Redfield, whereas those grown on nitrate (NO_3) had low N (0.88 pg cell^{-1}) and chlorophyll *a* content, and a high C:N (25:1). Cell size may also decrease with increasing nutrient limitation and effect cellular N content. Verity *et al.* (1992) and Menden-Deuer and Lessard (2000) compared cellular C and N in a variety of phytoplankton taxa and found that in general, small cells have higher C and N densities (per cubic micrometer) than larger cells. For example, among the prymnesiophytes, *Emiliana huxleyi* with a biovolume of 70 μm^3 has a N density of 47 fg μm^{-3} whereas the larger *Pleurochrysis carterae* with a biovolume of 648 μm^3 has a N density of 17 fg μm^{-3} (Verity *et al.*, 1992). Irradiance and temperature may also affect cell N content. At low light more photosynthetic apparatus protein complexes (RUBISCO and D1) are needed, which decreases the C:N and increases the N:P ratios (Beardall *et al.*, 2001; Dortch *et al.*, 1985). At photoinhibitory levels of irradiance, however, proteins decrease (Geider *et al.*, 1996; Geider and La Roche, 2002; Raven, 1984). Berges *et al.* (2002) found that as temperature increases, diatom *Thalassiosira pseudonana* protein, total cellular N, and biovolume remain constant, but cell C and chl increase, which causes an increase in C:N.

Despite their dominance and important ecological role in large open ocean habitats, there are few data on the chemical composition of photosynthetic, nitrogen fixing cyanobacteria. *Prochlorococcus* spp. have N contents ranging from 2.2 to 9.4 fg N cell^{-1} and a C:N ranging from 5.0 to 9.9 in nutrient replete conditions (Bertilsson *et al.*, 2003; Heldal *et al.*, 2003). As with most other phytoplankton species, the C:N increases with increasing nutrient limitation (Bertilsson *et al.*, 2003). *Synechococcus* spp. have higher N contents ranging from 17 to 50 fg N cell^{-1}; however, they have similar C:N and N cell density as *Prochlorococcus* spp. (Bertilsson *et al.*, 2003; Heldal *et al.*, 2003). Nitrogen fixing species have high N:P stoichiometry due to the need for more light harvesting machinery for energy to power the fixation process (Klausmeier *et al.*, 2004). For example, *Trichodesmium* spp. have an N:P of 42–125 (Letelier and Karl, 1996). Only a few studies have determined protein content of cyanobacteria. Vargas *et al.* (1998) found protein makes up 37–52% of the dry weight of 12 strains of filamentous cyanobacteria, and Cuhel and Waterbury (1984) determined the average cellular protein content (96 fg protein cell^{-1}) and C:protein (3.1) of *Synechococcus* Clone 7803.

2.1.2. Bacteria

Heterotrophic bacteria (and autotrophic cyanobacteria, described above) are consumed directly by protozoan microflagellates and ciliates (Azam et al., 1983; Strom, 2000). Although too small to be consumed directly by most mesozooplankton except for the mucous-web feeders (e.g., larvaceans, Deibel and Lee, 1992), bacteria colonizing fecal pellets, marine snow, or other such detritus are indirectly consumed by detritivores (Steinberg, 1995). Heterotrophic bacteria utilize a wide range of dissolved organic N substrates (e.g., amino acids, proteins, urea, nucleic acids) with highly variable biochemical compositions, and can compete with phytoplankton and utilize inorganic N (e.g., ammonium) to meet their N requirements when organic N is limited (Kirchman, 2000). Despite the variable C:N stoichiometry of the substrates they catabolize, bacteria maintain a strict, nutrient-rich stoichiometry with C:N between 4 and 7 (Bratbak, 1985; Fukuda et al., 1998; Goldman et al., 1987; Lee and Fuhrman, 1987; Makino and Cotner, 2004; Nagata, 1986). Natural marine bacteria contain 1.6–35 fg N cell^{-1}, with coastal bacteria having higher C and N contents relative to oceanic bacteria (Fagerbakke et al., 1996; Fukuda et al., 1998; Vrede et al., 2002). Additionally, small cells have higher N densities relative to larger cells in similar environmental conditions (Kroer, 1994). Bacteria are N rich mainly due to their peptidoglycan cell membrane and high growth rates, which reflect high nucleic acid (14.5% N) and high protein (17% N) contents (Simon and Azam, 1989). Bacteria contain between 60 and 330 fg protein cell^{-1} (Zubkov et al., 1999), which can be nearly 63% of a bacteria cell's dry weight (Simon and Azam, 1989). Although bacteria typically maintain a narrow range of C:N, changes in growth rates and available nutrients can alter the C:N. For example, C:N ratio increased from exponential to stationary growth phases in Vibrio natriegens (Fagerbakke et al., 1996; Vadstein and Olsen, 1989) due to a decrease in RNA and protein cell content (Maaloe and Kjeldgaard, 1966). Additionally, the C:N reached 12 in nutrient-limited isolate marine bacteria in a study by Vrede et al. (2002). Finally, as bacteria become starved, water is lost from the cell (Kerkhof and Ward, 1987), which decreases cell volume, increases C:volume ratio, and increases the cellular C:N (Simon and Azam, 1989; Troussellier et al., 1997).

2.1.3. Zooplankton

Many zooplankton feed omnivorously or carnivorously, thus they are both the consumer and the consumed. Marine zooplankton have a rigid stoichiometry, similar to heterotrophic bacteria. The biochemical composition of a variety of marine crustacean and gelatinous zooplankton is shown in Table 26.1, and is averaged by major taxa in Fig. 26.2. For crustacean zooplankton as a group, the average body N content is 10% of dry weight and C:N ratio (by weight) is 5 (Fig. 26.2A; see also Ventura, 2006). Nitrogen content in marine copepods ranges from 4.6 to 23.5% of body dry weight and C:N ratio ranges from 1.7 to 16.6 (Table 26.1, the majority of which listed are calanoids), but on average are also 10% and 5, respectively (Fig. 26.2B). On average, body N content and C:N is similar across all the major crustacean taxa, as well as the polycheates (Fig. 26.2B). Proteins are the most abundant organic compound in copepods, and are found mainly in

Table 26.1 Biochemical composition of some crustacean and gelatinous zooplankton

Taxa and species	Body dry weight or AFDW[a] average or range (mg)	Total N (% DW)	Protein (% DW)	C:N (by weight)	N:P (by weight)	References[b]
Crustacea **Copepods**						
Acartia clausi	0.003–0.007	10.3–23.5	—	1.7–7.5[c]	3.7–9.3[c]	Cataletto and Fonda Umani, 1994; Gismervik, 1997
A. tonsa	0.004–0.012	8.7–16.4	27.7	2.6–4.2[c]	—	Ambler, 1985; Libourel Houde and Roman, 1987
Acartia sp.	0.002–0.005	12.1–12.7	—	3.8–5.4[c]	3.0–11.7[c]	Walve and Larsson, 1999; Pertola *et al.*, 2002
Anomalocera patersoni	—	11.7	—	4.0	—	Gorsky *et al.*, 1988
Calanus cristatus	2.6–8.9	5.9–7.6	50.2	5.1–10.0	—	Omori, 1969; Ikeda, 1972
C. finmarchicus	0.3	9.3	30.1–44.5	4.9–16.6	13.3	Mayzaud and Martin, 1975; Tande, 1982; Båmstedt and Ervik, 1984
C. glacialis	0.4–0.7	9.7–11.2	48.6	4.0–5.2	—	Ikeda and Skjoldal, 1989; Ikeda, 1972
C. helgolandicus	—	11.2	—	4.1	—	Gorsky *et al.*, 1988
C. hyperboreus	1.9–4.0	6.7–7.4	—	7.8–9.1	—	Ikeda and Skjoldal, 1989
C. lighti	0.1	12.7	—	3.8	—	Omori, 1969
C. pacificus	0.3	7.8–11.2	42.7–54.5	4.1–7.5	—	Omori, 1969; Willason *et al.*, 1986
C. plumchrus	1.3	7.0	53.5	8.8	—	Omori, 1969; Ikeda, 1972
C. propinquus	0.8–1.0	12.3–12.5	45.1–59.1	3.4–3.5	16.6–18.1	Ikeda and Mitchell, 1982; Ikeda and Hing Fay, 1981
Calanus spp.	—	—	—	4.2–4.6[c]	9.5–12.6[c]	Gismervik, 1997
Candacia aetiopica	0.06	12.6	—	3.7	—	Omori, 1969
C. armata	—	10.2	—	3.6	—	Gorsky *et al.*, 1988

C. columbiae	0.4	11.2	—	4.2	—	Omori, 1969
Centropages typicus	—	10.9	—	3.5	—	Gorsky *et al.*, 1988
Centropages spp.	—	—	—	5.0–5.9[c]	5.9–11.3[c]	Gismervik, 1997
Chiridius armatus	0.6	—	46.1	—	—	Båmstedt, 1978
Disseta palumboi	0.5	10.7	—	4.8	—	Omori, 1969
Eucalanus bungii bungii	1.1	7.6	52.5	6.5	—	Omori, 1969; Ikeda, 1972
Euchaeta elongata	—	—	50.8	—	—	Dagg and Littlepage, 1972
E. marina	—	11.0	—	5.2	—	Gorsky *et al.*, 1988
Eurytemora affinis	—	12.0–12.7	—	3.9–5.3[c]	3.4–11.3[c]	Walve and Larsson, 1999; Pertola *et al.*, 2002
Gaidius variabilis	0.2–0.4	9.6–10.0	—	4.9–5.1	—	Ikeda and Hirakawa, 1998
Labidocera actifrons	0.3	12.9	—	3.5	—	Omori, 1969
Metridia gerlachei	0.2–0.3	11.1–11.4	59.9	3.8–4.0	18.1–20.9	Ikeda and Mitchell, 1982; Ikeda and Hing Fay, 1981
M. longa	0.4	10.4	30.2	4.5		Ikeda and Skjoldal, 1989; Båmstedt and Ervik, 1984
M. okhotensis	0.5	5.8	—	11.0	—	Omori, 1969
Neocalanus cristatus	—	5.2–8.0	—	6.8–8.7	—	Lindsay, 2003
N. plumcrus	0.4	6.9	—	8.2	—	Ikeda and Hirakawa, 1998
Oithona atlantica	0.003	9.8	—	4.8	—	Ikeda and Hirakawa, 1998
Paraeuchaeta birostrata	3.2	7.1	—	8.2	—	Omori, 1969
P. elongata	1.3–2.5	8.4–8.5	—	6.2–6.7	—	Ikeda and Hirakawa, 1998
P. sarsi	—	7.2	—	8.2	—	Lindsay, 2003
Pleuromamma sp.	—	11.2–13.6	—	3.9	—	Lindsay, 2003
P. xiphias	0.2	13.1	—	3.6	—	Omori, 1969
Pontellina plumata	0.03	12.2	—	3.6	—	Omori, 1969
Pseudocalanus sp.	0.003–0.02	7.0–12.3	—	4.0–8.6[c]	7.2–27.1[c]	Gismervik, 1997; Walve and Larsson, 1999
Rhincalanus nasutus	0.1	9.9	—	5.3	—	Omori, 1969
Spinocalanus antarcticus	—	4.6–4.9[d]	—	8.2–8.7	—	Kosobokova *et al.*, 2002

(*Continued*)

Table 26.1 Biochemical composition of some crustacean and gelatinous zooplankton (*continued*)

Taxa and species	Body dry weight or AFDW[a] average or range (mg)	Total N (% DW)	Protein (% DW)	C:N (by weight)	N:P (by weight)	References[b]
Copepod spp., Chukchi Sea	—	—	—	6.0–8.5	—	Thibault *et al.*, 1999
Copepod spp., Gulf of Mexico 0.1–9.0		—	29.6–67.7	—	—	Morris and Hopkins, 1983
Copepod spp., Sargasso Sea	—	8.2–11.2	—	4.3[c]	12.1[c]	Beers, 1966
Amphipods						
Cyphocaria challengeri	3.1	6.1	—	7.5	—	Omori, 1969
Cyphocaris sp.	81.4	7.0	—	6.8	10.0	Ikeda, 1988
Euthemisto libellula	—	—	49.4	—	—	Ikeda, 1972
Hyperia gaudichaudii	105.3	7.1	—	6.6	7.7	Ikeda and Mitchell, 1982
Parathemisto gaudichaudii	3.6–13.1	7.4–8.6	34.6–40.3	4.2–5.6	10.5–11.6	Ikeda and Mitchell, 1982; Ikeda and Hing Fay, 1981
P. japonica, juvenile	0.5	8.2	—	5.9	—	Omori, 1969
P. libellula	3.0	8.1	—	4.8	—	Ikeda and Skjoldal, 1989
Phronima sedentaria	—	6.5	—	4.1	—	Gorsky *et al.*, 1988
Phrosina semilunata	—	7.1	—	4.2	—	Gorsky *et al.*, 1988
Platyscelus serratulus	2.2	4.4	—	6.0	—	Omori, 1969
Primno abyssalis	13.0–16.7	9.3–9.4	—	4.0–4.3	—	Ikeda and Hirakawa, 1998
Scina crassicornis	—	6.5	—	4.5	—	Gorsky *et al.*, 1988
Themisto japonica	3.1	8.9	—	4.2	—	Ikeda and Hirakawa, 1998
Viblia antarctica	12.1	8.2	—	4.9	9.5	Ikeda and Mitchell, 1982
Ostracods						
Conchoecia pseudodiscophora	0.03–0.04	5.7–7.3	—	5.5–8.3	—	Ikeda, 1990
Conchoecia sp.	—	7.0–9.0	—	4.9–6.0	—	Lindsay, 2003

Discoconchoecia pseudodiscophora	0.1	7.8	—	6.6	—	Kaeriyama and Ikeda, 2004
Gigantocypris mulleri	48.0–160.1	8.2–9.3	—	3.6–3.9	20.5–23.3	Ikeda, 1988
Orthoconchoecia haddoni	0.3–0.5	8.7–9.2	—	4.6–5.3	—	Kaeriyama and Ikeda, 2004
Metaconchoecia skogsbergi	0.1	9.4	—	4.2	—	Kaeriyama and Ikeda, 2004
Euphausiids						
Euphausia krohnii	—	10.0	—	3.1	—	Gorsky *et al.*, 1988
E. pacifica	14.0	10.7	46.4–73.7	3.6	—	Omori, 1969; Mauchline, 1980 and references within
E. pacifica, juvenile	1.5	10.1	—	3.9	—	Omori, 1969
E. similes	2.7–7.0	9.7–14.2	—	3.8–4.0[c]	—	Lindsay, 2003
E. superba	18.0–252.6	9.5–10.3	43.3	3.6–4.7	9.2–11.1	Ishii *et al.*, 1987; Ikeda and Hing Fay, 1981
E. triacantha	82.8	10.7	—	4.4	13.4	Ikeda, 1988
Meganyctiphanes norvegica	—	11.0	—	3.4	—	Gorsky *et al.*, 1988
Nematoscelis megalops	—	9.7	—	3.7	—	Gorsky *et al.*, 1988
Stylocheiron abbreviatum	—	8.5	—	4.1	—	Gorsky *et al.*, 1988
Tessarabrachion oculatus	14.5	10.0	—	4.7	—	Omori, 1969
Thysanoessa inermis	4.6–34.8	7.4–10.0	41.5	4.8–7.4	—	Ikeda and Skjoldal, 1989; Mauchline, 1980 and references within
T. raschii	—	—	78.6	—	—	Ikeda, 1972
Euphausiid spp.	0.7–47.2	—	43.4–52.5	—	—	Morris and Hopkins, 1983
Shrimps						
Acanthephyra quadrispinosa	—	9.5	—	4.8	—	Lindsay, 2003

(Continued)

Table 26.1 Biochemical composition of some crustacean and gelatinous zooplankton (*continued*)

Taxa and species	Body dry weight or AFDW[a] average or range (mg)	Total N (% DW)	Protein (% DW)	C:N (by weight)	N:P (by weight)	References[b]
Bentheogennema borealis	—	6.6	—	8.8	—	Lindsay, 2003
Sergestes similis	—	11.0	—	3.9	—	Childress and Nygaard, 1974
Sergia prehensilis	—	11.0	—	3.9	—	Lindsay, 2003
Mysids						
Boreomysis arctica	13.5	—	38.0	—	—	Båmstedt, 1978
Eucopia hanseni	—	7.3	—	5.9	—	Gorsky et al., 1988
Gnathophausia gigas	127.3	7.3	30.6	5.7	6.6	Ikeda, 1988; Childress and Nygaard, 1974
Leptomysis lingvura	—	11.1	—	3.4	—	Gorsky et al., 1988
Siriella aequiremis	1.0	11.0	—	3.9	—	Omori, 1969
S. armata	—	11.4	—	3.8	—	Gorsky et al., 1988
S. thompsoni	0.5–0.8	—	46.9	—	—	Morris and Hopkins, 1983
Polychaetes						
Glycera rugosa	—	—	56.2	—	—	Giese, 1966
Phoronopsis viridis	—	—	29.4	—	—	Giese, 1966
Sabella starki magnificans	—	—	31.7	—	—	Giese, 1966
Tomopteris carpenteri	9.4–137.9[e]	8.2–8.7	32.5[e]	4.0–4.5	19.3–23.7	Ikeda and Mitchell, 1982; Clarke et al., 1992
T. septentrionalis	—	—	73.8	—	—	Ikeda, 1972
Polychaete spp.	—	4.4–11.2	—	3.3[c]	8.9[c]	Beers, 1966
Gelatinous Zooplankton						
Ctenophores						
Beroe cucumis	29.7[a]	1.1	—	—	—	Curl, 1962
B. ovata	43.2–441.6[f]	1.0	8.3[f]	3.8	—	Kremer et al., 1986; Anninsky et al., 2005

Beroe sp.	92.2–236.6[e]	—	10.9[e]	—	—	Clarke *et al.*, 1992
Cestum veneris	—	0.7	—	4.4	—	Gorsky *et al.*, 1988
Eurhamphaea vexilligera	—	0.2	—	3.7	—	Kremer *et al.*, 1986
Mnemiopsis leidyi	16.8–31.2[f]	—	3.8[f]	—	—	Anninsky *et al.*, 2005
Mnemiopsis sp.	25.0[a]	0.2	—	—	—	Curl, 1962
Ocyropsis maculata	—	0.6	—	3.5	—	Kremer *et al.*, 1986
Ocyropsis spp.	—	0.3	—	3.9	—	Kremer *et al.*, 1986
Pleurobrachia sp.	18.1	—	12.1	—	—	Ikeda and Hing Fay, 1981
Scyphozomedusae						
Atolla wyvillei	270.6–2233.7[e]	4.0	17.0[e]	3.9	—	Clarke *et al.*, 1992
Aurelia aurita	21.0[a]	1.3–1.4	2.1–28.6	3.3–5.3	10.0–68.3	Larson, 1986; Schneider, 1988; Lucas, 1994
Cassiopea xamachana	35.8[a]	2.2	—	5.4	—	Lutcavage and Lutz, 1986; Arai, 1997
Chrysaora fuscenscens	—	2.0	—	3.5	—	Larson, 1986
Cyanea capillata	31.4–37.0[a]	0.4–3.7	9.6	3.4–4.0	—	Curl, 1962; Larson, 1986; Bailey *et al.*, 1995; Arai, 1997
Pelagia noctiluca	26.3–31.0[a]	1.4–4.0	10.9–19.8	2.9–4.2	7.7–18.0	Curl, 1962; Morand *et al.*, 1987; Gorsky *et al.*, 1988; Malej, 1989; Malej, 1991; Arai, 1997
Poralia rufescens	27.0[a]	0.1	0.2	4.3	—	Bailey *et al.*, 1995; Arai, 1997
Thaumatoscyphus atlantica	—	6.3	—	4.0	—	Larson, 1986

(*Continued*)

Table 26.1 Biochemical composition of some crustacean and gelatinous zooplankton (*continued*)

Taxa and species	Body dry weight or AFDW[a] average or range (mg)	Total N (% DW)	Protein (% DW)	C:N (by weight)	N:P (by weight)	References[b]
Hydromedusae						
Aglantha digitale	14.0–42.0[a]	4.3–4.7	—	3.4–3.9	—	Larson, 1986; Ikeda and Skjoldal, 1989
Botrynema brucei	59.4–212.0[e]	8.2	7.5[e]	4.5	—	Clarke *et al.*, 1992
Calycopsis borchgrevinki	60.6–209.7[e]	8.2	11.0[e]	4.5	—	Clarke *et al.*, 1992
Diphyes antarctica	22.8–45.6[e]	8.2	13.4[e]	4.5	—	Clarke *et al.*, 1992
Eperetmus typus	31.0[a]	2.5	—	3.4	—	Larson, 1986
Eutonina indicans	23.0[a]	2.5	—	3.6	—	Larson, 1986
Gonionemus vertens	48.0[a]	4.1	—	3.3	—	Larson, 1986
Halicreas papillosum	—	7.0	—	4.6	—	Ikeda, 1974
Mitrocoma cellularia	19.0[a]	0.8	—	3.5	—	Larson, 1986
Phialidium gregarium	23.0[a]	2.5	—	3.8	—	Larson, 1986
P. lomae	21.0[a]	1.7	—	4.0	—	Larson, 1986
Sarsia princeps	27.0[a]	1.8	—	3.7	—	Larson, 1986
Stomotoca atra	36.0[a]	2.6	—	3.7	—	Larson, 1986
Hydromedusae spp.	—	1.3–6.9	—	2.5[c]	16.9[c]	Beers, 1966
Chaetognaths						
Eukrohnia hamata	5.8	—	39.1	—	—	Båmstedt, 1978
Sagitta bipunctata	—	12.9	—	4.0	—	Gorsky *et al.*, 1988
S. elegans	78.4[a]	7.8–15.1	54.2–84.0	2.6	18.4	Curl, 1962; Ikeda, 1972; Mayzaud and Martin, 1975
S. enflata	—	9.1	—	5.0	—	Gorsky *et al.*, 1988
S. minima	—	11.8	—	4.3	—	Gorsky *et al.*, 1988
S. setosa	—	10.0	—	3.9	—	Gorsky *et al.*, 1988
Chaetognath spp.	—	6.3–9.4	—	3.6[c]	12.5[c]	Beers, 1966

Pteropods

Cavolina longirostris	4.0	3.5	—	6.3	—	Omori, 1969
Cleodora sulcata	54.8	8.5	—	4.3	16.7	Ikeda and Mitchell, 1982
Clione antarctica	25.3	7.7	31.2–34.8	5.2	29.6	Ikeda and Mitchell, 1982; Ikeda and Hing Fay, 1981
C. limacina	16.5–76.4	4.7–5.7	52.7	5.0–5.9	—	Ikeda and Skjoldal, 1989; Ikeda, 1972
Euclio pyramidata	5.0	2.9	—	7.0	—	Omori, 1969
Limacina antarctica	6.5	8.1	39.9–45.4	4.1	15.3	Ikeda and Mitchell, 1982; Ikeda and Hing Fay, 1981
L. helicina, juvenile	0.1	6.0	—	4.9	—	Omori, 1969
L. inflata	0.1	1.5	—	11.1	—	Omori, 1969
Pteropod spp.	—	2.7–4.2	—	6.9[c]	10.9[c]	Beers, 1966

Salps

Ihlea punctata	—	1.7	—	3.7	—	Gorsky et al., 1988
I. racovitzai	10.9	2.8	—	3.6	17.5	Ikeda and Mitchell, 1982
Pegea confoederata aggregate	0.7–69.0	1.0[g]	8.0	4.8	—	Madin et al., 1981
Pyrosoma atlanticum	—	2.8	—	4.0	—	Gorsky et al., 1988
Salpa fusiformis solitary	22.9[a]	0.5–2.7	10.7[e]	3.9–4.3	10.8	Clarke et al., 1992; Curl, 1962; Le Borgne, 1982a
S. maxima aggregate	0.9–281.0	2.5[g]	8.0	4.6	—	Madin et al., 1981
S. thompsoni aggregate	—	1.5	—	4.0	10.1	Iguchi and Ikeda, 2004
S. thompsoni solitary	—	1.3	—	3.9	11.8	Iguchi and Ikeda, 2004

Larvaceans

Fritillaria pellucida	—	13.6	—	4.6	—	Gorsky et al., 1988
Megalocercus huxleyi	—	—	22.4	—	—	Skjoldal, 1981
Oikopleura dioica	—	11.3	—	5.3	—	Gorsky et al., 1988

Table 26.1 Biochemical composition of some crustacean and gelatinous zooplankton (*continued*)

Taxa and species	Body dry weight or AFDW[a] average or range (mg)	Total N (% DW)	Protein (% DW)	C:N (by weight)	N:P (by weight)	References[b]
O. gaussica	0.8–1.1	—	35.4	—	—	Ikeda and Hing Fay, 1981
O. longicauda	—	13.9	—	3.6	—	Gorsky *et al.*, 1988
Siphonophores						
Chelophyes appendiculata	—	6.8	—	3.5	—	Gorsky *et al.*, 1988
Siphonophore spp.	—	1.0–4.4	—	3.7[c]	21.1[c]	Beers, 1966

For some species, data were compiled using multiple references. Polychaetes are included as a separate grouping.

— = Not determined, or not reported in cited reference. When C:N (by weight or atoms) was not available in the reference, it was calculated using body C and N values provided.

[a] AFDW, ash-free dry weight.

[b] Species listed in the reference were sampled from the following environments: Gismervik (1997)—Oslofjord, Norway; Cataletto and Fonda Umani (1994)—Gulf of Trieste, N. Adriatic Sea; Ambler (1985)—East Lagoon, Galveston, TX; Libourel Houde and Roman (1987)—Chesapeake Bay; Walve and Larsson (1999)—Baltic Sea; Gorsky *et al.* (1988)—Ligurian Sea, NW Mediterranean; Omori (1969)—N Pacific; Ikeda (1972)—Bering Sea, N Pacific Ocean; Mayzaud and Martin (1975)—St. Margaret's Bay, N Atlantic; Tande (1982)—Balsfjorden, Norway; Båmstedt and Ervik (1984)—west coast of Norway; Ikeda and Skjoldal (1989)—Barents Sea, Arctic; Ikeda and Mitchell (1982)— Antarctica (epipelagic); Ikeda and Hing Fay (1981)—Antarctica; Båmstedt (1978)—Korsfjorden, Norway; Dagg and Littlepage (1972)—Haro Strait and Saanich Inlet, British Columbia; Walve and Larsson (1999)—Baltic Sea; Ikeda and Hirakawa (1998)—S Japan Sea; Lindsay (2003)—Sagami Bay, Japan; Kosobokova *et al.* (2002)—Arctic Ocean; Thibault *et al.* (1999)—Chukchi Sea, Arctic Ocean; Morris and Hopkins (1983)—Gulf of Mexico; Beers (1966)—Sargasso Sea; Ikeda (1988)—Prydz Bay, Antarctica (mesopelagic); Ikeda (1990)—Japan Sea; Kaeriyama and Ikeda (2004)—W North Pacific Ocean; Mauchline (1980)—various (review); Ishii *et al.* (1987)—Antarctica; Childress and Nygaard (1974)—S California; Giese (1966)—Antarctica; Clarke *et al.* (1992)—Southern Ocean; Curl (1962)—New York (continental shelf); Anninsky *et al.* (2005)—Black Sea; Kremer *et al.* (1986)—Bahamas; Larson (1986)—Saanich Inlet, BC; Schneider (1988)—Kiel Bight, Baltic Sea; Arai (1997)—various (review); Lutcavage and Lutz (1986)—Biscayne Bay, FL; Bailey *et al.* (1995)—Cape Hatteras, North Carolina; Morand *et al.* (1987)—Ligurian Sea, NW Mediterranean; Malej (1989)—Gulf of Trieste, N. Adriatic Sea; Malej (1991)—Gulf of Trieste, N. Adriatic Sea; Ikeda (1974)—various; Madin *et al.* (1981)—N Atlantic; Le Borgne (1982)— E tropical Atlantic Ocean; Iguchi and Ikeda (2004)—Antarctica; Skjoldal (1981)—Great Barrier Reef, Australia.

[c] Original C:N, N:P converted from molar to weight according to formulas: C:N (weight) = (atomic C:N) × (12/14); N:P (weight) = (atomic N:P) × (14/31).

[d] Determined assuming dry weight = carbon content × 2.5.

[e] Calculated from Clarke *et al.* (1992; tables).

[f] Determined assuming dry weight = 2.4% wet weight (Finenko *et al.*, 2001).

[g] Dry weight calculated from ash-free dry weights using carbon content ranges of 50–5000 μg for *P. confoederata* and 100–30,000 μg for *S. maxima* (Madin *et al.*, 1981).
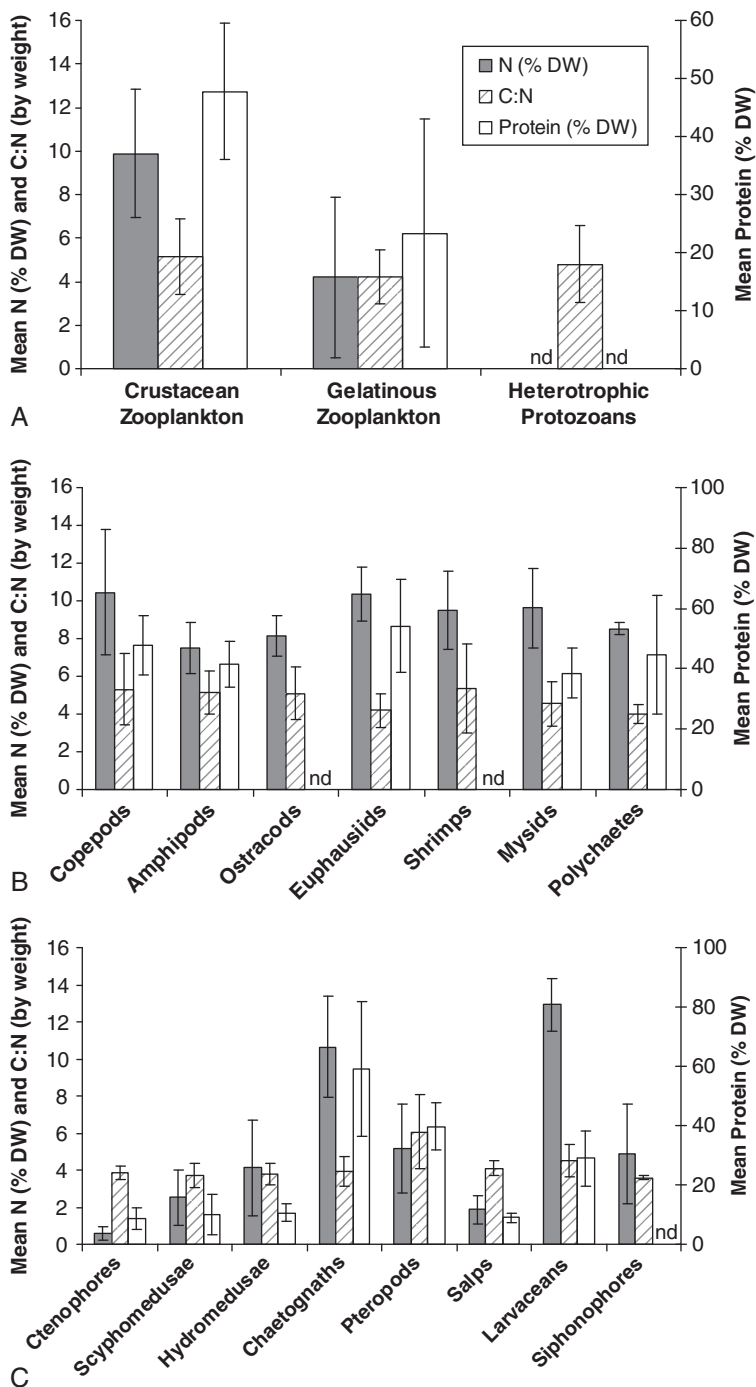
Figure 26.2 Summary of literature data on nitrogen and protein content, and C:N ratio of major zooplankton taxa, from Tables 26.1 and 26.2. (A) Major zooplankton groups, (B) Crustacean taxa and polychaetes, (C) Gelatinous taxa. Bars represent mean ±1 standard deviation. nd indicates not determined.

muscle tissue (Båmstedt, 1986). Proteins and free amino acids together have been shown to account for most of copepod body N content (67 and 7%, respectively, for *Calanus finmarchicus*, Ventura, 2006). Protein content in marine copepods ranges from 27.7 to 67.7% of body dry weight (Table 26.1), and on average 48% for copepods overall and for crustacea as a group (Fig. 26.2B; see also Ventura, 2006). Crustacean zooplankton contain a large diversity of amino acids, with highest concentrations of glutamic acid, aspartic acid, lysine, leucine, and glycine (Cowey and Corner, 1963; Parsons *et al.*, 1984). Free amino acids contribute less to body dry weight (e.g., on average 5–11% of body dry weight in mysiids, euphausiids, and marine calanoid copepods, Ventura, 2006) than proteins in the crustacean zooplankton.

Gelatinous zooplankton have lower body N and protein content compared to crustacean zooplankton, but a similar C:N (Fig. 26.2A–C). Table 26.1 shows the range of body N as a proportion of dry weight in a variety of gelatinous zooplankton which, for example, includes: ctenophores (0.2–1.1%), schyphomedusae (0.1–6.3%), hydromedusae (0.8–8.2%), pteropods (1.5–8.5%), and salps (0.5–2.8%), and on average is 4% for all gelatinous taxa (Fig. 26.2A). Gelatinous zooplankton protein content ranges from 0.1% of body dry weight in the scyphozoan medusa *Poralia rufescens* (Bailey *et al.*, 1995) to 2.1–28.6% in *Aurelia aurita* (Lucas, 1994) (Table 26.1) and is generally higher than both lipid and carbohydrate content in scyphomedusae (Arai, 1997). Overall mean protein content for gelatinous zooplankton is 23% of dry weight (Fig. 26.2A), and for some groups, such as the medusae and ctenophores, is noticeably lower than this overall mean (Fig. 26.2C). However, isolated tissues in medusae, such as gonads and tentacles, can contain higher carbon, nitrogen, protein, carbohydrate, and lipid content (as proportion of dry weight) relative to the whole organism (Larson, 1986; Lucas, 1994). Interestingly, Pacific leatherback sea turtles, which primarily depend on gelatinous zooplankton for food, have been observed consuming only tentacle and gonadal tissue, presumably targeting these higher nutritional parts of the medusae (Benson, unpublished).

Studies of the biochemical composition of marine protozoa are extremely limited, and are summarized in Table 26.2. Nitrogen content in marine ciliates and heterotrophic dinoflagellates ranges from 60 to 131,000 pg N cell^{-1} (mean = 1274 pg N cell^{-1}—excluding the large dinoflagellate *Noctiluca scintillans*), and C:N ratio (by weight) ranges from 3 to 13 (mean = 4.4). Ohman and Snyder (1991) determined the protein content of the ciliate *Uronema* sp. to be 310 pg cell^{-1}. Marine flagellates have lower cellular N contents (0.3–19 pg N) compared to ciliates and heterotrophic dinoflagellates, but higher N density (0.1–0.2 pg N μm^{-3} for flagellates vs. 0.04 pg N μm^{-3} for ciliates and heterotrophic dinoflagellates— excluding *N. scintillans*) and similar C:N (4.1–5.5, by weight for flagellates).

Although zooplankton tend to maintain a rather rigid biochemistry, intra- and interspecific differences may arise due to physical characteristics (e.g., water content, body size), developmental stage, as well as location (e.g., latitude) and season. Increased water content, and thus decreased muscle tissue, leads to a decrease in protein and N content in zooplankton (Childress and Nygaard, 1974; Morris and Hopkins, 1983). This may explain the lower N and protein content in gelatinous zooplankton relative to crustaceans as described above. The effect of water content on proteins can also be seen within taxonomic groups. For example, *Calanus pacificus*

Table 26.2 Biochemical composition of microzooplankton (heterotrophic protozoans)

Taxa and species	Cell or lorica[a] volume (μm^3)	Average or range (pg C cell^{-1})	Average or range (pg N cell^{-1})	C:N (by weight)	Reference[b]
Ciliates					
Favella sp. (culture)	316,293[a]	19,193	4701	4.1	Verity and Langdon, 1984
Laboea strobila	47,000–97,000	13,000–38,000[c,d]	2900–3200[d]	4–13	Putt and Stoecker, 1989
Mesodinium pulex	—	330[e]	79[e]	4.2	Broglio *et al.*, 2003
Stenosemella steini (field)	60,085[a]	3670	896	4.1	Verity and Langdon, 1984
S. ventricosa (field)	275,495[a]	12,302	2920	4.2	Verity and Langdon, 1984
Strobilidium spiralis	76,000	10,500–14,000[c,d]	3500[d]	3–4	Putt and Stoecker, 1989
Strombidium capitatum	47,000	4200–11,200[c,d]	1400[d]	3–8	Putt and Stoecker, 1989
S. sulcatum	—	910–1220[e]	220–290[e]	4.1–4.2	Broglio *et al.*, 2003
Tintinnopsis acuminata (culture)	9198[a]	790–866	169–188	4.6–4.7	Verity and Langdon, 1984; Verity, 1985a
T. baltica (field)	—	3375	856	3.9	Verity and Langdon, 1984
T. parva (field)	14,467[a]	1249	275	4.5	Verity and Langdon, 1984
T. rapa (field)	17,627[a]	1531	337	4.5	Verity and Langdon, 1984
T. tubulosoides (culture)	92,182[a]	5032	1148	4.4	Verity and Langdon, 1984
T. vasculum (culture)	141,617[a]	8520	1797	4.7	Verity and Langdon, 1984
T. vasculum (culture)	—	8337–8940	1730–1901	4.6–4.8	Verity, 1985a
T. vasculum (field)	173,309[a]	9975	2173	4.6	Verity and Langdon, 1984
Uronema sp.	169–2102[f]	230	60	3.8	Ohman and Snyder, 1991
Dinoflagellates					
Gymnodinium dominans	—	230	63	3.7	Broglio *et al.*, 2003
Noctiluca scintillans	2.01×10^8	353,000	131,000	2.8	Tada *et al.*, 2000
Oxyrrhis marina	4745	469	89	5.3	Menden-Deuer and Lessard, 2000

(*Continued*)

Table 26.2 Biochemical composition of microzooplankton (heterotrophic protozoans) (*continued*)

Taxa and species	Cell or lorica[a] volume (μm³)	Average or range (pg C cell⁻¹)	Average or range (pg N cell⁻¹)	C:N (by weight)	Reference[b]
Flagellates					
Bodo designis	54	12.60	3.10	4.1	Eccleston–Parry and Leadbetter, 1995
Jakoba libera	75	14.15	2.58	5.5	Eccleston–Parry and Leadbetter, 1995
Monas sp.	15	1.45[g]	0.32[g]	4.5	Børsheim and Bratbak, 1987
Paraphysomonas imperforata	212	32.49	7.42–19.00[h]	4.5	Goldman *et al.*, 1985; Eccleston-Parry and Leadbetter, 1995
Stephanoeca diplocostata	35	5.42	1.10	4.9	Eccleston–Parry and Leadbetter, 1995

When C:N was not available in the reference, it was calculated using body C and N values provided.

— = Not determined, or not reported in cited reference.

[a] Lorica volume given (lorica can be considerably larger than the cell it encases).

[b] Species listed in the reference were sampled from the following environments: Verity and Langdon (1984)—Narragansett Bay, RI; Broglio *et al.* (2003) and Eccleston-Parry and Leadbetter (1995)—Batch cultures; Putt and Stoecker (1989)—either Perch Pond or Vineyard Sound, MA; Ohman and Snyder (1991)—Southern CA coast; Tada *et al.* (2000)—Seto Inland Sea, Japan; Børsheim and Bratbak (1987)—Raunefjorden, Norway; Goldman *et al.* (1985)—Vineyard Sound, MA.

[c] C ranges calculated using N cell content and C:N from Putt and Stoecker (1989).

[d] Data are from various irradiance levels.

[e] C and N calculated using conversion factors from Putt and Stoecker (1989) or Menden-Deuer and Lessard (2000).

[f] Measurements made in stationary phase.

[g] Values calculated from Børsheim and Bratbak (1987), their Table 1, and text.

[h] N cell content calculated from equation in Goldman *et al.* (1985) for exponential growth.

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copepods have 42.7–54.5% body dry weight as protein (Willason *et al.*, 1986), and some *Eucalanus* spp., with higher water content, have 4–5.5% body dry weight as protein (Flint *et al.*, 1991; Mauchline, 1998). Relationships between body length and N content for marine copepods have been made using linear regression models and generally show an increase in N content with body length (Cataletto and Fonda Umani, 1994; Uye, 1982). Additionally, there are likely small compositional differences between juvenile and adult developmental stages (Sterner and Hessen, 1994), and copepods that are reproducing and utilizing their lipid storage have lower C:N compared to non-reproducing copepods (Ventura and Catalan, 2005).

A general regional trend in copepod biochemical composition is an increase in N content and a decrease in C:N from high to low latitudes (Fig. 26.3; Båmstedt, 1986); thus, eastern tropical Atlantic copepods have a lower C:N (4.5–5.4; Le Borgne, 1982A) than polar copepod species (6.8; Conover and Huntley, 1991). Interestingly, the same overall mean N content (7% of dry weight, calculated from Madin *et al.*, 2001 and Landry *et al.*, 2001) and C:N (5.1) for mixed zooplankton is found at two long-term mesozooplankton time series in the N. Atlantic (Bermuda Atlantic Time-series Study, BATS) and N. Pacific (Hawaii Ocean Time-series, HOT) oligotrophic central gyres (Landry *et al.*, 2001; Madin *et al.*, 2001). In addition, chemical composition among different zooplankton taxa in the Sargasso Sea and N. Pacific (at similar latitudes) are nearly indistinguishable. Beers (1966) determined the range of percent N as dry weight for a variety of Sargasso Sea
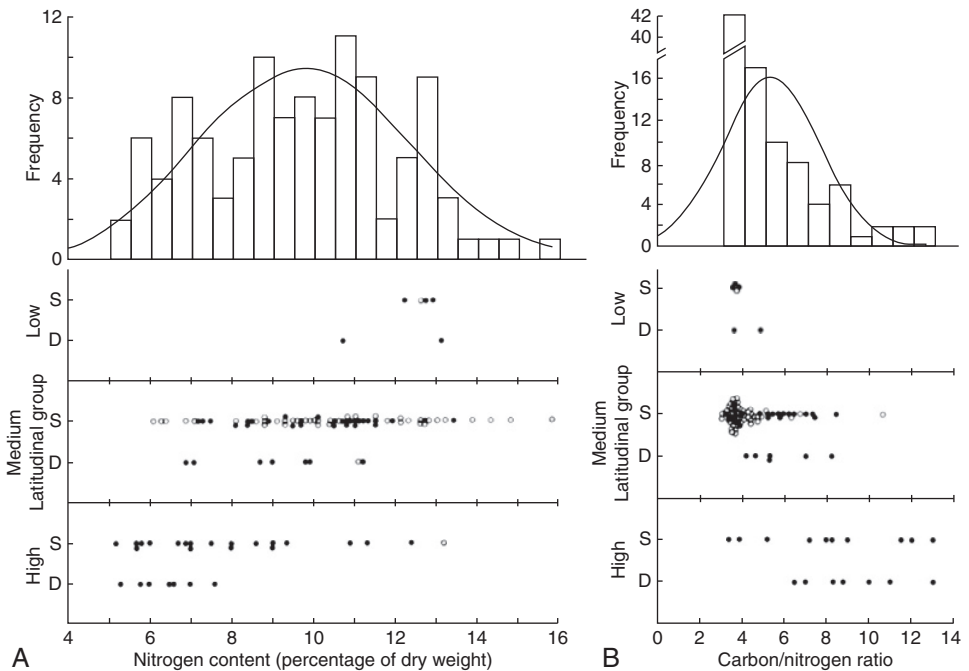
Figure 26.3 Nitrogen contents (A) and C:N ratio (B) of copepod species from low, medium, and high latitudes. S, shallow-water species; D, deep-water species. (Adapted from Båmstedt, 1986).

zooplankton to be 8.2–11.2% for copepods, 9.4–10.5% for euphausiids and mysids, and 6.2–9.4% for chaetognaths. Similarly, N as % dry weight for North Pacific copepods, euphausiids, and chaetognaths is 5.1–13.1%, 10.0–10.7%, and 10.7–11.1%, respectively (Omori, 1969). Seasonal differences in zooplankton biochemical composition are also predictable, especially for copepods in temperate and high latitudes, which experience high seasonality (Costa *et al.*, 2006; Sterner and Hessen, 1994; Tande, 1982). *C. finmarchicus*, for example, displayed a decreasing C:N (from 13.0 to 7.9 in copepodite stages IV and V, males and females) and an increase in protein from late winter until the spring spawning period. C:N then increased throughout the summer until early winter for female copepods due to increasing lipid content in preparation for the overwintering period (Tande, 1982). During overwintering, copepod body lipid (primarily wax esters) declines as reserves are metabolized (Campbell *et al.*, 2004; Costa *et al.*, 2006; Jónasdóttir, 1999; Tande, 1982). In another example, *Acartia tonsa* seasonal N content ranged from 8.7 to 16.4% of body dry weight and C:N ranged from 3.0 to 4.9, with the highest N content and lowest C:N in spring, and the opposite in summer (Ambler, 1985).

2.1.4. Detritus

Detritus can be an important food and N source for zooplankton (Dilling *et al.*, 1998; Hessen *et al.*, 1990; Mayzaud *et al.*, 1998; Roman, 1977, 1984, 1980; Steinberg, 1995). A majority of the particulate matter in marine systems is composed of detritus (Volkman and Tanoe, 2002), which consists of non-living biogenic material such as senescent phytoplankton, zooplankton fecal pellets, and abandoned larvacean houses (e.g., Alldredge and Silver, 1988). In coastal regions, allochthonous organic matter input (Cai *et al.*, 1988; Goñi *et al.*, 2003) as well as non-living macrophyte organic matter (Mann, 1972) are also important components of detritus. The nutrient content of detritus depends on the detrital source (plankton community composition), age, depth, particle size, and bacterial remineralization activities (Tenore *et al.*, 1982). Copin-Montegut and Copin-Montegut (1983) conducted a study comparing particulate organic matter in the Atlantic, Indian, and Antarctic oceans and the Mediterranean Sea. The average surface C:N among these sites was 5.6 (by atoms) and varied less than 20% between sites, while N:P was more variable ranging from 18 to 23. Although the concentrations of particulate matter decreased with depth, the C:N and the percent of refractory carbon in the particles slightly increased. Caron *et al.* (1995) found Sargasso Sea detrital C:N (by weight) is 7.3–7.4. However, detrital C:N may be much higher when derived from N-limited algae (Tezuka, 1989). Detrital N consists of protein as well as amino sugars (chitin), humic acids and inorganic clay particles bound with N, and mucopolysaccharides released by bacteria (Roman, 1983). In a study conducted by Mayzaud *et al.* (1998), protein content in *Thalassiosira weissflogii* detritus was 32 pg cell^{-1}, equal to only about half of protein content in live *T. weissflogii* cells. Similarly, Roman (1984) found live *T. weissflogii* cells contained 6 × more N, 12 × more protein, 9 × more fatty acid, a low near Redfield C:N, and more types and higher concentration of amino acids compared to detritus from *Thalassia testudinum*. Detrital particles also serve as a hotspot of microbial activity and are colonized by N-rich bacteria (Kiørboe, 2003; Kiørboe *et al.*, 2002), which increase bulk N content and decrease

C:N of detrital particles (Tenore *et al.*, 1982). However, detritus with low nitrogen and phosphorus content (high C:N, C:P) will be decomposed much slower than nutrient-rich detritus, as the gross growth efficiency of bacteria decreases with increasing C:N of detritus (Goldman *et al.*, 1987).

2.2. Nitrogen requirements: Effects of food quantity and quality

Feeding and growth rates, egg production and hatching success, and fecal pellet production are affected by both quantity and quality of food supply (Abou Debs, 1984; Besiktepe and Dam, 2002; Frost, 1972; Tang and Taal, 2005). Changes in food quality can create a mismatch in stoichiometry between predator and prey (Sterner and Hessen, 1994) and effect all of the above parameters, as well as body N content, and ultimately zooplankton production and biomass. In fact the nutritional status of phytoplankton (N cell quotas), not the phytoplankton biomass, may be the major bottom-up determinant of zooplankton biomass (Hessen, 1992; Sterner and Hessen, 1994) except in periods of low food availability (Jónasdóttir *et al.*, 2002).

Food quality may be based on prey size, shape, mobility, and chemical and physical properties (Stoecker *et al.*, 1986). The C:N of food reflects its protein: lipid ratio (Omori and Ikeda, 1984; Urabe and Watanabe, 1993); thus, an organism may be protein limited, but not necessarily N-limited. In addition, phytoplankton with lower C:N are not always nutritionally high-quality food because they may be deficient in some essential organic compounds, such as polyunsaturated fatty acids and specific amino acids (Ahlgren *et al.*, 1990; Anderson and Pond, 2000; Urabe and Watanabe, 1993). This is evident in trophic upgrading studies, which have focused on the importance of microzooplankton in mediating the transfer of essential nutrients from phytoplankton to zooplankton greater than 200 μm (Klein Breteler *et al.*, 1999; Tang and Taal, 2005; Veloza *et al.*, 2006).

2.2.1. Effects on feeding and growth rates

Although studies have extensively investigated food quality effects on feeding and growth rates, the results are variable (Cowles *et al.*, 1988; Harris *et al.*, 1986; Kiørboe, 1989; Roman, 1983; Stoecker and Sanders, 1985). Many previous studies have shown zooplankton may maximize the uptake of limiting nutrients, such as N and protein, to support growth (Sterner and Hessen, 1994). Both microzooplankton and mesozooplankton have been shown to have higher ingestion and growth rates when feeding on prey items of higher quality (Cowles *et al.*, 1988; Libourel Houde and Roman, 1987; Stoecker *et al.*, 1986). For example, the copepod *A. tonsa* increases its maximum ingestion rate of N and protein feeding on algal prey with relatively higher N and protein content (dinoflagellate *Amphidinium carteri* vs. senescent diatom *T. weissflogii*) (Libourel Houde and Roman, 1987).

Alternatively, zooplankton may increase N and protein ingestion rates to compensate for lower quality food (Darchambeau and Thys, 2005; Harris *et al.*, 1986; Kleppel, 1983; Mayzaud *et al.*, 1998; Plath and Boersma, 2001; Sterner and Hessen, 1994). Mayzaud *et al.* (1998) found *A. tonsa* copepod protein ingestion rates ranged from 4.2 μg protein ind^{-1} h^{-1} for live diatom prey (*T. weissflogii*) and 4.5 μg protein ind^{-1} h^{-1} for live dinoflagellate prey (*Prorocentrum micans*), to 12.0 μg protein ind^{-1} h^{-1}

for a 50:50 mix of live diatom and detritus formed from senescent *T. weissflogii*. The saturation concentrations of prey (prey concentration at maximum ingestion rate) were 344, 221, and 731 μg protein l^{-1}, respectively, which suggests that *A. tonsa* ingested more cells in the lower protein detritus mixture in order to maximize the intake of protein. However, these saturation concentrations of prey are much higher than those found previously for the copepod *Eucalanus pileatus* feeding on the diatom *Rhizosolenia alata* (62–125 μg protein l^{-1} or 10–20 μg N l^{-1}; Mayzaud *et al.*, 1998; Paffenhöfer and Van Sant, 1985) and for the copepod *Neocalanus plumchrus* feeding on *T. weissflogii* (73 μg protein l^{-1}; Dagg and Walser, 1987). Harris *et al.* (1986) also found ingestion rates varied with prey N and protein content. The copepod *Calanus helgolandicus* feeding on *Cryptomonas maculata* on average ingested 0.098 μg N ind^{-1} h^{-1} and 0.747 μg protein ind^{-1} h^{-1}, while *C. helgolandicus* feeding on *T. weissflogii* ingested 0.202 μg N ind^{-1} h^{-1} and 1.243 μg protein ind^{-1} h^{-1}. Although both diets sustained copepod body protein (60 μg protein ind^{-1}), ingestion rates for N and protein were higher for *T. weissflogii* in order to compensate for the lower N and protein densities of *T. weissflogii* (0.014 and 0.081 pg protein μm^{-3}) compared to *C. maculata* (0.037 pg N μm^{-3} and 0.296 pg protein μm^{-3}) (Harris *et al.*, 1986). Additionally, the phagotrophic microflagellate *Paraphysomonas imperforata* has a lower N ingestion rate (63 pg N ind^{-1} d^{-1}) when N-rich bacteria are present in their food mixture, as opposed to an all-algal diet (80 pg N ind^{-1} d^{-1}) (Goldman *et al.*, 1985).

Lastly, zooplankton may not alter ingestion rates even under high variability of food quality (Kiørboe, 1989). There is, however, a high correlation between food quality and zooplankton growth. For example, zooplankton growth per unit of food biomass decreases as prey algae become increasingly nutrient limited (Kiørboe, 1989; Sterner, 1993). These processes require further investigation in order to fully understand how food quality affects feeding and growth.

2.2.2. Effects on egg production and hatching success

Studies investigating the role of food quality on egg production have found a high correlation between protein and essential fatty acid content of the food source and egg production rate of copepods (Abou Debs, 1984; Jónasdóttir, 1994; Jónasdóttir *et al.*, 2002; Kleppel and Hazzard, 2000; Kuijper *et al.*, 2004; Sterner and Hessen, 1994; Thor *et al.*, 2002). Klein Breteler *et al.* (2005) show that N limitation in phytoplankton decreases both cell protein and the essential fatty acid content, compounds that are essential in the nutrition of reproducing female copepods. Indeed, egg production rates of *Acartia* spp. decreased when these copepods were fed prey items lower in total N, protein, and certain fatty acids (Jónasdóttir, 1994). High prey N conconcentration, however, does not always yield high egg production; in fact, the prey C:N ratio is an important factor in copepod egg production (Augustin and Boersma, 2006; Kuijper *et al.*, 2004). In a dynamic energy budget model, Kuijper *et al.* (2004) show copepod N gross growth efficiency of egg production (the efficiency with which N can be used for producing eggs) increases with increasing prey C:N, reaching a peak with a C:N between 10 and 15. Experiments indicate *Acartia* spp. produces more eggs when fed algae with a C:N of 9.1 compared to algae with a C:N of 4.5 (Augustin and Boersma, 2006). These studies suggest that a moderate C:N ratio (10–15) can maximize both egg

production and N gross growth efficiency of the egg production, whereas copepods feeding on prey with a low C:N have a low efficiency for utilizing the N for egg production and instead use the excess N for maintenance.

A number of studies show diatoms decrease copepod fecundity and egg viability (Ban *et al.*, 1997; Miralto *et al.*, 1999; Poulet *et al.*, 1994; Uye, 1996). Ianora and Poulet (1993) compared egg production and viability of the copepod *Temora stylifera* feeding on two different diets: the dinoflagellate *Prorocentrum minimum* and the diatom *Thalassiosira rotula*. Egg production was similar between the two diets; however, the total number of viable eggs was three times higher with the dinoflagellate diet. This increased hatching success may be due to the higher carbon, nitrogen, protein, amino acid, vitamin, and fatty acid content of the dinoflagellate diet relative to to the diatom diet (Ianora and Poulet, 1993). Additionally, Ban *et al.* (2000) noticed a high percentage (20–40%) of hatched *Pseudocalanus newmani* eggs produced deformed nauplii when diatoms were the major food source for the copepod. The deleterious effects of diatoms on copepod fecundity and egg viability has recently been questioned, however, due to lack of negative correlation of *in situ* hatching success and diatom abundance and dominance in the field (Irigoien *et al.*, 2002; Jónasdóttir *et al.*, 1998; Pond *et al.*, 1996). It is suggested that not all diatoms negatively affect copepod egg viability and, in natural conditions, copepods can compensate for lack of nutrition in certain diatoms (those that are N-limited or low in proteins and fatty acids) by feeding on other prey items such as dinoflagellates and heterotrophic protozoans (Irigoien *et al.*, 2002; Paffenhöfer, 2002).

More recent studies have focused on the effects of toxic phytoplankton species on feeding, egg production, and hatching success of copepods, and again the results are variable. In studies conducted by Lincoln *et al.* (2001) and Palomares-García *et al.* (2006), there were no differences in feeding rates, egg production, or hatching success in copepods feeding on either a toxic diatom or dinoflagellate or their non-toxic counterparts. On the other hand, these parameters were strongly negatively affected when copepods fed on the toxic strain of haptophyte *Prymnesium patelliferum* (Nejstgaard and Solberg, 1996). These studies did not examine the chemical composition of the prey items, however, as many toxins are nitrogenous (e.g., paralytic shellfish toxin, domoic acid), their synthesis may be greatly affected by N limitation (Flynn and Flynn, 1995; Flynn *et al.*, 1994). Ingestion rates of the copepod *Acartia margalefi* and the heterotrophic dinoflagellate *Oxyrrhis marina* were lower when feeding on a toxic *Karlodinium* sp. vs. a non-toxic *Gymnodinium* sp. Furthermore, ingestion rates were also lower when feeding on a P-limited *Karlodinium* sp. compared to a non-nutrient limited *Karlodinium* sp. (Vaqué *et al.*, 2006). Future studies examining the effect of harmful phytoplankton N and toxin composition on copepod production would improve our understanding of the variability characteristic of studies thus far.

2.2.3. Effects on body nitrogen content

Zooplankton body N may also be affected by food quantity and quality. For example, Ikeda (1974) found the protein content of starved *Calanus cristatus* (now *Neocalanus cristatus*) decreased by 45% over 7 days. However, Andersen and Hessen (1991) did not see a change in the N:dry weight of cladocerans *Daphnia longispina*, *Bosmina longispina*, and *Holopedium gibiberum* in different food conditions, thus the effect of food quality on body N content requires further investigation.

2.2.4. Response to variable food quality

Zooplankton have many adaptations to respond to variable food quality. Zooplankton can use chemoreception to detect high quality food, and thus feed selectively on those food items (Cowles *et al.*, 1988; Paffenhöfer and Van Sant, 1985; Stoecker *et al.*, 1981; Stoecker *et al.*, 1986; Urabe and Watanabe, 1993). For example, the copepod *E. pileatus* ingested live *Rhizosolenia alata* diatom cells 1.2–3 times faster than dead *R. alata* cells (Paffenhöfer and Van Sant, 1985). Additionally, *A. tonsa* selectively fed on actively growing *T. weissflogii* with higher protein, dissolved free amino acids, and total N content as opposed to slow growing, high C:N *T. weissflogii* cells (Cowles *et al.*, 1988). Microzooplankton may also exhibit selective feeding. Stoecker *et al.* (1986) found the ciliate *Balanion* prefers dinoflagellates (2 *Heterocapsa* species) over cryptophytes (*Chroomonas salina*), chlorophytes (*Dunaliella tertiolecta*), and prymnesiophytes (*Isochrysis galbana*) because they support the fastest ciliate growth (3 divisions d^{-1} at 15°C). Zooplankton may also vertically migrate to areas of higher quality food (Sterner *et al.*, 1993), such as the primary production maximum where phytoplankton cells are actively growing (Cowles *et al.*, 1988) and richer in N. The ability to feed selectively on a more nutritious food decreases the elemental imbalance between prey and consumer.

Many zooplankton are opportunistic feeders and thus can feed omnivorously on both phytoplankton and microzooplankton such as heterotrophic ciliates and flagellates. Marine protozoa are increasingly realized to be an important part of mesozooplankton diets, either as a nutritional supplement for what can not be acquired from a strict phytoplankton diet, or as the primary food source (Landry and Calbet, 2004 and references therein). Carnivory and omnivory may be metabolically beneficial, as animal prey C is assimilated more efficiently than phytoplankton prey C, and N is assimilated more efficiently than C (Gifford, 1991; Tang and Dam, 1999). Stoecker and Egloff (1987) found that protozoans were an important food source for adult copepods, even in the presence of high concentrations of phytoplankton, suggesting a preference for higher food quality. *A. tonsa* copepods preferentially fed on the tintinnid ciliate *Favella* sp. over a higher concentration (in total C and N) of dinoflagellates *Heterocapsa triquetra* (Stoecker and Sanders, 1985), perhaps due to the higher quality (lower C:N) of the tintinnid compared to the dinoflagellate. Additionally, *A. tonsa* copepods had higher clearance rates for a variety of ciliates and a rotifer species over algae even under high algal concentrations (Stoecker and Egloff, 1987). Omnivory and carnivory is advantageous when phytoplankton prey items are nutrient limited and/or do not contain essential amino acids or polyunsaturated fatty acids necessary for zooplankton growth (Kleppel, 1983). This is especially true in detritus-based food webs where allochtonous organic matter is high in C relative to N (Stoecker and Capuzzo, 1990). Previous studies have shown that protozoan diets may enhance growth and survival of predators and also increase egg production, most likely due to their typically lower C:N and higher levels of essential nutrients such as polyunsaturated fatty acids (PUFAs), sterols, and amino acids, compared to phytoplankton (Gifford, 1991; Goldman *et al.*, 1985; Kleppel *et al.*, 1988; Putt and Stoecker, 1989; Stoecker and Capuzzo, 1990; Stoecker and Egloff, 1987; Stoecker and Sanders, 1985; Verity and Langdon, 1984).

3. Metabolism

3.1. Egestion

A proportion of the nitrogen ingested by zooplankton is assimilated, or taken up into the organism's tissues, while the rest is egested as feces. Fecal pellet production is an important mechanism for N cycling and transport to deep water and the sediments. The cycling of N and the amount of N transported from the euphotic zone to depth via fecal pellet production is dependent upon both the N composition of the pellets as well as on leaching of soluble nitrogenous products from pellets.

The elemental composition of fecal pellets can be affected by the amount, type, and quality of prey that is consumed (Urban-Rich *et al.*, 1998). The N content of fecal pellets has been measured for a variety of zooplankton feeding on natural diets and on unialgal cultures of different concentrations. Table 26.3 (also see Morales, 1987) shows examples of N content and C:N ratio of feces from a number of zooplankton species feeding on various food sources. The N content of feces ranges over three orders of magnitude, from about 13 ng N per pellet for small copepods (*Acartia clausi*), up to 32 μg N per pellet for large salps (*Salpa maxima*). The C:N ratios of freshly egested fecal pellets are generally higher than algal food, indicating preferential assimilation of N over C (e.g., Paffenhöfer and Knowles, 1979; Downs and Lorenzen, 1985; Morales, 1987; Table 26.3). Food quality also effects fecal pellet C:N ratio. During early phytoplankton bloom conditions, the C:N of *A. tonsa* pellets was lower (~4) than during the late bloom (~7), again suggesting copepods preferentially conserve N to maintain appropriate body tissue C:N ratio (Butler and Dam, 1994). However, Madin and Deibel (1998) note that the relatively high C:N ratios of salp fecal pellets, from about 10–20, could also result from the presence in fecal pellets of carbon-rich mucus from the salps' internal feeding net, as opposed to indicating greater absorption of N from ingested food. The superfluous feeding hypothesis (Beklemishev, 1962) predicts that at high food density the fecal pellet C:N ratio should be most similar to the prey C:N, as assimilation decreases at very high food concentration. However, several studies have shown that increasing food quantity does not necessarily decrease fecal pellet C:N ratio as predicted (Butler and Dam, 1994; Morales, 1987; Table 26.3). Several studies have also measured the composition of organic N constituents in feces. Protein constitutes 11.5%, and lipids 1.4%, of the dry weight of salp fecal pellets (Madin, 1982), and protein 4.5–6.2%, and lipids 0.4–0.5%, of the dry weight of euphausiid fecal pellets (Youngbluth *et al.*, 1989). Poulet *et al.* (1986) found the composition of dissolved free amino acids (DFAA) in copepod fecal pellets was dependent upon the type of food ingested, with four amino acids combined (ASP, SER, GLY, and LEU) on average making up 63% of the total DFAA in fecal pellets of four different species of copepods. The diversity of DFAA in fecal pellets was higher than in the surrounding seawater or in their phytoplankton diet, suggesting contribution of DFAAs from the cells of the copepods themselves (Poulet *et al.*, 1986) or from copepod gut bacteria.

Fecal pellets can contain unabsorbed digestive products that then leak into the surrounding water, providing a potentially important source of DOM (Jumars *et al.*, 1989; Lampitt *et al.*, 1990). Fecal pellets may leach relatively more DOM when they

Table 26.3 Composition of zooplankton fecal pellets

Producer	Food conditions	Fecal pellet composition			Fecal pellet C:N	Reference
		Carbon	Nitrogen	Units		
Copepods						
Palaemonetes pugio	*Nitzchia closterium*	20	4.5	% f.p. dry weight	4.5	Johannes and Satomi, 1966
Acartia clausi	Coccolithophorids	133	13–28	ng per f.p.	—	Honjo and Roman, 1978
	Natural (both 2×10^5 cells l^{-1})	96–187	15–38	ng per f.p.	—	Honjo and Roman, 1978
Undinula vulgaris and small copepods	Natural (30–40 µgC l^{-1}, 4–6 µg N l^{-1})	—	—	—	15–16	Gerber and Gerber, 1979
Eucalanus pileatus	*Rhizosolenia alata* (100 µgC l^{-1}, C:N = 5)	—	—	—	15	Paffenhöfer and Knowles, 1979
Pontella meadii	Natural	12	2–3	% f.p. dry weight	4.8	Turner, 1979
Small copepods	Natural	271	50	µg per mg f.p. dry weight	5.4	Small *et al.*, 1983
Large copepods	Natural	324	58	µg per mg f.p. dry weight	5.6	Small *et al.*, 1983
Temora stylifera	*Hymenomonas elongata* (C:N = 6)	154	18	ng per f.p.	8.5	Abou Debs, 1984
Calanus pacificus	*Thalassiosira weissflogii* (C:N = 6)	—	—	—	7.5	Downs and Lorenzen, 1985
A. tonsa and *Temora* sp.	*Thalassiosira* sp. (C:N = 9–13)	—	—	—	10–16	Checkley and Enzeroth, 1985
	Isochrysis galbana (C:N = 8–14)	—	—	—	8–25	Checkley and Enzeroth, 1985

Pseudocalanus spp. and *Temora longicornis*	*Thalassiosira weissflogii* (2×10^2–2×10^4 cells ml^{-1}, C:N = 5.5)	250	30	µg per mg f.p. dry weight	7-9	Morales, 1987
A. tonsa	*Thalassiosira weissflogii* exponential phase (314 and 2405 cells ml^{-1})	25 and 24	7.8 and 5.8	µg per f.p. volume (µm$^3 \times 10^{-7}$)	3.2 and 4.3	Butler and Dam, 1994
	Stationary phase (266 and 1954 cells ml^{-1})	3.7 and 3.7	0.6 and 0.6	µg per f.p. volume (µm$^3 \times 10^{-7}$)	6.1 and 7.1	Butler and Dam, 1994
Pleuromamma xiphias	Natural	416	77	ng per f.p.	6.3	Schnetzer and Steinberg, 2002
Euchirella messinensis	Natural	131	26	ng per f.p.	5.9	Schnetzer and Steinberg, 2002
Euphausiids						
Meganyctiphanes norvegica	Natural	2.6 and 5.8	0.46 and 0.58	µg per f.p.	5.7 and 10.0	Youngbluth *et al.*, 1989
		5.1 and 11.0	0.9 and 1.1	% f.p. dry weight	—	
Thysanopoda aequalis/ Euphausia brevis	Natural	1.2	0.24	µg per f.p.	5.7	Schnetzer and Steinberg, 2002
Salps						
Salpa maxima (aggregate stage, large)	Natural	301[a]	27[b]	µg per f.p	11.1	Madin, 1982
Salpa maxima	Natural	24.4	1.7	% f.p. dry weight	14.5	Madin, 1982
Salpa maxima	Natural	407[c]	31.8[d]	µg per f.p	12.8	Caron *et al.*, 1989

(Continued)

Table 26.3 Composition of zooplankton fecal pellets (*continued*)

Producer	Food conditions	Fecal pellet composition			Fecal pellet C:N	Reference
		Carbon	Nitrogen	Units		
S. cylindrica	Natural	69[c]	7.8[d]	µg per f.p	8.9	Caron *et al.*, 1989
Cyclosalpa pinnata (aggregate stage)	Natural	98[a]	8.5[b]	µg per f.p	11.5	Madin, 1982
C. affinis	Natural	69[c]	9.5[d]	µg per f.p	7.3	Caron *et al.*, 1989
Pegea socia (aggregate stage)	Natural	30[a]	2.4[b]	µg per f.p	12.5	Madin, 1982
P. bicaudata	Natural	183[c]	14.3[d]	µg per f.p	12.8	Caron *et al.*, 1989

Table adapted and updated from Morales (1987).

Units for carbon and nitrogen reported differ among studies and are given separately. Only studies that reported fecal pellet nitrogen composition are listed. f.p. = fecal pellet.

– = Not determined, or not reported in cited reference.

[a] Mean carbon value.

[b] Nitrogen calculated from carbon using given C:N.

[c] Carbon value assumed from AFDW given in reference.

[d] Nitrogen calculated from AFDW using given C:N.

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are newly defecated (Jumars *et al.*, 1989) or DOM losses may be on time scales of hours to days (Strom *et al.*, 1997; Urban-Rich, 1999). The rapid release of DOM via fecal pellet leaching retains the DOM in the euphotic zone, leaving less total POM for vertical transport. Copepods can also enhance DOM leakage by disrupting or breaking apart their own pellets and leaving the pellet vulnerable to physical and microbial degradation (Lampitt *et al.*, 1990). Urban-Rich (1999) and Thor *et al.* (2003) found that *Calanus* spp. and *A. tonsa* fecal pellets, respectively, can leach between 34 and 50% of the total C content of the fecal pellet as DOM in the first 48 hours of egestion. In the only study that has examined DON leakage from fecal pellets, Roy and Poulet (1990) found a rapid decrease in copepod fecal pellet total DFAA concentration within the first 3–5 days of incubation. The amount of DOM leaching from fecal pellets can be a function of food concentration and food type. A higher amount of DOC was leached from copepod fecal pellets when the copepods were feeding on higher concentrations of food (Urban-Rich *et al.*, 1998). Copepod fecal pellets leached higher amounts of DOC when copepods were feeding on dinoflagellates (compared to diatoms) (Thor *et al.*, 2003), and copepod fecal pellets are susceptible to leach higher amounts of DOC when copepods feed on heterotrophs (compared to phytoplankton) (Urban-Rich *et al.*, 1998). Studies examining the relative coupling of C and N during fecal pellet leaching are sorely needed. Daly *et al.* (1999) suggested that C and N release by copepods resulted in quantitatively different fluxes of DOC and DON, which ultimately resulted in highly non-Redfield ratios within those pools.

3.2. Sloppy feeding

Sloppy feeding, the physical breakage of phytoplankton cells, or of prey animals, due to zooplankton grazing can be responsible for the generation of significant amounts of DOM (Dagg, 1974; Jumars *et al.*, 1989; Lampert, 1978; Møller and Nielsen, 2001). The conditions that favor production of DOM via sloppy feeding are when cells are too large to be ingested whole and when prey density is high, such as during phytoplankton blooms (Møller, 2005; Møller, 2007; Møller and Nielsen, 2001; Roy *et al.*, 1989). Most studies to date have solely investigated release of DOC by sloppy feeding. For example, Lampert (1978) found 3–17% of the initial algal C ingested by the cladoceran *Daphnia* feeding on diatoms, flagellates, and green algae was lost as DOC due to sloppy feeding. Measurements of the fraction of phytoplankton POC ingested by copepods lost as DOC via sloppy feeding include 54–69% (Møller and Nielsen, 2001), 49% (Møller *et al.*, 2003), and 7–36% (Møller, 2007). The latter study also demonstrated experimentally that DOC production by sloppy feeding increases as the size of the prey increases relative to the size of copepod grazers (Møller, 2007). Only two studies directly investigating the mechanism of sloppy feeding on N release have been conducted (Roy *et al.*, 1989; Vincent *et al.,* 2007). An investigation of sloppy feeding by copepods feeding on the large diatom *Coscinodiscus* showed that despite significant breakage of the diatom cells, there was little accumulation of DFAA detected (Roy *et al.*, 1989). As *Coscinodiscus* cells have high internal DFAA concentration, the low accumulation DFAA produced by sloppy feeding was attributed to the rapid uptake of DFAA by bacteria and live *Coscinodiscus* cells in the experiments (Roy *et al.*, 1989). In more recent experiments of copepods

feeding on ^{15}N-labelled diatoms, DON release via sloppy feeding was equal to about half of copepod DON excretion. However, only 2–9% of the phytoplankton PON ingested was released as DON via both pathways (i.e., excretion and sloppy feeding; phytoplankton direct release was minimal) (Vincent *et al.*, 2007).

Increased release of amino acids (Furhman, 1987; Roman *et al.*, 1988; Poulet *et al.*, 1991; Williams and Poulet, 1986) or bulk DON (Hasegawa *et al.*, 2000) as a result of zooplankton feeding has been demonstrated, although the proportion of N release attributed to sloppy feeding (vs. excretion or other mechanisms) was unknown. A positive correlation was found between the vertical distribution and abundance of zooplankton and the concentration of amino acids with depth (Poulet *et al.*, 1991; Williams and Poulet, 1986). Although the exact mechanism was not investigated, it was presumed that the correlation was a result of zooplankton sloppy feeding and excretion (Poulet *et al.*, 1991; Williams and Poulet, 1986). Using an isotope dilution approach, Fuhrman (1987) found DFAA release by copepods was comparable to direct release by microplankton. In mesocosm experiments with copepod grazers, release rates of both ammonium and DFAAs were higher in treatments with copepods versus those with microplankton alone (Roman *et al.*, 1988). Other investigations measuring bulk DON release have also found increased release of DON in the presence of grazers, however, release rates when normalized to zooplankton biomass were minor compared to microplankton (<94 μm) (Hasegawa *et al.*, 2000).

3.3. Assimilation

The assimilated portion of food ingested is that which is absorbed through the gut wall and taken up into the animal's tissues, with the rest of the food egested as feces. The assimilation efficiency (AE) = $(I-E)/I \times 100\%$, where I is the ingestion rate and E is the egestion (defecation) rate. Assimilation efficiency varies with prey type, with AE for herbivorous species generally ranging from 60 to 95%, and carnivorous species higher, at more than 90% (Parsons *et al.*, 1984). N is assimilated at a much higher efficiency than carbon, especially in food with lower N and protein contents (Cowie and Hedges, 1996; Hassett and Landry, 1988; Kiørboe *et al.*, 1985; Landry *et al.*, 1984). A review of the literature on copepod assimilation efficiency also notes that on average copepods tend to assimilate nitrogen (AE = 77%) more efficiently than carbon (AE = 63%) (Tang and Dam, 1999; Tang, unpublished). This trend is also reported for salps (*Cyclosalpa bakeri*), with an AE of 71% for nitrogen and 61% for carbon (Madin and Purcell, 1992). The gammarid amphipod *Calliopius laeviusculus* feeding on copepods, however, had a similar assimilation efficiency for nitrogen (89%) and carbon (90%) (Dagg, 1976). Landry *et al.* (1984) found the assimilation efficiencies for both N (73.9–92.5%) and C (68.5–85.4%) for *C. pacificus* feeding on *T. weissflogii* were highest at low food concentrations, most likely due to increased digestive enzyme activity and ingestion capacity. As discussed elsewhere in this chapter (see Sections 3.1. and 3.6.), a preferential assimilation of nitrogen over carbon from ingested food may indicate a N deficiency in the food, thus the animal must assimilate more N relative to carbon to meet its nitrogen requirements. It should be noted, however, that a preferential assimilation of N over C does not necessarily indicate a N deficiency in the food–which always has more C than

N—but rather that zooplankton have evolved to be more efficient at assimilating N compounds which they need for growth.

3.4. Excretion

3.4.1. Products, rates, and pathways of nitrogen excretion

3.4.1.1. *Inorganic*   Inorganic N in the form of ammonium is considered the primary nitrogenous excretory product of mesozooplankton (e.g., Bidigare, 1983) and micro-zooplankton (Caron and Goldman, 1990), a trait shared by most aquatic invertebrates and teleost fishes (Regnault, 1987). Ammonium is produced as an endpoint of the catabolism of proteins and amino acids, via the oxidative deamination of glutamate through the enzyme glutamate dehydrogenase (GDH) (Bidigare, 1983; Le Borgne, 1986; Regnault, 1987). In copepods, excretion is through the maxillary glands which are located in the head region, and in the euphausiids excretion is through the gills (Ruppert and Barnes, 1996). In gelatinous zooplankton such as the cnidarians (e.g., medusae), nitrogenous waste diffuses through the body surface (Ruppert and Barnes, 1996). In marine protozoa waste is excreted via a contractile vacuole, or via diffusion (Weatherby, 1929).

Ammonia excretion rates of zooplankton have been extensively reported in the literature. A number of compilations and reviews (e.g., Ikeda *et al.*, 2001 for epipelagic copepods; Madin and Deibel, 1998 for salps and doliolids; Huntley and Nordhausen, 1995 and Ikeda and Mitchell, 1982 for Antarctic zooplankton; Atkinson and Whitehouse, 2000 for Antarctic krill) report equations to allow prediction of excretion rates for a given animal as a function of body mass and temperature (or on body mass alone). Table 26.4 lists examples of individual animal ammonia excretion rates across a variety of zooplankton taxa and environments. Excretion rates are highly dependent on animal size, temperature, feeding history, and other factors which are addressed in Section 3.4.2. Figure 26.4 shows the relationship between animal dry weight and ammonia excretion for epipelagic copepods from a wide range of environments (Ikeda *et al.*, 2001). When comparing multiple taxa, it is most useful to report excretion rate per unit animal body weight (e.g., per mg wet weight or dry weight, or dry weight of nitrogen or carbon), or for gelatinous zooplankton, body volume (per milliliter) is also used. Studies have shown that mass-specific ammonia excretion rates of gelatinous zooplankton (e.g., salps, cnidarians, ctenophores) are about an order of magnitude lower than other (e.g., crustacean) taxa if dry weight is used as the body mass unit (Schneider, 1990). However, if carbon is used as a body mass unit, the weight-specific ammonia excretion rates of gelatinous zooplankton are comparable to the crustacea (Schneider, 1990), as carbon is a significantly smaller percentage (<10%) of gelatinous zooplankton dry weight than of crustacean dry weight (C ~40% of dry weight).

As shown in Table 26.4, individual animal ammonia excretion rates (µg N ind^{-1} h^{-1}) range over 10 orders of magnitude, from 2.7×10^{-8} to 4.5×10^{-4} for protozoa, to 0.003 for small tropical copepods, up to 546 for large scyphozoan medusae. Weight specific ammonia excretion (µg N mg dry wt^{-1} h^{-1}) is generally highest for the smallest organisms, ranging from 0.2–178 for protozoans, to 0.01–4.51 for copepods, to 0.001–0.33 for larger crustacean zooplankton, and to 0.006–4.7 for gelatinous zooplankton. For copepods, ammonia excretion as a proportion of body N per day on average is generally <10%, with the exception

Table 26.4 Example of ammonia excretion rates for a variety of zooplankton taxa in different environments

Taxa and species	Environment	Temp (°C)	Body dry weight range (mg)	Excretion rate (μg N ind^{-1}h^{-1})	Excretion rate (μg N mg dry wt^{-1}h^{-1})	% Body N excreted d^{-1}	Reference
Microzooplankton (protozoa)							
Ciliates							
Colpidium campylum	Cultures	—	1.1×10^{-5} [a]	3.24×10^{-6} [b]	0.3	36	Nardone, 1949; Nardone and Wilbur, 1950
Cyclidium sp.	N. Central Pacific isolate	22	7.2×10^{-8} [a]	3.5×10^{-6}–1.3×10^{-5} [b]	48–178	5760–21,360	Berman _et al._, 1987
Didinium sp.	Cultures	—	2.0×10^{-4} [a]	3.0×10^{-4} [b]	1.5	180	Weatherby, 1929
Euplotes vannus	North Sea isolate	25	1.5×10^{-4} [c]	4.5×10^{-4} [b]	3.0	360	Gast and Horstmann, 1983
Paramecium aurelia	Cultures	27	2.0×10^{-5} [a]	2.0–8.0×10^{-5} [b]	1.0, 4.0	120–480	Soldo and Wagtendonk, 1961
Strombidium sulcatum	Mediterranean	12	1.7×10^{-6} [a]	4.3×10^{-7}–3.5×10^{-6} [b]	0.25–2.0	30–240	Ferrier-Pagès and Rassoulzadegan, 1994
Tintinnopsis acuminata	Narragansett Bay, Massachusettes (MA) isolate	5–15	1.8×10^{-6} [a]	7.9×10^{-6}–1.8×10^{-5} [b]	4.3–10	516–1200	Verity, 1985a
T. vasculum	Narragansett Bay, MA isolate	5–15	2.8×10^{-5} [a]	4.3–8.5×10^{-5} [b]	1.5–3.0	180–360	Verity, 1985a
Flagellates							
Bodo caudatus	Cultures	25	5.0×10^{-8} [a]	1.5×10^{-7} [b]	3.0	180	Lawrie, 1935
Monas sp.	Lake Kinneret, Israel isolate	3–30	7.0×10^{-9}	4.5×10^{-8}–3.6×10^{-7} [b]	6.4–51	384–3060	Sherr _et al._, 1983
Ochromonas sp.	Baltic Sea isolate	22	2.0×10^{-9} [a]	5.6×10^{-8} [b]	28	1680	Andersson _et al._, 1985
Paraphysomonas imperorata	Vineyard Sound, MA isolate	20–24	4.2×10^{-8} [a]	1.3–8.5×10^{-7} [b]	3–20	180–1200	Goldman _et al._, 1985
Pseudobodo sp.	Mediterranean	12	9.6×10^{-9} [a]	2.7–8.2×10^{-8} [b]	2.8–8.5	168–510	Ferrier-Pagès and Rassoulzadegan, 1994

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Crustacean Mesozooplankton
Copepods

12 Species	Inshore Great Barrier Reef	27–29	0.2–8.3×10^{-2}	0.3–5.1×10^{-2}	0.24–4.51	5.7–108	Ikeda *et al.*, 1982
13 Species	Inshore Great Barrier Reef	22	0.8–31×10^{-2}	0.5–8.8×10^{-2}	0.11–1.04	2.6–24.9	Ikeda *et al.*, 1982
16 Species	Tropical-various	24–29	0.006–0.7	0.4–16.4×10^{-2}	0.2–2.2	4.8–52.8	Ikeda *et al.*, 2001
16 Species	Subtropical-various	17.3–24	0.007–0.5	0.5–10.2×10^{-2}	0.2–0.9	4.9–20.6	Ikeda *et al.*, 2001
Pleuromamma xiphias	Sargasso Sea	21–26	0.5–0.6	0.2	0.41–0.42	14.2–14.6	Steinberg *et al.*, 2002
9 Species	Sargasso Sea	21–29	0.006–0.84	0.3–10.3×10^{-2}	0.26–1.42	5.9–36.3	Verity, 1985b
12 Species	Boreal-various	5.6–15.9	0.003–1.59	0.2–16.4×10^{-2}	0.02–0.71	0.5–16.9	Ikeda *et al.*, 2001
4 Species	Arctic	−0.3–2.3	0.35–3.95	1.3–5.1×10^{-2}	0.01–0.07	0.3–1.6	Ikeda *et al.*, 2001
4 Species	Antarctic	−0.2 to–1.7	0.22–1.08	1.4–11.3×10^{-2}	0.05–0.12	1.2–2.8	Ikeda *et al.*, 2001
Gaussia princeps	Mesopelagic, east Pacific	5.5	7.0	0.12	0.02	0.12	Quetin *et al.*, 1980

Amphipods

Anchylomera blossevillei	Sargasso Sea	20–26	2.4–5.7	0.8–1.1	0.20–0.33	6.9–11.4	Steinberg *et al.*, 2002
Paracallisoma coecus	Mesopelagic, east Pacific	5.5	50	0.05	0.1×10^{-2}	0.03	Quetin *et al.*, 1980
3 Species	Antarctic	−0.8 to–1.1	3.6–105.3	0.27–2.64	0.04–0.08	1.0–2.1	Ikeda and Mitchell, 1982

Euphausiids

Thysanoessa macrura	Antarctic	−0.5	9.96	0.1	0.01	0.5	Huntley and Nordhausen, 1995
Euphausiia superba[d]	Antarctic	0–2	17–73	—	0.02–0.04	2[e]	Atkinson and Whitehouse, 2000
E. superba	Antarctic	−1 to–1.1	27–353	1.2–10.3	0.03–0.04	0.7–1	Ikeda and Mitchell, 1982
Bentheuphausia amblyops	Mesopelagic, east Pacific	5.5	60	0.3	0.5×10^{-2}	0.12	Quetin *et al.*, 1980
3 Species	Sargasso Sea	20–21	3.0–7.8	0.4–1.1	0.14–0.15	4.6–5.2	Steinberg *et al.*, 2002

(*Continued*)

Table 26.4 Example of ammonia excretion rates for a variety of zooplankton taxa in different environments (*continued*)

Taxa and species	Environment	Temp (°C)	Body dry weight range (mg)	Excretion rate (μg N ind^{-1}h^{-1})	Excretion rate (μg N mg dry wt^{-1}h^{-1})	% Body N excreted d^{-1}	Reference
Ostracods							
Conchoecia sp.	Antarctic	−0.5	0.33	0.02	0.06	2.6	Huntley and Nordhausen, 1995
Shrimps							
Sergestes atlanticus	Sargasso Sea	21–24	9–23	2.4–3.5	0.15–0.26	5.1–9.0	Steinberg *et al.*, 2002
11 Species	Mesopelagic, east Pacific	5.5	110–840	0.75–15.1	0.1–5.1×10^{-2}	0.03–1.2	Quetin *et al.*, 1980
Mysids							
3 Species	Mesopelagic, east Pacific	5.5	280–930	3.88–57.1	0.01–0.09	0.3–2.1	Quetin *et al.*, 1980
Gelatinous Zooplankton							
Chaetognaths							
Sagitta spp.	Inshore Great Barrier Reef	26.5–29.0	0.07–0.17	0.02–0.06	0.31–0.37	7.5–9.0	Ikeda *et al.*, 1982
Sagitta spp.	Inshore Great Barrier Reef	22.0	0.11–0.33	0.05–0.08	0.15–0.54	3.6–12.9	Ikeda *et al.*, 1982
Ctenophores							
Pleurobrachia sp.	Inshore Great Barrier Reef	26.5–30.0	0.69–0.60	0.03–0.23	0.05–0.38	1.2–9.2	Ikeda *et al.*, 1982
Pleurobrachia sp.	Inshore Great Barrier Reef	22	4.22	0.06	0.01	0.3	Ikeda *et al.*, 1982
Mnemiopsis leidyi	Chesapeake Bay	18–28	7–391	0.7–5.5	0.02–0.06	16.5–18.4	Nemazie *et al.*, 1993
Beroe sp.	Antarctic	−0.8	401.6	2.21	0.01	0.6	Ikeda and Mitchell, 1982
4 Species[f]	Bahamas, tropical Atlantic	25	6–1500	—	0.6–3.2×10^{-2}	5–8	Kremer *et al.*, 1986
3 Species	Subtropical, tropical Atlantic	23–29	—	—	0.23–0.28	—	Biggs, 1977

Heteropods

Carinaria sp.	Mesopelagic, east Pacific	5.5	40	1.96	0.05	1.2	Quetin *et al.*, 1980
Pterotrachea hippocampus	Subtropical, tropical Atlantic	23–29	—	—	0.7	—	Biggs, 1977
Polychaetes (gelatinous species)							
Naiades sp.	Sargasso Sea	19	19.3	2.1	0.11	3.7	Steinberg *et al.*, 2002
Tomopteris carpenteri	Antarctic	−0.8 to−1.0	9.7–137.9	0.35–4.17	0.03–0.04	0.9–1.0	Ikeda and Mitchell, 1982
Pteropods							
3 Species	Antarctic	−1.0 to−1.1	6.5–54.8	0.38–2.4	0.04–0.06	1.2–1.7	Ikeda and Mitchell, 1982
2 Species	Subtropical, tropical Atlantic	23–29	—	—	0.05–0.12	—	Biggs, 1977
Salps							
6 Species	Various	21–28, 13.5–19.5[h]	—	0.07–14	—	6.4–55.6	Cetta *et al.*, 1986
2 Species	Antarctic	−1.0 to−1.1	10.9–114.6	0.15–0.81	0.01	86–200	Ikeda and Mitchell, 1982
5 Species	Subtropical, tropical Atlantic	23–29	—	—	0.18–0.71	—	Biggs, 1977
Scyphomedusae							
Pelagia noctiluca	Palau, Western Caroline Islands	21	—	—	$2.7\text{–}5.7\times10^{-2}$	3.3	Muscatine and Marian, 1982
Aurelia aurita	Western Baltic	15	1857–19,928	91–546	$1.7\text{–}5.5\times10^{-2}$	3.9–9.7	Schneider, 1989
Chrysaora quinquecirrha	Chesapeake Bay	18–28	13–2830	0.28–124.5	$1.0\text{–}8.5\times10^{-2}$	4.9–5.6	Nemazie *et al.*, 1993
2 Species	Subtropical, tropical Atlantic	23–29	—	—	$0.5\text{–}2.8\times10^{-1}$ [g]	—	Biggs, 1977
Siphonophores							
Sphaeronectes gracilis	Catalina Island, coastal California	13–14	6.3[i]	0.05–0.1	$0.9\text{–}1.5\times10^{-2}$	3–5	Purcell and Kremer, 1983

(*Continued*)

Table 26.4 Example of ammonia excretion rates for a variety of zooplankton taxa in different environments (*continued*)

Taxa and species	Environment	Temp (°C)	Body dry weight range (mg)	Excretion rate (μg N ind^{-1}h^{-1})	Excretion rate (μg N mg dry wt^{-1}h^{-1})	% Body N excreted d^{-1}	Reference
12 Species	Subtropical, tropical Atlantic	23–29	—	—	0.2–6.2$\times$10$^{-1\,g}$	—	Biggs, 1977
Hydromedusae							
Clytia spp.	Bay of Villefranche-sur-mer	15–21	0.5–14.5$\times$10^{-2}	0.02–0.55	1.1–4.7	6–34	Matsakis, 1992
3 Species	Subtropical, tropical Atlantic	23–29	—	—	0.9–3.5 $\times$ 10^{-1}	—	Biggs, 1977

When percent body N excreted per day was not available in the reference, it was calculated using body N values provided. If body N data was not provided, we assumed body N = 10% body dry weight (DW) for crustacea, body N = 2% DW for ciliates and body N = 4% DW for flagellates (calculated from Tables 26.1 and 26.2).

— = Not determined, or not reported in cited reference.

[a] Individual mean cell volumes assumed from literature values and converted to wet weight (1 μm^3 = 1 pg wet weight) and dry weight (dry weight = 0.2 $\times$ wet weight) (Caron and Goldman, 1990).

[b] Excretion rate(μg N ind^{-1} h^{-1}) calculated by multiplying average excretion rate (μg N mg DW^{-1} h^{-1}) by average DW (mg).

[c] Body dry weight calculated from dividing average excretion rate(μg N ind^{-1} h^{-1}) by the average weight-specific excretion rate (μg N mg DW^{-1} h^{-1}).

[d] Data listed is from freshly caught krill in filtered seawater (Atkinson and Whitehouse, 2000).

[e] Maximum.

[f] See Table 26.3; Kremer *et al.* (1986).

[g] Excretion rates in Biggs (1977) are given in mg body protein^{-1}, this was converted to dry weight assuming body protein = 23% DW (for gelatinous zooplankton; Table 26.1).

[h] For *Salpa fusiformis*.

[i] Mean dry weight.
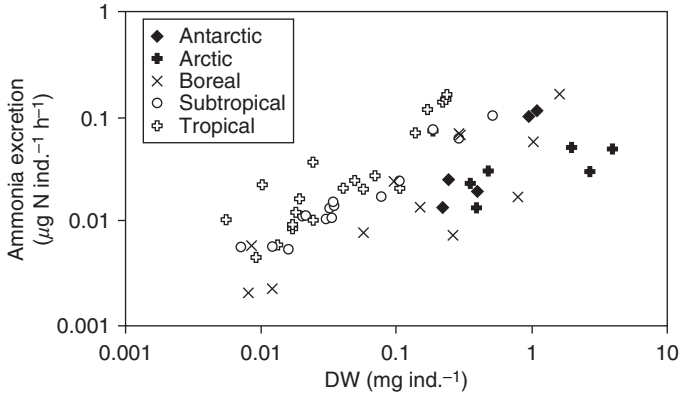
Figure 26.4 Scatter diagram showing the relationship between dry weight and ammonia excretion rate for epipelagic marine copepods. The data points represent mean rates for all copepod species from each type of environment, Antarctic, Arctic, boreal, subtropical, and tropical (temperature range: −1.7 to 29°C). (Adapted from Ikeda *et al.*, 2001).

of tropical species which is higher (on average ~20%, calculated from Ikeda *et al.*, 1982, 2001). In a global estimation of open-ocean mesozooplankton excretion in the epipelagic zone, Hernández-León *et al.* (2008) also show that specific ammonium excretion rates are highest in the tropics (~20–25% body N per day), and decrease rapidly toward the poles to <5%. Excretion as a proportion of body N per day for larger crustacean and gelatinous zooplankton, is also generally <10%, but is occasionally high for some taxa, such as the salps, which can turn over their entire body N content in a day (Table 26.4). At the far end of the spectrum are the marine protozoa which are capable of excreting ammonia at a rate hundreds to thousands of times their body weight per day, illustrating their importance in remineralization (Table 26.4).

3.4.1.2. *Organic*

DON, such as urea and amino acids, is generally considered to be excreted at lower, but substantial levels compared to ammonia (Bidigare, 1983). There are, however, few measurements of the relative importance of inorganic versus organic excretion by zooplankton, and rates of DON excretion vary greatly (Steinberg *et al.*, 2000, 2002). Indeed some studies indicate DON frequently exceeds ammonia as the primary nitrogenous excretory product (Miller and Glibert, 1998; Conover and Gustavson, 1999). The handful of studies to date that report excretion of DON by zooplankton indicate DON can make up a substantial proportion of the total dissolved nitrogen (TDN) excreted. Table 26.5 (updated from Steinberg *et al.*, 2002) shows a variety of planktonic crustaceans (copepods, amphipods, euphausiids, mysids, shrimp) with combined urea and amino acid excretion, or excretion of total DON, ranging from 7 to 89% of the TDN excreted (Note: The definition of TDN varies in these studies as is indicated in Table 26.5, but is normally given as ammonia, urea, and amino acids combined). Only a few studies have reported organic N excretion by gelatinous zooplankton. Combined urea and amino acid excretion, or excretion of total DON, for gelatinous zooplankton (ctenophores, pteropods, chaetognaths, salps, and siphonophores) ranges from 16 to 46% of the TDN excreted

Table 26.5 Proportion of total dissolved nitrogen (TDN; inorganic+organic) excreted as DON (the key below denotes how % TDN excreted as DON was measured for each study)

Taxa and species	% Urea	% Amino acids	% TDN excreted as DON	Reference
Microzooplankton				
Tintinnids	40[a]	—	—	Johansen, 1976, in Verity, 1985a
Crustacea				
Copepods				
Calanus chilensis	6–17	11–14	20–28[b]	Dagg *et al.*, 1980
Centropages brachiatus	53	—	53[b]	Dagg *et al.*, 1980
Eucalanus inermis	8–28	0	12–43[b]	Dagg *et al.*, 1980
Acartia clausi	30–54	5–15	16[c]	Mayzaud, 1973
A. tonsa	23	18–48	62–89[b]	Miller and Glibert, 1998
Calanus glacialis	1	—	29[b]	Ikeda and Skjoldal, 1989
C. hyperboreus	8[a]	6	7[b]	Ikeda and Skjoldal, 1989
C. helgolandicus	—	6	22[c]	Corner *et al.*, 1976c; Butler *et al.*, 1969b
Pleuromamma xiphias	—	—	21[c]	Steinberg *et al.*, 2002
Amphipods				
Amphipod	—	—	25[c]	Roger, 1982
Phronima sedentaria	—	—	49[c]	Mayzaud, 1973
Parathemisto libellula	—	—	20[b]	Ikeda and Skjoldal, 1989
Anchylomera blossevillei	—	—	24[b]	Steinberg *et al.*, 2002
Euphausiids				
Euphausiids	1	13	41–44[c]	Roger, 1982
Euphausiia pacifica	—	—	14[b]	Jawed, 1969
Meganyctiphanes norvegica	—	—	14[c]	Mayzaud, 1973
Nematobrachion flexipes	—	—	40[c]	Steinberg *et al.*, 2002
Thysanopoda aequalis and *Euphausia brevis*	—	—	26[c]	Steinberg *et al.*, 2002
Mysids				
Neomysis rayii	0	18	18[b]	Jawed, 1969

Shrimps				
Peneid shrimp	—	—	26–34[c]	Roger, 1982
Sergestid shrimp	—	—	29–32[c]	Roger, 1982
Caridean shrimp	—	—	21–45[c]	Roger, 1982
Sergestes atlanticus	—	—	29[c]	Steinberg *et al.*, 2002
Gelatinous Zooplankton				
Ctenophore				
Mnemiopsis leidyi	—	21	46[c]	Kremer, 1977
Pteropod				
Clione limacina	12	10	22[b]	Ikeda and Skjoldal, 1989
Cheatognaths				
Sagitta elegans	1	15	16[b]	Ikeda and Skjoldal, 1989
S. setosa	—	—	28[c]	Mayzaud, 1973
Salps				
Salpa fusiformis	—	—	41[c]	Mayzaud and Dallot, 1973
Thalia democratica	—	—	30[c]	Mayzaud and Dallot, 1973
Siphonophore				
Sphaeronectes gracilis	—	—	40[c]	Purcell and Kremer, 1983

Table is modified and updated from Steinberg *et al.* (2002).

% Urea, % total dissolved nitrogen excreted as urea; % amino acids, % total dissolved nitrogen excreted as amino acids; % TDN excreted as DON, % total dissolved nitrogen excreted as DON. Note: Most studies did not measure actual total dissolved nitrogen, but rather ammonia, urea, and amino acids.

—= Not determined, or not reported in cited reference.

[a] Urea/(ammonia + urea).

[b] (Urea + amino acid)/(ammonia + urea + amino acids).

[c] Total DON/TDN.

(Table 26.5). Mucus production by gelatinous zooplankton may be another important source of DON release. For example, Steinberg *et al.* (2002) found 66% of TDN was excreted as DON for gelatinous, mucus-releasing alciopid worms, and although DON production was not measured, the medusa *A. aurita* may release up to 7% of its assimilated C as DOC mainly through mucus production (Hansson and Norrman, 1995). LeBorgne (1982b, 1986) shows that the organic fraction of nitrogen excretion of a mixed zooplankton assemblage decreases with increasing weight percentage of copepods in the tropical Atlantic, suggesting gelatinous zooplankton and other taxa are relatively more important to DON excretion. There are even fewer data on microzooplankton DON excretion. Tintinnid urea release is 67% of ammonia release, equal to 40% of total N (ammonia + urea) release (calculated from Johansen, 1976, as referenced in Verity, 1985a) (Table 26.5). Studies to date do indicate marine protozoa can release a significant amount of ingested N as DON. Table 26.6 shows 1–22% of the total nitrogen ingested (note—not of total N excreted, as in Table 26.5) by flagellates and ciliates feeding on bacteria is released as amino acids.

The proportion of TDN that is excreted as DON and the DON composition may be affected by the quantity and quality of the food that is consumed (Miller and Roman, 2008; Miller and Glibert, 1998) (also see Section 3.4.2.3. below). For example, some studies show that zooplankton increase their organic, relative to inorganic, excretion of N when starved (Mayzaud, 1973; Miller and Roman, 2008). It has also been suggested that herbivores may be expected to excrete more urea than carnivores, as arginine (a precursor of urea) levels are higher in marine phytoplankton than in zooplankton (Bidigare, 1983). The composition of DON excreted may be dependent upon species, as shown by the relative amounts of different amino acids excreted by several mesozooplankton taxa (Webb and Johannes, 1967). DON may also be released in different patterns from ammonia. While ammonia is released continuously in crustacea (Regnault, 1987), amino acids can be released in high-concentration "spurts" lasting from 20 to 60 min (Gardner and Paffenhöfer, 1982). Steinberg *et al.* (2002) found a decrease or leveling off in DON excretion rates of migrating zooplankton, possibly due to this non-continuous release of DON.

Table 26.6 Proportion of total nitrogen ingested by microzooplankton (protozoa) released as dissolved amino acids

Taxa and species	% Total N ingested released as amino acids	Reference
Flagellates		
Paraphysomonas imperforata	1–22	Nagata and Kirchman, 1991
Ochromonas sp.	7	Andersson *et al.*, 1985
Pseudobodo sp.	10	Ferrier-Pagès *et al.*, 1998
Ciliates		
Strombidium sulcatum	16	Ferrier-Pagès *et al.*, 1998

3.4.2. Factors affecting excretion rates

3.4.2.1. Temperature
As described above in Section 3.4.1.1., temperature, along with body mass, is a dominant factor affecting excretion rate, and has been used extensively to predict excretion rates of marine zooplankton using multiple regression models (Ikeda, 1985; Ikeda *et al.*, 2001). Metabolic rate normally increases as temperature increases. The relationship between metabolic rate and temperature for marine zooplankton is described most commonly as the Q_{10} value

$$Q_{10} = \left(\frac{r_1}{r_2}\right)^{\frac{10}{(t_1 - t_2)}}$$

where r_1 and r_2 are the rate of excretion (or respiration) at temperature t_1 and t_2, respectively. The Q_{10}, the factor by which metabolic rates increase for a $10°C$ increase in temperature, is generally around 2 ± 0.5 for zooplankton ammonia excretion, but ranges between ~1 and 4 (e.g., Table II in Bidigare, 1983). Analysis of extensive metabolic datasets compiled for 38 species of copepods from habitats ranging from -1.7 to $29°C$, and a phylogenetically diverse range of epipelagic zooplankton from habitats -1.4 to $30°C$, revealed a range of Q_{10} values for ammonia excretion from 1.8 to 2.0 (Ikeda *et al.*, 2001), and 1.9 (Ikeda, 1985), respectively. Reported Q_{10} values for ammonia excretion of some gelatinous zooplankton include 2.1–2.3 for the salp *Salpa fusiformis* (Andersen and Nival, 1986), ~4 (Kremer, 1977) and 1.6 (Park and Carpenter, 1987) for the ctenophore *Mnemiopsis leidyi*, and 1.3–2.0 for the hydromedusae *Clytia* spp. (Matsakis, 1992). Little information is available on Q_{10} for microzooplankton excretion; the average Q_{10} for ammonia excretion for the microflagellate *Monas* sp. was 5.1 (range 0.7–20.5) (calculated from Table 3 in Sherr *et al.*, 1983). Rearrangement of the above equation by taking the natural logarithm of both sides makes Q_{10} useful for predicting excretion rates of zooplankton at different temperatures due to changes in depth (e.g., vertical migration) or seasons. Temperature can affect different metabolic processes differently, resulting in varying metabolic quotients (e.g., O:N, or oxygen consumption–ammonia excretion ratio, which also varies with the substrate being metabolized) and thus Q_{10} for excretion for a given organism is not always the same as the Q_{10} for respiration (LeBorgne, 1986).

3.4.2.2. Body size (weight)
Excretion (or respiration) rate (Y) can be related to body weight (W) according to the allometric equation:

$$Y = aW^b$$

where a is the species/temperature dependent constant and b is the size-dependent (or "body-mass scaling") constant (Bidigare, 1983). The coefficients a and b have been characterized well due to their usefulness in modeling (LeBorgne, 1986). For most zooplankton, weight-specific excretion rate (e.g., mg N mg dry weight^{-1} h^{-1}) decreases with increasing body weight (size) (e.g., Table 26.4), and b is less than 1, and usually in the range 0.7–0.9 (Vidal and Whitledge, 1982; Ikeda *et al.*, 2000).

Both coefficients vary with the weight unit applied (e.g., dry weight, carbon, or nitrogen) and type of metabolism (e.g., ammonia excretion, phosphate excretion, or respiration) being studied. Schneider (1990), in comparing ammonia excretion rates between gelatinous and nongelatinous zooplankton, found body carbon or nitrogen units were best for making broad interspecific comparisons as they reduced differences in body composition among the different taxa.

3.4.2.3. Nutritional level

The effects of food level and starvation on excretion rates of marine zooplankton have been reasonably well studied. Excretion rates tend to increase with increasing food availability and ingestion rate, and are also dependent upon the type of food. For example, excretion of both ammonia and urea by the copepod *C. helgolandicus* feeding carnivorously on barnacle nauplii increased with increasing food concentration and ingestion rate (Corner *et al.*, 1976), as did excretion of ammonia by the copepod *E. pileatus* feeding on diatoms (Gardner and Paffenhöfer, 1982), ammonia and phosphate excretion of *Metridia pacifica* feeding on algal cultures (Takahashi and Ikeda, 1975), and ammonia excretion of the ctenophore *Mnemiopsis mccradyi* feeding on copepods (Kremer, 1982). However, Miller and Landry (1984) found ammonia excretion rates of the copepod *C. pacificus* were relatively constant with increasing diatom density, implying this copepod increases its growth efficiency as ingestion rate increases. The copepod *T. stylifera* excreted twice as much ammonia when fed on a diet of the prymnesiophyte *Hymenomonas elongata* as when fed on a diet of the diatom *Phaeodactylum tricornutum* (Abou Debs, 1984), indicating food type plays a major role as well. Excretion rates of Antarctic krill acclimated to both filtered seawater and to excess food were 30% higher during feeding periods (Atkinson and Whitehouse, 2000). Similarly, excretion rates of Antarctic krill acclimated to filtered seawater and to six different types of food excreted 4.5 times more ammonia when feeding (Ikeda and Dixon, 1984). Thus, excretion rates measured during long incubations (e.g., 24 h) without food will likely underestimate excretion. In addition to excretion rates of fed animals being higher than starved, the relative proportion of metabolic substrate catabolized (i.e., lipids vs. proteins) can vary, resulting in different O:N, N:P, and O:P metabolic ratios in starved versus fed zooplankton (Ikeda, 1977; Mayzaud, 1973; Quetin *et al.*, 1980).

3.4.2.4. Other miscellaneous environmental factors

While temperature, body size, and nutritional status of zooplankton play the largest role in affecting excretion, other factors such as light and salinity can also play a role. Light can play a significant, indirect role in excretion, and diel patterns in grazing and excretion have been documented. Ammonium excretion in surface waters of the vertically migrating copepod *Calanus helgolandicus* was 1.7 times higher at night than day (Harris and Malej, 1986). This pulsing and periodicity in ammonia input as a result of zooplankton diel migration may be significant for phytoplankton N uptake, especially in oligotrophic waters (Harris and Malej, 1986). Conversely, Miller and Glibert (1998) found ammonia excretion by the calanoid copepod *A. tonsa* was higher in the day, and excretion of urea and dissolved primary amines higher in the early morning and early evening. Pitt *et al.* (2005) found no net ammonia excretion by the scyphozoan medusae *Phyllorhiza punctata* during the day due to ammonia uptake by their

symbiotic zooxanthellae, but net ammonia excretion was measured at night (under nutrient-enriched conditions). A second scyphozoan species with no or few zooxanthellae, *Catostylus mosaicus*, excreted 1.5 times more ammonia during the day than night (Pitt *et al.*, 2005). Thus, the form of N excreted can also be tied to a diel cycle, and the diel pattern varies by species. This may partially be explained by changing phytoplankton metabolism and products of photosynthesis over the diel cycle, resulting in diel alterations in the ratio of phytoplankton protein:lipid: carbohydrate (Cuhel *et al.*, 1984; Morris, 1981). Thus phytoplankton N substrates ingested by herbivorous zooplankton may vary over a diel cycle (Cuhel *et al.*, 1984), which could in turn affect N excretion products. Finally, the interplay between zooplankton grazing and ammonia excretion, and phytoplankton ammonia uptake over the diel cycle can be more important than physical mixing as a generator of cyclic ammonia patterns in surface waters, as noted in the Southern Ocean (Priddle *et al.*, 1997).

Salinity effects on N excretion will be most pronounced in coastal or estuarine environments where organisms are exposed to tidal fluctuations in salinity. Although highly dependent on the osmoregulatory capabilities of the organism in question, studies on the effect of these kinds of salinity changes on zooplankton excretion rates tend to show ammonia excretion rates increase when animals are hyper-regulating (adjusting to less saline conditions, as in an ebb tide), and decrease when they are hypo-regulating (adjusting to more saline conditions, as in a flood tide) (Regnault, 1987). Ammonia excretion and oxygen consumption of the under-ice arctic amphipod *Onisimus glacialis* increased with decreasing salinity (Aarset and Aunass, 1990). Changes in salinity have been reported to cause ionic shifts in estuarine scyphomedusae affecting their size and weight (Wright and Purcell, 1997), thus care must be taken with reporting size or weight-specific excretion of gelatinous zooplankton in differing salinities. Finally, to our knowledge, other effects such as hydrostatic pressure, which can affect oxygen consumption rates (e.g., Childress, 1977) have not been investigated for zooplankton N excretion. The toxic effects of high external ammonia concentration on excretion rates, while investigated for some benthic crustaceans and fish (see Regnault, 1987 for review), to our knowledge have not been investigated for marine zooplankton.

3.5. Growth and reproduction

For completeness we include mention of zooplankton growth and reproduction (which together equal secondary production) in this chapter as a pathway of N metabolism; however, much of the recent literature focus on growth is on carbon-based models of weight-specific growth (for copepods see Hirst and Bunker, 2003; Hirst and Lampitt, 1998; Hirst and Sheader, 1997; Huntley and Lopez, 1992; for the most recent synthesis of metazoan invertebrate zooplankton, including copepods, see Hirst *et al.*, 2003; for microzooplankton see Calbet and Landry, 2004; Landry and Calbet, 2004), and we have previously addressed some of the N-based food quality factors that effect egg production in Section 2.2.2. Zooplankton growth rate is related to body weight or size. An extensive literature review of data on growth and ingestion rates of zooplankton of a broad size range (from protozoans to

metazoans) indicates biomass-specific growth rate scales negatively to weight (smaller organisms grow faster), with the maximum growth rate for protozoans nearly 10 times higher than that for metazoans (Hansen *et al.*, 1997). Hirst and Lampitt (1998) and Hirst *et al.* (2003) used a multiple linear regression model to predict copepod growth rate from temperature and body size (based on 2528 published copepod growth rates, in the latter study, with accompanying body weight and temperature data; note—other studies, e.g., Hirst and Bunker, 2003, have included chlorophyll *a* as a descriptor too). The resulting overall multivariate regression equation that relates intrinsic growth rate, g (d^{-1}) to temperature, T (°C), and copepod body weight, BW (μg C ind^{-1}) from the more recent study is $\log_{10} g = 0.0345[T] - 0.128[\log_{10}BW] - 1.529$ (Hirst *et al.*, 2003). In this compilation, any N-specific growth rates from the literature were assumed to be equal to carbon-specific rates (Hirst and Lampitt, 1998; Hirst *et al.*, 2003), thus estimates of N secondary production for copepods could be made using this model as well by applying a body dry weight C:N ratio. (This assumes regardless of the size and growth rate the copepod has a fixed C:N).

Comparisons between the major metazoan zooplankton taxa indicate that in general the gelatinous zooplankton (with the exception of chaetognaths) have higher weight-specific growth rates than copepods and other crustacea of similar body C weight, with thalacians (e.g., salps, larvaceans) having the highest overall growth rates (d^{-1}) of any taxa (Hirst *et al.*, 2003 and references therein). These high growth rates presumably lead to high N biomass production in gelatinous zooplankton blooms (although gelatinous zooplankton have relatively lower body N content than crustacea, see Section 2.1.3.). Finally, with the exception of Landry and Calbet (2004), there is a dearth of information on microzooplankton production in the oceans. In their study, a synthesis of phytoplankton growth and microzooplankton grazing rates coupled with estimates of gross growth efficiency indicated microzooplankton secondary production is equal to 21–35% (and higher when multiple steps in the grazing chain are considered) of primary production, and that as a group micro-grazers are deriving the majority of their production from consuming phytoplankton (compared to feeding on bacteria). Considering the varying elemental compostion of phytoplankton and bacteria, this has interesting implications for cycling of N and other elements in the microbial food web, as addressed next.

3.6. Stoichiometery of zooplankton nitrogen metabolism with other elements

Due to the high plasticity of phytoplankton prey items and the contrasting strict stoichiometry of herbivores, zooplankton elemental composition, and that of its food, regulate the elemental ratio of nutrients excreted. Thus, a change in the zooplankton taxa or food source can cause wide changes in the quantity and composition of bioavailable regenerated products (Caron and Goldman, 1990; Gismervik, 1997; Pertola *et al.*, 2002; Sterner and Elser, 2002; Strom *et al.*, 1997). For example, a consumer with low N and high P body content feeding on prey with high N and low P content will retain more P and excrete more N and thus favor P limitation in the plankton. Conversely, a consumer feeding on N-limited food would retain more N and excrete more P (Fig. 26.5; Sterner, 1990; Touratier
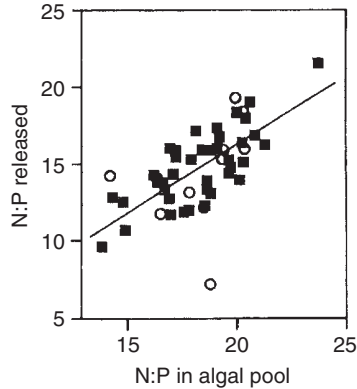
Figure 26.5 The ratio of nitrogen to phosphorus (N:P) excreted by mesozooplankton (squares) and microzooplankton (circles) plotted against the N:P in the algal pool. Data from Le Borgne, 1982a. (Adapted from Sterner, 1990).

et al., 2001). This has been shown for tropical copepods feeding on a natural plankton assemblage (Le Borgne, 1982a) and for microflagellates feeding on diatoms grown under varying nutrient conditions (Andersen *et al.*, 1986; Goldman *et al.*, 1985, as summarized by Caron and Goldman, 1990).

Stoichiometric theoretical models have been implemented in order to further understand consumer-driven nutrient recycling processes (Andersen, 1997; Elser and Urabe, 1999; Hessen and Andersen, 1992; Sterner, 1990; Touratier *et al.*, 2001). The various combinations of parameters used in these models differ, such as assimilation efficiency of individual nutrients, food quality, quantity of food supply, heterotrophic growth efficiency, and physical parameters such as temperature; however, the models all agree that the stoichiometry of nutrients released from zooplankton is mainly a function of both prey and grazer elemental composition. Anderson and Hessen (1995) have described the theoretical minimum of food C:N based on assimilation efficiencies, net growth efficiency for carbon, and C:N of herbivore and nitrogenous compounds as the threshold elemental ratio (TER). For example, if food C:N = TER, herbivore production is not limited by either C nor N. However, if food C:N > TER, herbivore production is likely limited by N. Additionally, models of the influence of C:N ratios in zooplankton food on C:N ratios in zooplankton biomass and fecal pellets indicate that simply considering elemental (i.e., C:N) composition is not adequate. Rather, considering the biochemical makeup of the ingested food (proteins, lipids, carbohydrates) is important to accurately predict C:N ratios in fecal pellets from that of ingested food, as the assimilation efficiency of a given element depends on the biochemical form in which the element is present (Anderson, 1994; Anderson and Hessen, 1995).

What is clear from studies to date is that non-Redfield excretion and recycling of elements is the norm for zooplankton (Steinberg *et al.*, 2002). For example, a variety of Barents Sea zooplankton exhibit wide ranges of ratios of respiration and inorganic excretion: C:N (range 4–44), N:P (range 2–45), and C:P (range 59–910) (calculated from Table 3 in Ikeda and Skjoldal, 1989). Respiration and inorganic excretion

ratios, as well as organic excretion ratios (DOC:DON) for diel vertically migrating zooplankton in the Sargasso Sea are also non-Redfield (Steinberg *et al.*, 2002). Ingestion of prey in surface waters at night and subsequent non-Redfield stoichiometry metabolism at depth during the day (''active flux'') by these migrators may contribute to nutrient-ratio anomalies present below the euphotic zone in the western Sargasso Sea (Anderson and Pondaven, 2003; Steinberg *et al.*, 2002). Small *et al.* (1983) showed that Pacific tropical zooplankton excrete 38–49% more ammonium N than respired C, relative to body concentrations of N and C, respectively. However, the excess N lost through ammonium excretion was balanced by a like amount conserved during fecal pellet production, yielding a relatively constant body C:N ratio (Small *et al.*, 1983). A more complete understanding the stoichiometry of zooplankton N metabolism will be necessary to fully model the role of zooplankton in ocean nutrient and carbon budgets.

4. Conclusions and Future Directions

Considerable progress in our understanding of the role of marine zooplankton in nitrogen cycling has been made in the last two decades since the first edition of this book. A compilation of ammonia excretion rates alone would be enough to fill an entire book chapter, but even here there are considerable gaps in our knowledge. We are now at the stage where large data sets are being compiled, and it is possible, for example, to reasonably predict ammonia excretion as a function of body mass for a given organism and temperature using regression equations—and avoid doing experimental incubations. These individual, size-specific excretion rates can be scaled up to a community rate if total biomass and size structure of the community is known. However, most of these data, and the algorithms developed from them, are available for crustacean mesozooplankton (e.g., copepods), and considerably less data is available for other groups.

A common theme throughout this review is that more information is needed on the role of marine protozoa, gelatinous zooplankton, and of taxa living below the euphotic zone in N consumption and metabolism. Consumption of phytoplankton and bacteria by microzooplankton are key processes in N transformations in the ocean (e.g., Nagata, 2000; Strom, 2000). Microzooplankton are an important component of the diet of many mesozooplankton, and provide nitrogenous nutrition that can not be gained from phytoplankton alone. In this light, we still know comparatively little about the biochemical composition of marine protozoans, and measurements on ammonia or DON release from microzooplankton are limited. Such information will provide important new perspectives on the stoichiometry of consumer-driven nutrient recycling. Experiments with the delicate gelatinous zooplankton are tricky, as damage can yield erroneous rates of metabolism, and many important gelatinous zooplankton are too large (e.g., scyphozoan medusae) to easily incubate. However, these key members of the planktonic food web are important in biogeochemical cycling, and we need a better understanding of their contribution to N consumption and metabolism. Most work on N cycling by zooplankton has been

carried out on epipelagic organisms. We know little about nitrogenous nutrition and metabolism of zooplankton of the mesopelagic zone, which include full-time residents as well as vertical migrators that actively transport N to and from depth, or on effects of environmental factors such as hydrostatic pressure on N metabolism of the very deep-living organisms.

The processes governing how food quality and quantity affects feeding, growth, and reproduction of zooplankton are complicated, and while significant progress has been made, gaps still remain. Previous studies show effects of food quantity and quality effects on egg hatching success are quite variable, due to species-specific differences in food nutritional quality, toxicity, or some other unexplored cause. Because egg production and hatching success are key determinants of zooplankton secondary production, future studies are needed to further explore the sources of this variability. While a number of studies have found differences in consumer feeding and growth rates based on dead versus live phytoplankton, or phytoplankton versus microzooplankton prey, future studies may need to consider multiple trophic levels to account for quality changes up the food web due to trophic upgrading (Klein Breteler *et al.*, 1999; Tang and Taal, 2005; Veloza *et al.*, 2006).

Studies of the relative magnitude and timing of the various N release mechanisms—excretion versus fecal pellet leaching versus sloppy feeding are needed. While N excretion rates for a variety of zooplankton have been extensively measured, only a few studies have directly investigated the mechanism of fecal pellet leaching or sloppy feeding on N release, thus little is known of the relative importance of each process to N cycling. The effects of food quantity and quality on N release by fecal pellet leaching or sloppy feeding are also not well known, and investigation of the relative coupling of C and N and P by these various release mechanisms would be beneficial.

Given the importance of excretion of DON by zooplankton to the microbial loop, more studies measuring organic excretion rates should be a priority, particularly for the protozoa and gelatinous zooplankton for which there are only a handful of measurements. The role of zooplankton community composition in DON cycling also deserves further attention, as some taxa (e.g., gelatinous zooplankton) may be relatively more important than others (e.g., crustacea) in the release of DON (LeBorgne, 1982b, 1986). Even less is known about the composition of DON released by zooplankton feeding and metabolism, and studies on both the composition and lability of this material are needed. Concerning the latter, experiments designed to explore the use of zooplankton-produced DON by the bacterial community (e.g., Richardot *et al.*, 2001) will provide valuable insight on the role of zooplankton in fueling the microbial loop.

As a general note on experimental work in studying N consumption and excretion, most of the methods that were in use two decades ago for measuring N consumption and excretion rates are still in use and valuable today. Normally zooplankton must be confined in bottles in order to resolve changes in ammonia or DON concentration, for example. Improvements upon this method could be made by employing some of the new nanomolar ammonia sensor technology (Li *et al.*, 2005; Masserini and Fanning, 2000) to detect small changes in ammonia concentration in excretion experiments, and allow for lower densities of animals to be used in

incubations. The measurement of N consumption and release in a natural planktonic assemblage with multiple trophic levels is still difficult, and currently isotopic methods (see McCarthy and Bronk, in this volume) may be the only way to tease apart the various mechanisms and elucidate pathways for N release.

Finally, a high priority should be given to studies linking N consumption and release with other elements. Studies following the pathway of multiple elements from food, to incorporation into zooplankton consumers, to their metabolic products will yield a wealth of information on biochemical nutritional requirements of zooplankton and the role of zooplankton in affecting the stoichiometry of nutrient cycling in the sea.

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Nitrogen-Fixing and Nitrifying Symbioses in the Marine Environment

Rachel A. Foster *and* Gregory D. O'Mullan

Contents

1. Introduction

The term ''symbioses'' was first defined loosely by De Bary (1879) as two or more differently named organisms living together. Although symbiotic interactions are ubiquitous in nature, few of the marine planktonic systems have been well characterized, and comparatively less is known of the functional role of the symbiont for the host and vice versa. Many of the planktonic symbioses are between eukaryotic hosts and cyanobacterial symbionts, or cyanobionts. Cyanobacteria are photosynthetic, and many are capable of nitrogen (N_2) fixation, thus often it is presumed

that the cyanobacterial partner functions as a carbon and/or nitrogen source for the host. In a parallel system involving sponges, often the microbial symbionts are a diverse assemblage of heterotrophs, lithotrophs, and phototrophs. One unifying character in sponge–microbe system is the exchange of nitrogen.

Nutrients are often the key limiting factors to primary production in the tropical seas, and symbioses are frequently observed in these types of oligotrophic habitats. From his many microscopy observations, Norris (1967) speculated that a considerable part of the biota in the open ocean were involved at one time or another in a consortium, either temporary or more permanent. Forming a symbiotic association might then be considered an ecological adaptation to life in the oligotrophic ocean.

Compared to their terrestrial counterparts (see Rai *et al.*, 2000), marine symbiotic systems are greatly under-sampled, and thus the many intricacies of these unique relationships remain largely unresolved. Difficulties in isolating and identifying these symbioses have been the primary problems in attaining useful information about them. Without epifluorescence microscopy most of the planktonic associations would go unnoticed. With blue and green excitation, however, the cyanobionts exhibit fluorescence patterns distinct from their photosynthetic (diatom) and heterotrophic (dinoflagellate) partners (Fig. 27.1). In sponge–microbe and other invertebrate associations, the symbionts are more difficult to identify by standard microscopy due to the complexity of the mixed assemblage. For example, microscopy provided some evidence that nitrifying bacteria may associate with *Vesicomya* clams, however the data was insufficient to differentiate between nitrifying and methanotrophic bacteria (Deming *et al.*, 1997).

For the purpose of this text on symbioses as they relate to the marine nitrogen cycle, we will first emphasize the more common open ocean diatom–diazotrophic associations (DDAs), then summarize the recent advances in our understanding of sponge–nitrifying microbial associations, and provide brief introductions to a few other relevant symbioses. In addition, we recommend, the chapter by O'Neil and Capone (Chapter 21) for details on algal–animal symbioses of Coral Reefs as they relate to the nitrogen cycle, Chapter 4 by Carpenter and Capone on Nitrogen Fixation and Chapter 5 by Ward on Nitrification.

2. Diatom–Diazotrophic Associations

2.1. Hosts and cyanobionts

Some of the earliest reports of planktonic symbiosis describe the association of a heterocystous cyanobacterium, *Richelia intracellularis*, with various diatoms, including *Rhizosolenia* (Ostenfeld and Schmidt, 1901), *Hemiaulus* spp. and *Guinardia cylindrus* (Sündström, 1984; Taylor, 1982; Villareal, 1992). Up to 13 different species of *Rhizosolenia* have been reported with *Richelia* symbionts; however some authors (Sournia, 1970; Sündström, 1984) questioned the host identity, argued that many were misidentified, and proposed that most of the *Rhizosolenia* species were just varieties of *R. clevei*.
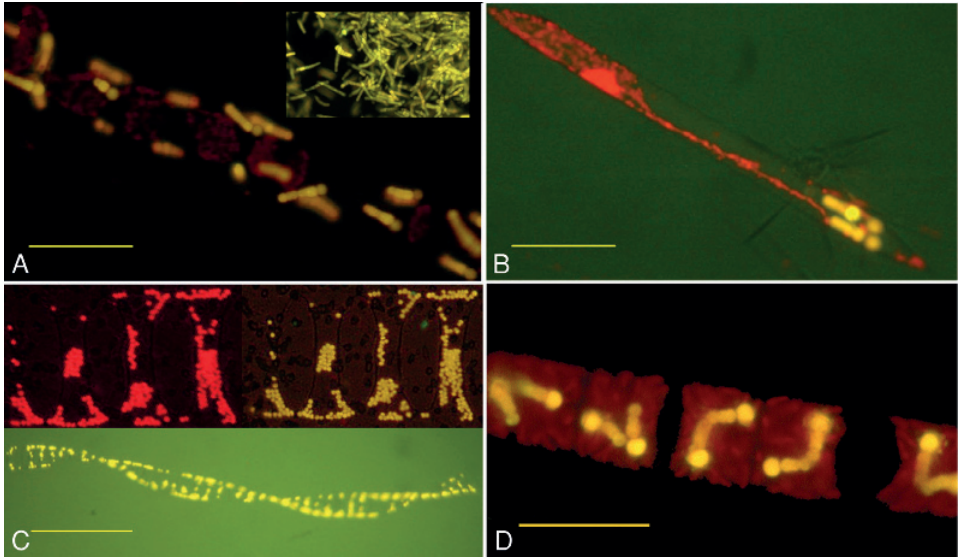
Figure 27.1 Epi-fluorescent micrographs of the better-studied diatom–diazotrophic associations (DDAs) common to the World's oligotrophic Oceanic Basins. (A) Blue excitation of a *Chaetoceros compressus* chain with attached heterocystous cyanobiont, *Calothrix rhizosoleniae*; inset is *Calothrix* SC01 isolate of Foster and Zehr. (B) A *Rhizosolenia clevei* filament with several *Richelia intracellularis* trichomes at its apical end. Note the chloroplast of host diatom fluoresces red, while cyanobiont excites yellowish-orange under blue light. (C) From top to bottom: Green and blue excitation of a diatom host, *Climacodium frauenfeldianum* with intracellular coccoid cyanobionts. Bottom is a blue excitation of symbioses in free-floating form. (D) A chain of *Hemiaulus membranaceus* diatoms with pairs of *Richelia intracellularis* symbionts in each host. Scale bar 20 μm. All photographs from Foster except 1D was provided by D.A. Caron.

Two of the most common *Hemiaulus* species reported with symbiotic *R. intracellularis* are *H. hauckii* and *H. membranaceus*. A third symbioses, occurs between *Richelia* and *H. sinensis*. In *Hemiaulus* host diatoms, it is not known where the symbiotic *Richelia* reside, whereas in *Rhizosolenia* spp. hosts, the *Richelia* remains as an extracellular endosymbiont residing between the plasmalemma and silica wall of the diatom host (Janson *et al.*, 1995; Taylor, 1982; Villareal, 1990). In *Hemiaulus* diatoms, typically there are two trichomes (series of cells comprised of a few vegetative cells and one terminal heterocyst) per host cell, and in *Rhizosolenia* species occasionally 1–32 *Richelia* trichomes have been observed (Sündström, 1984; Villareal, 1990).

Lemmerman (1905) was one of the first to depict the unique association of another heterocystous cyanobacterium, *Calothrix rhizosoleniae*, attached to the spines of a *Chaetocoeros compressus* diatom. Norris (1961) noted that the cyanobionts only attach transversely to the intercellular spaces of the diatom with the heterocyst closest to the host diatom. Others report the same symbiont as *R. intracellularis* (Gómez *et al.*, 2005; Janson *et al.*, 1999; Karsten, 1907; Norris, 1961), thus there is

conflicting taxonomy. For simplicity, here we report the symbiont attached to *Chaetocoeros* diatoms as a *Calothrix*. There have been a few other reports of a *Richelia* symbiont growing epiphytically on the spines of *Bacteriastrum* diatoms (Carpenter, 2002; Carpenter and Foster, 2002; Rai *et al.*, 2000; Villareal, 1992).

A few other symbioses have been described between a heterocystous cyanobacterium of similar morphology to *Anabaena* and *Nostoc* cells residing with *Coscinodiscus* sp. and *Roperia tessellata* diatoms, respectively (Taylor, 1982; Villareal, 1992). Interestingly, Carpenter (2002; Plate IIb) found the cyanobionts of a *Coscinodiscus* sp. diatom collected near Zanzibar similar in cell morphology and diameter to a unicellular *Synechocystis* sp.

Recently, Carpenter and Janson (2000) also reported that the open-ocean chain forming diatom, *Climacodium frauenfeldianum,* typically contains numerous coccoid cyanobacteria (Fig. 27.1). A similar sized cyanobiont has been described in the freshwater diatom, *Rhopalodia gibba* (Prechtl *et al.*, 2004). The *R. gibba* diatoms are unique since the cyanobionts reside intracellularly and the diatoms have the capacity to fix nitrogen (Floener and Bothe, 1980). It is likely that the cyanobiont partners are fixing the nitrogen since all eukaryotes lack the nitrogenase enzyme (enzyme required for N_2 fixation).

2.2. Cultivation, transmission, and cell divisions

Few have attempted to isolate and cultivate these consortia, and to the best of our knowledge only T. Villareal (1989) was successful for several months in culturing a *Rhizosolenia-Richelia* symbiosis. The division cycles of the host *Rhizosolenia* and *Richelia* were asynchronous in culture, and as such several asymbiotic hosts were observed (Villareal, 1989). Transmission from host to daughter cell is typically vertical, however asymbiotic hosts and free-living *Richelia* observed in the field and culture suggest horizontal transmission as well.

Taylor (1982) described the details of the vertical transmission in the *Rhizosolenia-Richelia* symbioses when he observed several *Richelia* cells migrating to opposite valves of host cells prior to host division. Villareal (1989) estimated the trichome migration at approximately 5 μm s^{-1} and to be independent of host cytoplasmic streaming. The details of symbiont transfer in *Hemiaulus* and *Chaetoceros* have not been clearly described. Gómez *et al.* (2005) observed free trichomes of *Richelia* (note Gómez *et al.* (2005) identify symbionts of *Chaetoceros* as *Richelia*) and suggested that the free filaments, which originate from *Rhizosolenia* (*clevei*) diatoms, colonize senescent *Chaetoceros compressus* diatoms and subsequently spread out after replication. This speculation is not supported by evidence presented by Janson *et al.* (1999) and Foster and Zehr (2006), which showed high sequence divergence between the various symbionts of the different host diatoms, and thus a high degree of host specificity, or in other words, each *Richelia/Calothrix* strain is specific to one host genus (section 2.3).

In Fall 2004, several chains of *Chaetoceros compressus* chains were hand-picked from the subtropical Pacific (station ALOHA) that had several symbiotic *Calothrix* cells attached to the host diatoms spines (Foster and Zehr unpubl.). We were

successful in culturing the symbiont, and the isolate, *Calothrix* SC01, has been maintained free living (without the diatoms) in nitrogen-deplete media. This isolate has been subject to a few experiments, including a phylogenetic study of the precursor gene for the nitrogenase (*nifH*) enzyme (Foster and Zehr, 2006) and several acetylene reduction (AR) assays (proxy for N_2 fixation).

2.3. Specificity and symbiont phylogenetic diversity

In contrast to the co-occurring and free-living cyanobacteria that reside in the open ocean, there are far fewer studies on the phylogentic diversity of the symbiotic *Richelia/Calothrix* and the other open ocean consortias. Difficulties in collection, isolation, and separation from the other phytoplankton populations, have been the primary obstacles. However recent studies (Foster and Zehr, 2006; Foster *et al.*, 2006; Janson *et al.*, 1999) using single-cell approaches were successful with molecular genetic analyses and allowed sequence data to be matched back to particular populations. Subsequently, the sequence data has been directly applicable to other assays (i.e., Quantitative PCR), which estimate cell densities for target phylotype (*Richelia* associated with *Rhizosolenia*) using gene copy abundances (see below, section 2.5).

Janson *et al.* (1999) were first to report on the high host specificity of *Richelia* symbionts associated with four of the DDAs. In their study, the *hetR* gene, a gene that functions in heterocyst and akinete differentiation (Buikema and Haselkorn, 1991; Leganés *et al.*, 1994), was amplified from individual host samples containing several filaments of *Richelia* associated with *Rhizosolenia clevei*, *H. hauckii*, *H. membranaceus*, and *Chaetoceros* sp. The symbiotic specimens were collected from two cruises, one in the Caribbean Sea and one in the South Pacific Ocean. Janson *et al.* (1999) inferred a high degree of host-symbiont specificity since the symbiont sequences from the different host genera were highly divergent (sequence similarity <85%). In addition, the *hetR* nucleotide sequences derived from *Richelia* symbionts associated with *H. membranaceus* sampled in the Atlantic and Pacific Oceans were nearly identical (98.9% identical), suggesting genetic relatedness was not dependent on geographical location (Janson *et al.*, 1999).

A second phylogenetic investigation of the same DDAs by Foster and Zehr (2006) corroborated the results of Janson *et al.* (1999) for the *hetR* gene and analyzed the phylogenetic diversity of two additional genes, *nifH* and 16S rRNA. NifH is a functional gene marker, and encodes the iron subunit of dinitrogenase reductase, the enzyme responsible for N_2 fixation. In this later study, sequence identity was highest (98.2%) amongst the16S rRNA sequences, and more divergent for the *hetR* (83.8%) and *nifH* (91.1%) sequences. This study also identified three previously unidentified heterocystous-like *nifH* sequence groups, which were recently reported from station ALOHA, het-1, het-2, and het-3 (Church *et al.*, 2005; Zehr *et al.*, 2007), as the *Richelia* associated with *Rhizosolenia clevei*, *H. hauckii*, and *Calothrix* symbiont of *Chaetoceros* sp., respectively.

In addition, Foster and Zehr (2006) found a parallel divergence in the *nifH* sequences as Janson *et al.* (1999) reported for *hetR* sequences. In the study by Janson *et al.* (1999), they found that the *hetR* sequence associated with a *Richelia-H.*

hauckii was different than the *hetR* sequence of a *Richelia* associated with a *H. membranaceus*. Thus, the specificity was on a host species level. A similar pattern resulted in the *nifH* phylogenetic data presented by Foster and Zehr (2006), which suggested the further delineation of the het-2 group into het-2A and het–2B, to represent *Richelia* associated with *H. hauckii* and *H. membranaceus*, respectively. The same host species level of specificity may occur within the *Rhizosolenia* sp. hosts, however it has not been investigated.

There have been a few phylogenetic studies of other planktonic symbioses other than the *Richelia*-Diatom symbioses. These however use a 16S rRNA phylogeny and rely on the high similarity between the resultant sequences to known nitrogen fixers as potential evidence for diazotrophy rather than looking for a nitrogen-fixing gene (i.e., *nifH*) directly. A few of these studies are briefly reviewed here.

Carpenter and Janson (2000) reported the 16S rRNA phylogeny of the cyano-bacterial symbionts that reside within the diatom, *Climacodium frauendulum*. They found a high sequence identity (>98%) between the cyanobiont 16S rRNA sequences and a 16S rRNA sequence derived from the unicellular diazotroph, *Cyanothece* sp. ATCC 51142. A similar 16S rRNA sequence was retrieved from the freshwater diatom, *Rhopalodia gibba* (Prechtl *et al.*, 2004). NifD gene sequences were also amplified from *R. gibba,* which were closely related to *Cyanothece* ATCC 51142 *nifD* sequences. In another 16S rRNA study by Foster *et al.* (2006) several sequences similar to16S rRNA sequences of *Cyanothece* sp. 51142 were recovered from a single *Histiones* (Dinoflagellate) sp. host containing symbiotic cells similar in morphology and cell diameter to *Cyanothece*. In the open ocean, *Crocosphaera watsonii*, which is similar in cell diameter size (3–5 μm) and physiology (i.e., temporal segregation of N_2 fixation) to *Cyanothece* ATCC 51142, is a common cell type, and is likely the cyanobiont for many of the above-mentioned symbioses between a marine eukaryote and a coccoid cyanobacterium (Foster, pers. obs.).

2.4. Host–symbiont interactions

In symbiotic systems, like these where the association appears quite intimate or the symbiont population occupies a majority of the host cell volume, the relationship is assumed necessary (Douglas, 1998) and/or beneficial. The benefit of the DDA relationship is not fully understood nor characterized, and because N_2 fixation has been measured when the DDAs are present, it is presumed that some of the nitrogen fixed by the symbiont is transferred to the host diatom. To date, there are only a few studies that have attempted to understand the nature of the symbiosis between the *Richelia* symbiont and the host diatom.

In a micro–autography study, field collected *Rhizosolenia-Richelia* symbioses were incubated with ^{14}C-labelled bi-carbonate. Higher density of silver grains localized on the symbiotic *Richelia* trichomes than on the host *Rhizosolenia* fila-ments, suggesting that the *Richelia* were actively photosynthesizing and the host diatom were inactive (Weare *et al.*, 1974). An equally plausible explanation for less silver grains on the *Rhizosolenia* host is that some of the fixed and labeled photosynthetic products were transferred to the host from the symbiont. Similar scenarios of carbon, and nitrogen transfer, are well documented in terrestrial

symbioses with heterocystous symbionts, i.e., *Azolla-Anabaena*, Lichen–*Nostoc* symbioses (Rai *et al.*, 2000). Weare *et al.* (1974) also speculated that the metabolically active host diatoms act as a source of inorganic nutrients, i.e., phosphate, for their symbionts.

In culture, Villareal (1990) measured growth and nitrogenase activity (acetylene reduction) in the *Rhizosolenia-Richelia* symbioses, and demonstrated light saturation kinetics in both activities. In addition, he demonstrated preliminary evidence for excretion of fixed nitrogen to the surrounding medium (details described in Villareal, 1990), and suggested the extracellular location of the symbiont (between the frustule and the plasmalemma) is mechanistic for nutrient transfer to the medium. Further elucidation of host-symbiont interactions, transfer, and benefit/cost of the relationship is a challenging, yet warranted subject for future investigation.

Janson *et al.* (1995) also verified the previous work of others (Taylor, 1982; Villareal, 1990) that the *Richelia* cyanobionts were always located outside of the host (*Rhizosolenia*) cytoplasm. Using immuno-cytochemistry coupled with transmission electron microscopy (TEM), Janson *et al.* (1995) demonstrated the localization of anti-bodies to nitrogenase and Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), in the heterocyst and vegetative cells, respectively, of the *Richelia* symbionts of *Rhizosolenia clevei*. They suggested that ammonia assimilation was potentially repressed in the *Richelia* cells, since they found low localization of antisera to the ammonia assimilation enzyme, glutamine synthetase (GS). It should be noted that immunogold assays do not indicate enzyme activity rather only show presence of the enzymes when the cells were fixed. Thus the results from Janson *et al.* (1999) only suggests *Richelia* symbionts have the potential to function as nitrogen and/or carbon sources for their respective hosts.

Although the *Calothrix* cyanobionts of *Chaetoceros* are attached to the diatom spines, the cyanobionts are only located at the intercellular spaces and attached transversely at the heterocyst (Norris, 1961), which could be seen as a morphological adaptation for nitrogen transfer. Others (e.g., Villareal, 1990) have suggested that the extracellular location of the *Richelia* symbionts with *Rhizosolenia clevei* is mechanistic for nitrogen transfer.

After several months in isolation without the *Chaetoceros* host, our cultured isolate, *Calothrix* SC01, started to change its cell character. For example, the trichome length extended, intercalary heterocysts were observed, and several trichomes appeared to branch. We interpreted such changes in the symbiont trichome and cell integrity as due to loss of control by the diatom host over the symbiont since the symbiont is in a free-living state. These latter observations also suggest that the free-living trichome potentially looks different than that which is observed when it lives symbiotically, and thus could be easily misidentified/overlooked in the field.

Others (Foster *et al.*, 2007; Kimor *et al.*, 1978; Villareal, 1989) have reported that vegetative cells degrade first, and often the heterocysts are the last part of the *Richelia* trichome to remain in a host diatom, which could also suggest host control or some sort of cell signaling between host and symbiont. Gómez *et al.* (2005) observed *Calothrix* symbionts associated with *Chaetoceros* diatoms which lacked chloroplasts suggesting that the host diatoms were senescent; quite possibly though the symbiotic

Calothrix act as a source of fixed carbon to their hosts. Transfer of fixed nitrogen and/or carbon remains undocumented in all the DDAs; it has only been inferred from growth of the *Rhizosolenia-Richelia* symbioses in N-free media in culture (Villareal, 1989).

2.5. Geographical distribution and cell abundances

Generally speaking, *R. intracellularis* associated with *Hemiaulus* spp. have higher abundances in the Atlantic Ocean (Carpenter *et al.*, 1999; Foster *et al.*, 2007; Villareal, 1991, 1994), and *R. intracellularis* associated with *Rhizosolenia* sp. are more commonly reported from the North Pacific central gyre (Ferrario *et al.*, 1995; Mague *et al.*, 1974; Venrick, 1974; Wilson *et al.*, 2008). Villareal (1994) reported the occurrence of *Hemiaulus-Richelia* symbiosis was 5–254 times more abundant in the North Atlantic, Caribbean Sea, and Bahama Islands than the *Rhizosolenia-Richelia* symbiosis. There are no observations of the *Chaetoceros-Calothrix* symbioses in the subtropical and tropical Atlantic Ocean, and some have suggested its geographical limitation to the Pacific and Indian Ocean basins, however, we cannot discount that few have actually looked. Others have reported the DDAs distribution in the Indian Ocean (Norris, 1961), the Red Sea (Kimor *et al.*, 1992), and more recently in the Eastern Mediterranean Sea (Bar–Zeev *et al.*, 2008), and the western China Seas (Gómez *et al.*, 2005), which make *Richelia* and *Calothrix* the most widespread marine heterocystous cyanobacteria described.

There are a few exceptions where the DDAs are capable of penetrating coastal waters. For instance, Kimor *et al.* (1978) reported an ''unusual occurrence of *Richelia* associated with *Hemiaulus membranaceus*'' off the coast of California and one report of *Richelia* associated with *H. hauckii* and *H. membranaceus* in waters off Hawaii (Heinbokel, 1986). Recently, White *et al.* (2007) observed high abundances (100 L^{-1}) of the *Rhizosolenia-Richelia* symbioses in the Gulf of California. *Hemiaulus-Richelia* also occurs at Carrie Bow Cay, Belize (Villareal, 1994), and off the coast of Texas (Villareal pers. comm.).

Most heterocystous cyanobacteria dominate brackish and freshwater environments where they occur in the plankton and the benthos as free–living cells and are seldom found in the open ocean. Few report *Richelia* and *Calothrix* as free-living (Gómez *et al.*, 2005; White *et al.*, 2007), thus both are the exception and have made their successful transition to the open ocean as symbionts.

Some of the highest numbers for the *Hemiaulus-Richelia* symbioses were reported in the western tropical North Atlantic (WTNA). Carpenter *et al.* (1999) observed an extensive bloom off the NE coast of South America in autumn of 1996. They reported cell densities from 10^2 to 10^6 *Richelia* L^{-1}. Recently, in the same vicinity as the study of Carpenter *et al.* (1999), Foster *et al.* (2007) reported extremely high *nifH* gene copy (>10^5 copies L^{-1}) abundances (proxy for cell abundances) for *Richelia* associated *H. hauckii* and *Rhizosolenia clevei*. In addition, they found within the plume waters of the Amazon River runoff a positive correlation between salinity and the abundance of the *H. hauckii-Richelia* abundance (Foster *et al.*, 2007).

Church *et al.* (2005) were first to report the *nifH* gene expression for the het–1 and het–2 groups, which were later identified as *Rhizosolenia-Richelia* and *Hemiaulus-Richelia* symbioses (Foster and Zehr, 2006) from Station ALOHA in the N. Pacific Ocean. They found *nifH* expression for the het-1 group (*Rhizosolenia-Richelia* symbiosis) increased dramatically (10^2 to 10^6 *nifH* cDNA copies L^{-1}) in the early morning (04:00–06:00) and gradually declined throughout the late morning and evening. In addition, they detected *nifH* expression for the het-1 group (*Rhizosolenia-Richelia* symbiosis) down to 200 m at midday and midnight, indicating a very active DDA population throughout the water column during day and night periods.

The abundance reported by Venrick (1974) and Mague *et al.* (1974, 1977) in the North Pacific central gyre were limited to estimates of the *Rhizoslenia-Richelia* symbioses. An interesting oversight reported in these studies was that the diatom *H. hauckii* was also present. In fact, abundance of *H. hauckii* during the Fall 1969 were 250 cells ml^{-1} (Venrick, 1974) and Mague *et al.* (1974) recorded 4000 cells L^{-1}. These abundances for *H. hauckii* were not reported as symbiotic; the *Richelia* in a *H. hauckii* is extremely inconspicuous with light microscopy (Fig. 1), and it is likely that it was overlooked. Venrick (1974) noted that in winter months (Nov.-Feb.) the *Rhizosolenia-Richelia* densities were low, ~60 cells L^{-1}, and reached 10^3–10^4 cells L^{-1} during summer (June-Sept.). Mague *et al.* (1974) observed a subsurface maximum in the abundance *Rhizosolenia-Richelia* symbioses (60–80 cells L^{-1}) at 40 m in the N. Pacific gyre. It was also the depth of maximum acetylene reduction (see below, section 2.6).

2.6. Nitrogen fixation

Although there have been observations of large and expansive blooms of DDAs (Villareal, 1994; Carpenter *et al.*, 1999), there have been relatively few reports of the N_2 fixation or contribution of the DDAs to the global marine nitrogen budget. This is largely due to the difficulty in collection of the symbioses without compromising the integrity of the symbiotic complex and thus the physiological measures. A limited number of field studies (Carpenter *et al.*, 1999; Mague *et al.*, 1974; 1977; Venrick, 1974; Villareal, 1991; White *et al.*, 2007; Bar–Zeev *et al.*, 2008), and fewer culture experiments (Villareal, 1989; 1990) represent the only physiological measures of N_2 fixation by the DDAs.

Venrick (1974) measured primary production rather than N_2 fixation, and recorded the highest carbon fixation rates (154.4 mg C m^{-2}) during a summer bloom (average abundance 1.5×10^7 filaments m^{-2}) of *Rhizosolenia-Richelia* in the North Pacific central gyre. The carbon fixation rate represents the carbon fixed by both host diatom (*Rhizosolenia*) and symbiont (*Richelia*) since both are photosynthetic. She then used the average cell specific N_2 fixation rates reported earlier by Mague *et al.* (1974) to estimate the range (6.2–12.5 mg N m^{-2}) in daily N_2 fixation rate by *Rhizosolenia-Richelia*. Venrick (1974) extrapolated that 30–60% of excess productivity in the North Pacific central gyre was accounted for by the presence of a *Rhizosolenia-Richelia* bloom. Recently, a similar hypothetical estimate of N_2 fixation was provided by Foster *et al.* (2007), where they found that a dense

population of *H. hauckii* with symbiotic *Richelia* accounted for 89–100% of the N_2 fixation (8.1×10^5–7.5×10^6 fmol N L^{-1} day^{-1}) in the WTNA. Villareal (1991) did a similar calculation and noted that only 100 cells L^{-1} of the *Hemiaulus–Richelia* symbioses could provide 15% of the entire N_2 fixation. Although these calculations are a crude means of estimating the rate of N_2 fixation and have obvious bias, they do highlight the potential significant influence of DDAs on the nutrient and energy budgets of phytoplankton in the oligotrophic environments.

Mague *et al.* (1974) reported low (0.02–0.64 µg N mg N^{-1} h^{-1}), but comparable rates of N_2 fixation by *Rhizosolenia-Richelia* to free-living *Trichodesmium*. During the DDA bloom observed by Carpenter *et al.* (1999) in the WTNA, the *Hemiaulus-Richelia* added an average of 45 mg N m^{-2} day^{-1} to the water column, which far exceeded estimates of new nitrogen flux from below the euphotic. Most recently, White *et al.* (2007) estimated that N_2 fixation by *Richelia* associated with *Rhizosolenia*, and to a lesser extent by *Trichodesmium*, supplied 35–48% of the phytoplankton-based nitrogen demand in the central and eastern basins of the Gulf of California. Rates of N_2 fixation were recently estimated by the acetylene reduction technique on bulk water in the Eastern Mediterranean Sea. The highest rates of N_2 fixation (0.4–3.1 nmol N L^{-1} d^{-1}) were recorded in the summer and ∼40–70% of the total N_2 fixation was attributed to populations of symbiotic *R. intracellularis* (Bar-Zeev *et al.*, 2008). Besides the laboratory data of Villareal (1990; 1992), the above-mentioned studies represent the few field data for N_2 fixation by the DDAs and each demonstrate the obvious ecological importance of these diazotrophic populations.

The earlier works by Mague *et al.* (1974; 1977), Venrick (1974), and Kimor (1978) attempted to define some of the environmental factors and conditions that control the N_2 fixation activity and distribution of DDA populations. Some have argued that distribution and activity is largely controlled by latitude, temperature, nutrients (i.e., iron, phosphorous), and wind stress for the other co-occurring cyanobacteria.

Kimor *et al.* (1978) observed that the unusual occurrence of the symbiotic *H. membranceus* off the coast of southern California occurred during an unusually warm period (18.5°C) for that geographical region and season. Later, in Carrie Bow Cay, Belize, Villareal (1994) reported 98% of *Hemiaulus* sp. examined contained *Richelia*, and that symbiotic *Hemiaulus* were present as far north as 31°N in the Pacific, further evidence that the geographical range of these symbioses is capable of penetrating cooler and more subtropical boundaries.

In her 9-year field study in the North Pacific central gyre, Venrick (1974) reported that for most of the years *Richelia* associated with *Rhizosolenia* were relatively low (0.1–1 *Richelia* cell ml^{-1}) in abundance, however, in summer months when the upper water column stratified and nutrients were measurably low, symbiotic populations increased 1–2 orders of magnitude. Environmental parameters, i.e., nutrient concentrations, during bloom and non-bloom summers in the upper 45 m were indistinguishable, suggesting little evidence for a condition to initiate and perpetuate the blooms. Venrick (1974) proposed that the blooms were a localized phenomena occurring independently at various locations within the central Pacific

Ocean basin. Similarly higher rates of N_2 fixation in the eastern Mediterranean Sea were recorded during peak stratification (Bar-Zeev *et al.* 2008), thus it seems water column dynamics play an important role in bloom formation and sustenance.

Gómez *et al.* (2005) observed the *Chaetoceros-Richelia* (note these authors use an alternative nomenclature) symbioses was restricted to the transition zones between the slope waters and the Kuroshio Current in the western Pacific Ocean. They proposed that their distribution was related to local mixing of the Kuroshio Current with the coastal waters, where *Chaetoceros* is a dominant member of the neritic phytoplankton population.

Mague *et al.* (1974) found that highest biological fixation occurred in the summer months in the North Pacific central gyre when resident populations of the *Rhizosolenia-Richelia* symbioses began to increase. The surface waters were stratified and concentrations of phosphate and nitrate were undetectable. Enrichments of 0.5 to 5 µM orthophosphate to samples containing *Rhizosolenia-Richelia* concentrates increased acetylene reduction, but when concentrations >5 µM were added, activity decreased, and at 50 µM amendments the rate was equivalent to the initial. These results suggested that to some extent the symbioses were P limited. To date, there have been no other nutrient manipulation experiments. Since the nitrogenase enzyme complex has a high iron requirement, an interesting and open question would be the effect of increased iron on N_2 fixation rates.

Typically, diatoms thrive in colder waters with high nitrate concentrations. Thus it seems that the DDAs have gained a successful existence into the warm oligotrophic waters of tropical and subtropical seas by their symbiotic partners. There have been few observations or investigations for the presence of the DDAs in higher nutrient environments, i.e., rivers, estuaries, or in regions of intense upwelling. Abundance for two of the three DDA groups (het-1 & het-2) was recently reported in the Amazon River plume in the WTNA (Foster *et al.*, 2007), where elevated nutrients were measured. Cell abundances (5–120 cells L^{-1}) for symbiotic *Hemiaulus* and *Rhizosolenia* populations were recorded within lower salinity waters of the Orinoco River (Corredor, pers. comm). The same DDAs were detected near the Congo River plume in the eastern equatorial Atlantic (EEA) (Foster, unpubl.). Combined, these observations suggest that in the Atlantic Ocean, riverine inputs play an important role in the distribution of the DDAs. Hypothesized, but not yet investigated, is that rivers are the source of free-living *Richelia* populations, since most heterocystous cyanobacteria dominate in brackish and estuarine waters.

These earlier and more recent field measures all demonstrate the importance of these DDAs on the local conditions, however, in most experiments the collection of samples used towed nets, and thus are rather disruptive. Two experiments by Mague *et al.* (1974, 1977) found that preparing samples by concentration caused a significant (17–29%) reduction in acetylene reduction activity. It seems that more attention or creative sampling schemes need to be developed to accurately measure the N_2 (and likely carbon) fixation by these DDAs. Studies similar to those presented by Zehr *et al.* (2007) and Needoba *et al.* (2007), which combine $^{15}N_2$ uptake rates with quantitative PCR approaches for the target diazotrophs are a plausible alternative since assays are run on bulk water.

2.7. Implications

A recent model presented by Deutsch *et al.* (2007) estimates global N_2 fixation by applying an oceanic circulation model to the relative changes of nitrate and phosphate concentrations in the surface ocean. Their model predicts N_2 fixation in all the regions of the world's oceans where these DDAs occur and have been reported. A major shortcoming noted in the model was, "diazotrophs with both a high biomass N:P and an unusually high export efficiency, should they be found, would be underestimated by our approach." The DDAs have extremely high vertical fluxes (Schaerek *et al.*, 1999a,b), and represent an excellent example of a population that would be likely overlooked in this type of model.

DDAs are among the most unique phytoplankton populations because they have a dual function. Large and expansive blooms contribute directly to the vertical flux of organic matter to the deep sea (Schaerek *et al.*, 1999a,b), all the while being widespread and sometimes patchy in distribution in the euphotic zone where they provide fixed N to the co-occurring non-diazotrophic phytoplankton population. Although controversial and limited in direct scientific evidence, we assume that a majority of the carbon and presumably fixed nitrogen associated with the DDAs does in fact fall out below the euphotic into the mesopelagic. Thus the DDAs represent an important link in the biogeochemical cycling of both carbon and nitrogen in the world's oceans, and yet when compared to other larger diazotrophs, e.g., *Trichodesmium*, DDAs are often under-represented in nutrient budgets and far under-sampled.

3. Sponge–Nitrifier Associations

3.1. Sponge–microbe specificity and phylogenetic diversity

Sponges act as filter feeders capable of circulating thousands of liters of seawater through their osculum per day while feeding on organic particles and microorganisms from the water column (Pile, 1997; Vogel, 1977). Some sponges, primarily those in the class *Demospongia* (Vacelot and Donadey, 1977), are populated by microbial symbionts, mostly extracellular, that are able to avoid phagocytosis and digestion while residing in the sponge mesohyl matrix. The microbial density within the host biomass can far exceed that of seawater, reaching concentrations up to 10^{10} bacteria per gram of sponge wet weight (Hentschel *et al.*, 2006). For organisms that can avoid digestion, the host provides a favorable microbial habitat due to increased nutrient availability from the active pumping of seawater and release of ammonia, urea, and organic carbon as by-products (e.g., Davey *et al.*, 2002).

Microscopy and molecular genetic techniques have demonstrated that a single sponge often contains a very diverse microbial assemblage including bacteria (Hentschel *et al.*, 2002), archaea (Margot *et al.*, 2002; Preston *et al.*, 1996) and algae (Usher *et al.*, 2004; Wilkinson and Fay, 1979). These 16S ribosomal RNA surveys have detected microorganisms similar to known heterotrophs,

photoautotrophs, and chemolithoautotrophs. The nitrogen transformations attributed to these groups include N_2 fixation (e.g., Wilkinson and Fay, 1979), ammonia oxidation (e.g., Hallam *et al.*, 2006a,b), nitrite oxidation (e.g., Hentschel *et al.*, 2002), and nitrogen assimilation (e.g., Davy *et al.*, 2002). The phylogenetic diversity and species richness of the symbiont population found within a single host (Hentschel *et al.*, 2002; Hill *et al.*, 2006; Taylor *et al.*, 2004; Webster *et al.*, 2001) are unlike most known marine invertebrate-microbe symbioses which have comparatively low symbiont diversity (Steinert *et al.*, 2000). Nonetheless, the diversity of the sponge-microbe associations are often referred to as host specific. The bacterial symbionts appear to be distinct from the free-living bacterial populations in seawater and seemingly uniform bacterial populations have been detected in many geographically distant sponge species (Hentschel *et al.*, 2002; Hill *et al.*, 2006).

The diversity maintained in the sponge association may be partially attributed to the occurrence of asexual reproduction in sponges, allowing the establishment of a close association without requiring immediate incorporation of microbial cells into the germ line. Usher *et al.* (2005) reported that germ line incorporation does occur in at least some species and that cyanobacteria could be detected in both the egg and the sperm of *Chondrilla australiensis*. Sharp *et al.* (2007) further demonstrated that phylogenetically diverse, yet sponge specific, microbial lineages including bacteria and archaea could be found in *Corticium sp.* embryos. The stability of these complex associations over evolutionary time scales has only begun to be explored.

In summary, sponges form symbiotic associations with phylogenetically and metabolically diverse microbes. Although there is very limited evidence to document the direct benefits of the symbiosis, the microbes are thought to receive increased nitrogen for growth and the host to benefit from the removal of potentially toxic metabolites, i.e., ammonia and urea (Davey *et al.*, 2002). The metabolic versatility of these abundant and diverse microbial assemblages in combination with the increased flow rate provided by the filter feeding host creates a bioreactor that can have a large impact on the carbon and nitrogen cycles of a marine habitat.

3.2. Nitrification in sponges

The process of nitrification in marine sponges was first described by Corredor *et al.* (1988) by measuring the concentration of nitrate released by the coral reef sponges *Chondrilla nucula* and *Anthosigmella varians*. Multiple investigators have re-confirmed the release of nitrate from symbiont containing sponges (Diaz and Ward, 1997; Pile, 1996; Scheffers *et al.*, 2004) although the direct linkage of nitrate release to chemolithoautotrophy has not been established. It is assumed that ammonia released as a by-product of host metabolism is oxidized by microorganisms living within the sponge mesohyl matrix. Corredor *et al.* (1988) observed that the rate of nitrification was not equivalent for all host species and that it varied with symbiont composition. For example, *C. nucula*, a cyanobacteria containing sponge, released nitrate 200 times faster than *A. varians*, a zooxanthellae containing sponge. This difference in nitrate release may be driven by uncharacterized differences in the non-

cyanobacterial symbionts but the difference was generally attributed to bacterial symbioses. Similarly, Diaz and Ward (1997) found higher nitrification rates for sponges associated with cyanobionts, than non-cyanobacterial containing sponges. They found that in *Oligoceras violacea,* nitrite was primarily released, while in the other two spongs species (*Chondrilla nucula* and *Pseudaxinella zeai*) high concentrations of nitrate were released. This difference was attributed to an uncoupling of ammonia oxidation and nitrite oxidation in *O. violacea* but the composition of nitrifier symbionts was not examined. The potential nitrification rates for the sponge symbiont assemblages (up to 2650 nmol g^{-1} h^{-1}) were the greatest weight specific rates that have been reported and areal corrected rates were as much as four orders of magnitude greater than rates reported in coastal sediments (Diaz and Ward, 1997). In Curacao coral reefs, NOx efflux rates from cavities containing sponges were measured as 1.02–9.77 mmol m^{-2} day^{-1} (Scheffers *et al.*, 2004); and 1.9 m^{-2} day^{-1} (van Duyl *et al.*, 2006). These findings suggest that sponge-nitrifier assemblages can be responsible for a large input of oxidized nitrogen to habitats where these associations abound. Marine sponges are unlikely to play a large role in the global nitrogen cycle but in local habitats such as tropical coral reefs, where sponges are both abundant and diverse (Diaz and Rutzler, 2001), their activities could potentially play an important role in controlling the budget of ammonia and NOx (Diaz and Ward, 1997; Scheffers *et al.*, 2004; van Duyl *et al.*, 2006). Thus, the reported decline of sponge biomass (Wulff, 2006) could alter nitrogen cycling in these oligotrophic habitats.

3.3. Genomic studies of nitrifying symbioses

Although molecular analyses have revealed diverse populations of bacteria in sponges (Hentschel *et al.*, 2002; Hill *et al.*, 2006; Taylor *et al.*, 2004; Webster *et al.*, 2001), a single archaeal group was found to dominate the marine sponge *Axinella mexicana* (Preston *et al.*, 1996). This symbiont, *Cenarchaeum symbiosum*, is extremely abundant and can account for up to 65% of the total microbial biomass found within the host. When this association was first identified, the metabolic activity of *C. symbiosum* remained a mystery. Molecular phylogenetics identified *C. symbiosium* as a member of the planktonic, marine nonthermophilic *Crenarchaeota*. This group of *Archaea* is widely distributed in the marine environment (DeLong *et al.* 1992; Fuhrman *et al.*, 1992), is estimated to account for up to 20% of the oceans total picoplankton (Karner *et al.*, 2001) and isotopic analyses suggest that it is capable of autotrophic growth (Pearson *et al.*, 2001; Ingalls *et al.*, 2006).

Metagenomic analyses conducted in the Sargasso Sea identified a gene sequence similar to bacterial ammonia monooxygenase (*amoA*) on a genome scaffold that also contained an crenarchaeotal ribosomal gene. This finding initially implicated the oxidation of ammonia as a chemolithoautotrophic metabolism associated with *Archaea* (Venter *et al.*, 2004). This finding was rapidly followed by cultivation and characterization of *Nitrosopulmilus maritimus* (Konneke *et al.*, 2005), unequivocally demonstrating the oxidation of ammonia to nitrate as an archaeal process and linking this transformation to some members of this abundant marine group. Ammonia oxidation by *Crenarchaeota* is now thought to be ecologically important and widely

distributed in marine, freshwater, and terrestrial environments (Cavicchioli *et al.*, 2007; Francis *et al.*, 2005; Schleper *et al.*, 2005; Treusch *et al.*, 2005; Wuchter *et al.*, 2006), a process previously attributed only to bacteria.

Although *C. symbiosum* remains uncultivated, its abundance within *Axinella mexicana* allowed the use of genomic approaches to characterize its metabolic potential (Schleper *et al.*, 1998; Hallam *et al.*, 2006a,b). These analyses revealed two abundant *C. symposium* symbiont populations co-inhabiting the host. The gene content, order, and orientation for these sympatric populations suggests very little recombination in the evolution of these strains. Localized regions of sequence variation reveal a limited number of genes under strong selective pressure worthy of additional investigation and a subset of candidate genes likely involved in the symbiosis. The genome was ''remarkably distinct from those of other known *Archaea*'' and contained a large number of genes most similar to marine environmental sequences thought to be from free-living planktonic *Crenarchaeota* (Hallam *et al.*, 2006b). Future comparisons between the genomes of free-living *Crenarchaeota* and *C. symbiosum* will lead to an increased understanding of the symbiosis.

More importantly, the genomic analyses enabled by the enrichment of *C. symbiosum* in the tissue of *Axinella* provide an opportunity to learn about the closely related, planktonic *Crenarchaeota.* This group is now predicted to be the dominant marine nitrifiers, but eluded cultivation and characterization for many years. *Axinella*'s two symbiont genomes contain many of the genes required for autotrophy and appear to assimilate carbon using a modified 3-hydroxypropionate cycle. There is evidence of a partial oxidative tricarboxylic acid cycle for mixotrophic growth (Hallam *et al.*, 2006a). Most of the genes encoding proteins involved in chemolithotrophic ammonia oxidation have been identified (including ammonia monooxygenase, ammonia permease, and urease) but a homologue for hydroxylamine oxidoreductase, which encodes a key enzyme involved in energy production from ammonia oxidation, has not yet been identified (Hallam *et al.*, 2006b). This either suggests that the symbiont may utilize novel enzymatic reactions for the oxidation of ammonia or it requires re-evaluation of its role as a nitrifier. In either case, the elucidation of this pathway in *C. symbiosum* requires additional investigation and may shed light on alternative mechanisms for ammonia oxidation in both free-living and symbiotic taxa.

3.4. Implications

Sponge-nitrifier associations appear to play a quantitatively significant role in the nitrogen budget of localized marine environments such as tropical coral reefs. The importance of these associations is not limited to their biogeochemical impact in the environment but also extends to their use as a model system for laboratory analyses. The symbiotic association of *A. mexicana* and *C. symbiosum* allows us an alternative method of studying the abundant, but cultivation-resistant, free-living *Crenarchaeota* picoplankton. Further exploration of the *C. symbiosum* genome may help to uncover a new pathway for ammonia oxidation and factors regulating the newly discovered and globally significant archaeal ammonia oxidizers.

4. OTHER RELEVANT SYMBIOSES

4.1. Diazotrophs in the copepod gut

An interesting and non-traditional example of symbioses, which has received relatively little attention are zooplankters with associated anaerobic diazotrophs (Braun *et al.*, 1999; Zehr *et al.*, 1998). Two independent studies revealed that planktonic copepods are associated with microorganisms, which possess *nifH* sequences phylogenetically related to strictly anaerobic sulfate reducers and clostridia (Cluster III *nifH* sequences) (Braun *et al.*, 1999; Zehr *et al.*, 1998). In the study by Braun *et al.* (1999), they also detected ethylene production during acetylene reduction assays on sorted copepods, indicating an active diazotrophic community. In the study by Zehr *et al.* (1998), zooplankton derived *nifH* sequences were not recovered or similar to any of the sequences derived from parallel bulk water samples, suggesting that the nitrogen-fixing species were likely gut-associated and not associated with the copepod's skeleton. Thus, the invertebrate gut may provide an unexpected refuge of suitable conditions for anaerobic N_2 fixation. And furthermore, considering that copepods are amongst the most abundant grazers in the world's oceans, the presence of N_2 fixing microflora associated with their guts is potentially another underrepresented source of nitrogen to the oceans (Zehr *et al.*, 1998).

4.2. Shipworm–bacterial associations

Nitrogen fixation has also been reported from marine shipworms. Shipworms are bivalves, which live attached to wooden ships, in which they bore holes, and thus have a diet of wood alone. Cellulose is the principal component of wood, and is indigestible to animals. Certain bacterial species, however, contain the necessary enzymes to break down cellulose, and shipworms are often reported with gut associated bacterial symbionts.

   In an early study by Carpenter and Culliney (1975), a bacterium was isolated from the gut of a Sargasso Sea shipworm, *Teredora malleolus*. Under anaerobic conditions and growth in a liquefying cellulose medium, significantly high rates of N_2 fixation rates (up to 1.5 micrograms of nitrogen per milligram dry weight per hour) were recorded. Similarly high rates were also measured in three other coastal shipworms. In a later study, a novel bacterium was also isolated from 6 species of teredinid bivalves (shipworms). Similar to the earlier study, the novel isolate was capable of digesting cellulose and fixing nitrogen (Waterbury *et al.*, 1983). Both studies showed that N_2 fixation associated with the shipworms was significant and suggested that similar symbioses might occur in other organisms that ingest terrestrial plant material (Carpenter and Culliney, 1975).

   Later Distal *et al.* (1991, 2002a, 2002b) identified shipworm symbionts by 16S rRNA ribotyping and cultivated one of the symbiotic bacterium, *Teredinibacter turnerae*, from the gill region (Gland of Deshayes) of a Teredinidae shipworm. More recently it was shown using a multi-isotope imaging mass spectrometry

approach that $^{15}N_2$ is taken up by the bacterial symbionts and some evidence was presented for the transference of fixed N to the host (Lechene *et al.* 2007). Thus, the shipworm–bacterial consortiums represent yet another understudied symbiotic association related to the nitrogen cycle.

4.3. Ascidian–*prochloron* symbioses

There are several didemnids (ascidians) that have been reported with symbiotic *Prochloron* cells. *Prochloron*, a genus of photosynthetic prokaryotes, are found in the marine environment as free-living and also associated with marine invertebrates. The primary role of the *Prochloron* symbionts has been identified as an organic carbon source to their respective ascidian hosts. There is however, some controversial evidence that the nitrogen is also transferred from symbiont to host (Paerl, 1984). Others have investigated nitrogen budgets in ascidian-*Prochloron* colonies and have suggested that the host ascidian and symbiotic *Prochloron* efficiently recycle the nitrogen within the colony (Koike *et al.*, 1993), and thereby act more similar to a nitrogen trap (see below, section 4.4.). In nutrient poor environments where the ascidian colonies thrive, an efficient means of recycling nitrogen is probably essential for their survival, and warrants future investigations.

4.4. The sponge–phototroph nitrogen trap

The coral-zooxanthellae association is a frequently cited example of a successful symbiotic relationship that forms the foundation for a diverse and ecologically important habitat within tropical, oligotrophic environments. This association is successful due to its ability to tightly recycle nitrogen and carbon. The coupling between an endosymbiotic phototroph and its filter feeding invertebrate host, acts as a nutrient and particle trap (Cook, 1983; Hinrichsen, 1997; Rahav *et al.*, 1989; Wild *et al.*, 2004). Sponges are abundant in many oligotrophic environments (Wilkinson, 1983). For example, in the coral reef environment, estimates for the percent areal coverage by sponges are as high as 24% of high light, hard substrates and 54% of low light, rubble substrates (Diaz and Rutzler, 2001). Just like corals, sponges are known to specifically associate with certain phototroph symbionts (Wilkinson and Fay, 1979; Usher *et al.*, 2004).

Recent stable isotopic evidence demonstrated that sponge dissolved inorganic nitrogen (DIN) can be used by symbiotic algae and is sufficient to remove nitrogen growth limitation for the phototrophs (Davy *et al.*, 2002). The system is therefore analogous to the coral-zooxanthellae. It had already been demonstrated that cyanobacteria could provide photosynthetically derived organics to the sponge and were capable of supplying the majority of the host's energy requirement (Cheshire *et al.*, 1997). Interestingly, Trautman *et al.* (2000) observed that sponge–phototroph symbioses are often abundant in areas where corals are scarce, suggesting some degree of competition between these associations in the tropical oligotrophic environment or different responses to environmental conditions such as particle loading. Coral reefs are currently experiencing a sharp global decline and frequent bleaching of the symbiotic phototrophs (Hinrichsen, 1997). It is therefore necessary to understand if

a similar global decline is also occurring for the sponge–phototroph nitrogen trap (Wulff, 2006). If it is, what will be the impact of this decline on metazoan biodiversity in the oligotrophic environment and how does this decline relate to coupling of host and symbiont? This association may take on an altered role rapidly changing reef environment and is an important system for further study.

5. FUTURE OUTLOOK AND PERSPECTIVES

One of the largest obstacles in determining the overall importance of symbiotic associations to the global cycling of nitrogen is the lack of consistent rate measurements. For example, ranges in DDA abundances and N_2 fixation rates from a variety of studies around the world have been reported, however few of the studies measuring DDA N_2 fixation utilized the same means for rate normalization, i.e., biomass, cells, volume. This makes it quite difficult to estimate an overall contribution of these populations to the tropical and subtropical oceanic nitrogen budgets.

It seems that more attention or creative sampling schemes need to be developed to accurately measure the nitrogen (and likely carbon) cycling in the context of these planktonic and invertebrate associations. Studies similar to those presented by Zehr *et al.* (2007) and Needoba *et al.* (2007), which combine ^{15}N isotope rate measurements with quantitative PCR approaches provide a promising path forward. While molecular and microscopic characterization of nitrogen symbioses has helped to elucidate the diversity and distribution of symbioses, future work should target a consistent approach to relate these to their biogeochemical importance.

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ANALYTICAL METHODS FOR THE STUDY OF NITROGEN

Matthew D. McCarthy *and* Deborah A. Bronk

Contents

1. Introduction

The study of nitrogen (N) cycling requires sensitive and accurate methods to measure various N compounds. The objective of this chapter is to review existing methods to analyze dissolved and particulate fractions of N with an emphasis on those methodologies that are most commonly used or represent the state of the art, noting that N analysis does indeed seem to be an art at times. Many techniques are temperamental at best and it is not uncommon to find small fetishes devoted to keeping a given mass spectrometer running. This chapter was written to stand alone as a general review of methods; more detailed descriptions of specific methods can be found in other chapters in this book. We begin with a brief note on collecting and preserving samples. The first main section of the review then describes techniques to measure the concentrations of N pools, followed by various approaches to chemically characterize organic N fractions. The last major section reviews techniques to measure N fluxes, including uptake, release, nitrification, denitrification, and N_2 fixation. We conclude with recommendations for future research.

This chapter was written largely from the perspective of water column studies; most of the methods, however, could also be used more broadly to analyze sediments, particles, or pore water samples. The difference between a given method for these sample types is principally one of the detection limit required and matrix-specific analytical challenges. Analyzing water column samples can be difficult because concentrations can be very low. While concentrations are generally much higher in sediment pore waters, extracting the necessary volume of pore water can be challenging. With respect to rate measurements, most of the techniques outlined could be applied in both the water column and the sediment, and several largely sedimentary rate measurements are specifically discussed below. Tracer approaches in sediments can be especially problematic because the substrate is so heterogeneous that getting uniform mixing of the tracer can be impossible (e.g., Middleburg *et al.*, 1996); for a more detailed discussion of methods used in sediments see Chapter 19 by Joye and Anderson, this volume.

Before we begin, we provide a brief comment on units. In general, we recommend that concentrations of N compounds be presented as μmol N L^{-1} (i.e., μM, or some similar metric equivalent) and rates to be presented as μmol N L^{-1} time^{-1}. Historically the μg–at N unit has been used in the presentation of N uptake rates, particularly in culture work; 1 μmol of a compound is equal to 1 μg–at N for a

substrate like NH_4^+ but is equal to 2 µg-at N in the case of a dinitrogen substrate such as urea. Despite the opinion of some of our esteemed colleagues who view the unit as outdated (Luther, *personal communication*; Williams, 2004), we find the µg-at N unit to be useful to standardize substrate additions when measuring N flux rates using both inorganic and organic N that may have variable amounts of N per substrate.

2. Collection and Storage of Samples

Here we provide some very basic guidelines on collection and storage of samples. We stress, however, that each analyst should determine the optimal washing and sampling protocol for each of their own applications.

Nutrient samples are generally stored in plastic bottles such as HDPE or PETG. Prior to use, all plasticware should be washed with detergent (e.g., Liquinox®), rinsed with distilled water (DW) and soaked in an acid bath (most commonly 10% HCl) for a couple of hours, followed by a final rinse with copious amounts of high quality DW or equivalent. Glass bottles or vials are also often used and are the preferred choice for nutrients and organics, such as dissolved organic nitrogen (DON), and dissolved free and combined amino acids (DFAA, DCAA). Glassware has the advantage that it can be wrapped in foil and baked in a muffle oven (minimum of 450°C for 2 h) for unambiguous cleaning; glass also has the disadvantage, however, that it can break during shipping or freezing.

To filter or not to filter—that is the question! If one is measuring dissolved compounds, it is customary to filter the sample prior to analysis. In open ocean systems, however, filtration is often not done because the amount of particulate material is small, and the risk of contamination is large. In coastal or estuarine systems, however, filtration is a must. Filtration of nutrient samples is generally done through a glass fiber filter (i.e., GF/F®) with a nominal pore size of 0.7 µm, or a 0.2–0.45 µm polycarbonate filter (e.g., Supor® or Nuclepore®) (reviewed in Knelfelkamp *et al.*, 2007). Benefits of glass fiber filters are that they are inexpensive, have a high flow rate, and can be cleaned by baking (minimum of 450°C for 2 h). Polycarbonate filters should be used with care as they can introduce high blanks for some analyses. For example, Supor filters can produce high dissolved organic carbon (DOC) and DON blanks; rinsing a 47 mm Supor filter with 200 ml of DW is generally sufficient to lower the blank substantially (C. Carlson, *personal communication*).

Sample storage options include acidification, refrigeration, pasteurization, or freezing (−20 or −80°C). The principle behind acidification is to lower the pH sufficiently to inhibit microbial activity that could alter the concentration of the analytes; HPLC-grade phosphoric acid is often used. If acidification is chosen, the analyst should take the pH change into account prior to analysis because many methods are pH sensitive. Refrigeration should only be considered for samples filtered through a 0.2 µm filter (thus making the sample nearly abiotic). Pasteurization has been pursued as a storage option because of the risk that freezers could fail, as

well as the expense of storing large numbers of samples frozen for long periods of time (Aminot and Kérouel, 1998). Though not appropriate for ammonium (NH_4^+), presumably due to the potential for loss through volatilization, researchers may want to investigate its use for storage of nitrate (NO_3^-) and nitrite (NO_2^-) samples. For most samples, simple frozen storage is generally the most common method and is what we recommend, with some caveats. Care must be taken to allow space for expansion during freezing, and if using glass vials duplicate samples are highly recommended to allow for potential breakage. Also, if storing samples in a freezer, that freezer should be clean and free of meat or animal tissues and high organic sediments, particularly when storing samples for the more sensitive analyses such as DFAA.

3. MEASUREMENT OF NITROGEN CONCENTRATIONS

Nitrogen exists in the ocean at oxidation states from −3 to +5. There are three forms of fixed inorganic N: NO_3^-, NO_2^- and NH_4^+. Nitrate is the final oxidation product and is the dominant form of fixed N in the deep ocean. Nitrite generally occurs at very low concentrations because it is an intermediate in the processes of nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) and denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) and so seldom accumulates to a large degree. Concentrations of NH_4^+ are highly variable but tend to be near the limit of detection in open ocean surface waters. Each of the inorganic forms has a number of manual and automated methods of analysis. We discuss the most widely used below.

3.1. General principles of nutrient analysis

In general, nutrient analyses are done either manually or using an autoanalyzer. Space limits preclude the presentation of detailed protocols. An excellent general review of nutrient analysis as well as detailed method descriptions and protocols can be found in Grasshoff *et al.* (1999) and Sparks *et al.* (1996).

Many of the methods discussed below are colorimetric techniques. The basic principle behind these techniques is to add a series of reagents that will build a dye molecule containing the analyte in question. The higher the concentration of the analyte, the higher the concentration of dye that will form. The final step in the analysis is to measure the absorbance of the sample using a spectropho-tometer at a wavelength that represents the peak absorbance for the dye in question. The conversion of absorbance to a concentration is described by Beer–Lambert's Law.

A number of basic principles should always be followed regardless of the analysis. First, the concentration in the standard curve should bracket the concentrations of the samples being analyzed. Second, standards should be run at the same time and treated in an identical fashion to the samples being measured. Third, for many analyses, the matrix (e.g., freshwater or saltwater) of the sample will influence the absorbance measured. For this reason, standards should be prepared in a matrix that

matches the matrix of the samples. In the case of seawater samples, artificial or low nutrient seawater should be used in the preparation of the standards. In the case of estuarine samples, where the salinity can change dramatically from station to station, a salt correction is generally used (i.e., Grasshoff *et al.*, 1999). Fourth, the longer the cuvette (i.e., path length) used in the final analysis, the lower the limit of detection that can be reached. The use of long-path liquid waveguide capillary cells (e.g., meters long) has allowed great improvement in sensitivity and detection for a number of analyses (e.g., Zhang, 2000).

3.2. Ammonium (NH_4^+) analysis

Ammonium is present at very low concentrations (0.03–0.5 µM) in oceanic surface waters, at higher concentrations in coastal and estuarine waters (Sharp, 1983), and at concentrations orders of magnitude higher in sediment pore waters. In seawater, NH_4^+ exists as the acid base pair NH_4^+–NH_3 (ammonia); the pK_a of the pair is 9.3. The methods discussed here measure the sum of NH_4^+, the form that dominates at the pH of seawater ($\sim$<8.3), and NH_3, the volatile form that dominates under more alkaline conditions. There are many approaches to measuring NH_4^+, but we will focus on the two most widely used—phenol-hypochlorite and orthophtal-dialdehyde (OPA).

The most common approach to measuring NH_4^+ is a colorimetric one that uses the Bertholot reaction where a blue color is formed by phenol and hypochlorite in the presence of NH_4^+ (Searle, 1984). The reaction requires a catalyst or elevated temperatures to achieve the sensitivity needed for environmental samples. Sodium nitroprusside is commonly used as the catalyst (Solorzano, 1969); salicylate can also be used as the catalyst but the limit of detection is higher ($\sim$0.7 µmol L^{-1}) (Bower and Holm-Hansen, 1980). In either case, the reaction product is an azo dye, the absorbance of which is measured on a spectrophotometer at 630 nm.

To increase the sensitivity of the analysis, it is recommended that a 5 or 10 cm path length cell be used. The detection limit using a 10 cm cell is approximately 0.05 µM, with an upper limit of 40 µM without dilution. Use of a long-path liquid waveguide capillary cell (2 m) lowered the detection limit to 5 nM with a precision of 5% in the 10–100 nM range (Li *et al.*, 2005). Another approach used to decrease the limit of detection is to preconcentrate the NH_4^+ prior to analysis. In one technique, the indophenol is concentrated by extraction into n-hexanol; this method has a precision of 1.9 nM at concentrations ≤50 nM (Brzezinski, 1987). The indophenol can also be concentrated onto solid phase extraction (SPE) octade-cylsilane (C18) columns (Selmer and Sorensson, 1986), with the final concentration determined as outlined in Brzezinski (1987).

There are also a number of matrix issues that the analyst should bear in mind. In seawater there is a small salt effect, with lower absorbances observed in seawater versus distilled water for the same concentration of NH_4^+. Amino acids can cause an interference with the analysis, but at the concentrations of amino acids found in seawater the interference is generally insignificant (Solorzano, 1969); there is no interference with urea. Beware of analyzing samples with a pH higher than 11.0 because blanks become inconsistent; a pH problem is indicated if the sample has a

greenish, rather than blue, color. Also, samples from low salinity environments (<5) with high humic concentrations often have a tea color, which will interfere with the colorimetric protocol. One way to circumvent this problem is to precipitate the humics with a magnesium sulfate solution prior to analysis (Grasshoff et al., 1983). When samples cannot be run right away, another useful modification is the addition of the phenolic reagent immediately after sample collection to bind the NH_4^+. After the reagent is added, the samples can be stored in the refrigerator until analysis, circumventing the need to freeze the samples (e.g., Cochlan et al., 2002).

The phenol–hypochlorite method has also been adapted for use in microplate readers. The advantage is very small sample size (<300 µl) and extremely high sample throughput (~1000 samples per day). The limit of detection, however, is high (~1.6 µM), making it unsuitable for water column studies, although it may be applicable for sediment pore waters or highly eutrophic systems (Baudinet and Galgani, 1991).

The most common alternative to the phenol–hypochlorite method uses OPA. This technique was introduced in an automated version based on the conversion of NH_4^+ to NH_3, which is then allowed to diffuse across a membrane into a flowing stream of OPA to form a fluorescent product (Jones, 1991). This final product is then analyzed fluorometrically with a detection limit of ~1.5 nM (Jones, 1991). Advantages of the method are that it requires only a single reagent and it has a lower detection limit than the standard phenol–hypochlorite method. Furthermore, the salt effect is relatively small ($<3\%$ over a salinity range of 0–35 ‰) and there is no interference from primary amines. The technique has been modified to remove the gas diffusion cell, also with a reported detection limit of 1.5 nM (Kerouel and Aminot, 1997); a manual version has also been published (Holmes et al., 1999).

3.3. Nitrate (NO_3^-) and nitrite (NO_2^-) analysis

Nitrate is often near the limit of detection in surface waters (~0.05 µM) but increases to ~35 µM below the thermocline (Sharp, 1983). Nitrite is an intermediate in the processes of nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) and denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). Its presence can thus be diagnostic for these processes, but it seldom accumulates to concentrations >1 µM. Concentrations of NO_3^- and NO_2^- are often reported summed in the literature because their measurement is related. There are a number of methods that can measure NO_2^- directly, however, we are unaware of any method for the direct measurement of NO_3^-. To measure NO_3^-, it must first be reduced to NO_2^-, or some other more reduced form, such that the NO_2^- actually measured will include both preexisting NO_2^- plus the NO_2^- produced as a result of the reduction of NO_3^-. The concentration of NO_3^- is then calculated as:

$$[NO_{2+3}] - [NO_2^-] = [NO_3^-] \tag{28.1}$$

The most common method to measure NO_2^- is based on the formation of an azo dye formed when NO_2^- reacts with two aromatic amines added sequentially

(sulfanilamide and N-naphthylethylene-diamine dihydrochloride, NEDA, Strickland and Parsons, 1972). The resulting red azo dye is measured on a spectro-photometer with a linear standard curve up to 10 μM; sample concentrations greater than 10 μM should be diluted before being read. The precision of the method is ±0.01 μM (Strickland and Parsons, 1972). Application of a long-path liquid wave-guide capillary cell (2 m) can lower the detection limit for NO_2^- to 1 nM with a precision of 0.2% (Zhang, 2000).

The most common method for measurement of NO_3^- is cadmium reduction (i.e., the cadmium/copper amalgam method) with the chemiluminescence method the most common alternative. Both involve an initial step to reduce the NO_3^- to NO_2^-. In the former, reduction with copper coated cadmium can be done manually using a reduction column or, more commonly, an autoanalyzer (Hansen and Koroleff, 1999). After the reduction is complete the sample is treated for NO_2^- analysis as described above. The limit of detection of the technique is reported to be 0.05 μM (Strickland and Parsons, 1972); slight modifications to the automated procedure increased the precision of the NO_2^- and NO_3^- analyses to ±1.24 and ±2.87 nM, respectively (Raimbault et al., 1990). Application of a long-path liquid waveguide capillary cell (2 m) can lower the detection limit for NO_3^- to 2 nM with a precision of 0.8% (Zhang, 2000). The speed of automated NO_3^- analysis has also increased dramatically thus allowing higher sample through put, which makes fine scale underway sampling possible (e.g., Hales et al., 2004).

A useful alternative to cadmium columns is reduction of individual samples with spongy cadmium (Jones, 1984). Spongy cadmium is made by placing zinc sticks in 20% cadmium sulfate overnight, during which time the cadmium precipitates on the surface of the zinc. The spongy cadmium is then added to individual seawater samples with ammonium chloride and shaken for 90 min. The spongy cadmium has the advantage of having a much higher surface area than the granules used in cadmium columns. Advantages of the method include the ability to run a large number of samples simultaneously and stability in reductor efficiency over time. In addition, when sample concentrations vary over a wide concentration range, the spongy cadmium method can be used without the need to rerun samples using different calibrations, as is often required with autoanalyzers. This method is especially useful for sediment pore water samples that may have sulfide present, a compound that will reduce the efficiency or completely inactivate a cadmium reduction column. If the sulfide is not completely removed in a given sample only that sample will be compromised, rather than an entire reduction column. The limit of detection of the spongy cadmium technique is reported as 0.033 μM (Jones, 1984) but in practice limits of detection are closer to 0.1 μM, such that we reserve its use for samples from more eutrophic environments.

A common source of error in NO_3^- measurements is caused by a decrease in reductor efficiency over time, which has been reported as ∼1% per 4–6 h of autoanalyzer run-time (Garside, 1993). Reduction efficiency can decrease due to a decrease in surface area, precipitation of hydroxides on the cadmium, and loss of the copper coating. It is recommended that NO_2^- and NO_3^- standards be analyzed periodically during a run and the results used to correct for the loss in reductor efficiency (Garside, 1993). An efficient method to regenerate or reactivate cadmium

columns, manual or in autoanalyzers, is to pass a copper-EDTA complex through the column (Otsuki, 1978). This reactivation method has the advantage that it does not produce an excess of reduced colloidal copper that can clog the column, necessitating the column being unpacked each time it is regenerated.

The other commonly used method to measure NO_3^- and NO_2^- is chemiluminescence. In this technique NO_3^- and NO_2^- are reduced to nitric oxide (NO), which is then measured on a N oxide analyzer after its chemiluminescent reaction with ozone (Garside, 1982). The reduction of NO_3^- to NO_2^- is effected by injecting the sample into a heated reservoir containing an iron(II)-molybdate solution (Garside, 1982). The amount of NO produced is dependent on the volume of sample injected into the machine. Volumes up to 10 ml can be injected for low concentration samples. The range of the method was reported as 0.002–20 µM. The method is more sensitive than the cadmium reduction methods, with a precision of ± 0.002 µM NO_3^- and ± 0.0005 µM for NO_2^- (Garside, 1982). Vanadium(III) has now largely replaced the iron(II)-molybdate solution because it is a more reactive reducing agent, and retains its reducing power at much lower acidities and after repeated injections of seawater (Braman and Hendrix, 1989).

The trend toward monitoring over large spatial and temporal scales and the expense of analyzing discrete samples has greatly increased the interest in sensors that can be deployed within the environment. Nitrate has been the N form to receive the greatest interest in this regard because of its importance in controlling oceanic new production. There are now *in situ* instrument packages that can provide NO_3^- concentration measurements over a range of temporal scales while attached to a fixed location (days to months, e.g., Johnson and Coletti, 2002; Johnson *et al.*, 2007).

3.4. Total dissolved nitrogen/dissolved organic nitrogen analysis

The total dissolved nitrogen (TDN) pool consists of an inorganic N (NH_4^+, NO_3^-, and NO_2^-) fraction and an organic fraction. To calculate the DON fraction, the sum of the inorganic N concentrations are subtracted from the TDN concentration, with the residual being defined as DON.

$$DON = TDN - (NH_4^+ + NO_3^-/NO_2^-) \qquad (28.2)$$

The standard deviation of the DON concentration is calculated by propagating the error with the following equation:

$$s_{DON} = (s^2_{TDN} + s^2_{NH_4^+} + s^2_{NO_3^-/NO_2^-})^{1/2} \qquad (28.3)$$

where s^2 is the variance of the three measurements (Bevington, 1969). Measurements of DON thus have inherently large errors, since the final estimate of DON contains the combined analytical uncertainty of three analyses: TDN, NH_4^+, and combined NO_3^-/NO_2^-. This propagated uncertainty becomes an increasingly large problem in subsurface and deep ocean waters, where DON concentrations are small while dissolved inorganic nitrogen (DIN) concentrations are large.

A discussion of organic pools in general (DON plus DOC and dissolved organic phosphorus) is presented in Sharp (2002).

A cautionary note regarding statistics commonly used in nutrient chemistry—know the difference between Model I and Model II regressions (Laws and Archie, 1981). When one is plotting a standard curve, where the parameter on the x-axis is controlled, a Model I regression should be used. When comparing methods (e.g., as was done with the DON method intercomparison), where the same parameter is measured in a number of different ways, or with most field data, a Model II regression should be used (see Laws and Archie, 1981).

There are currently three methods commonly used to measure TDN concentrations: persulfate oxidation (PO) (Solorzano and Sharp, 1980; Valderrama, 1981); ultraviolet oxidation (UV, Armstrong and Tibbitts, 1968; Armstrong et al., 1966); and high temperature combustion (HTC, Suzuki and Sugimura, 1985); these three methods are reviewed in detail in Sharp et al. (2004). A comparison of the three methods was undertaken with 29 sets of analyses done on five field samples (Sharp et al., 2002a). The coefficient of variations for the five samples ranged from 19% to 46%, with the poorest replication observed on deep ocean samples. No significant differences were found between the different methods. Kjeldahl digestion is another method used to measure TDN that uses sulfuric acid to convert DON to NH_4^+. It has high blanks and low precision and is therefore not recommended for analysis of marine samples (D'Elia et al., 1977).

Analysis of TDN by the PO method involves adding a potassium persulfate oxidizing reagent to a sample and then autoclaving it for 0.5 h at 121°C and 15 lb/in^2 pressure. The oxidation converts the fixed N forms to NO_3^- and then the concentration of the NO_3^- is measured with one of the techniques described above. The PO method often suffers from high blanks that are a function of the relatively large persulfate addition required. If high blanks are obtained, recrystallization of the potassium persulfate once or twice can be done to exclude N contaminants in the crystals thus lowering the blanks (Hansen and Koroleff, 1999); this process, however, is time consuming. The required sample volume generally ranges from 5 to 20 ml, depending on the volume required for the subsequent NO_3^- analysis. Of all the methods, the PO technique has the lowest instrumentation cost. Samples can also be boiled if an autoclave is not available (Bronk et al., 2000). Readers are, however, cautioned against the use of Teflon or HDPE bottles. Teflon bottles are permanently misshapen during autoclaving and Teflon becomes pliable when heated repeatedly, resulting in loose caps and loss of sample. Bottles made of HDPE can be used, but must be autoclaved first because ~10% of them explode or leak during autoclaving. The safest method is to use sealed glass ampoules.

Analysis of TDN by the UV method requires an instrument that can hold samples at a fixed distance from a UV light source without allowing the samples to overheat (Armstrong et al., 1966). One common design for such an instrument was developed by Armstrong and Tibbitts (1968). Most commonly the method uses a 500–1200 W mercury vapor lamp. The sample tubes must be made of quartz to allow the passage of the UV radiation through the sample wall. Hydrogen peroxide (H_2O_2) is often used as an oxidant. Oxidation times generally vary from 18 to 24 h depending on the UV lamp; each new lamp should be tested to determine the

oxidation time required (Bronk *et al.*, 2000). The advantage of the UV technique is the small addition of H_2O_2 used, which results in generally lower blanks than the PO method. Disadvantages of the UV method includes the cost and effort of building a UV machine and the long oxidation time required. Automated approaches have been proposed that could increase sample throughput (Collos and Mornet, 1993; Le Poupon *et al.*, 1997).

With the HTC method, samples are combusted at high temperatures in the presence of oxygen. Though chemically simple, the HTC method requires great care to achieve reproducible results from day to day. In general the HTC method is not considered to have a salt effect (Walsh, 1989). Advantages of the HTC method are the small sample volume required and relatively low blank. The main disadvantage with this method is the expense of the equipment, and the time and effort required for set-up and troubleshooting.

3.5. Particulate nitrogen analysis

Concentrations of particulate nitrogen (PN) are commonly $<1\,\mu M$ in oceanic waters and $<10\,\mu M$ in coastal systems (Sharp, 1983). To measure PN, also known as particulate organic N (PON), a field sample is first filtered, commonly through a baked (450°C for a minimum of 2 h) Whatman GF/F filter (nominal pore size 0.7 μm); silver filters (Gordon and Sutcliffe, 1974) and Anapore® alumina silicate filters can also be used. The filter is then dried and analyzed with a CHN (carbon hydrogen nitrogen) elemental analyzer that combusts the filter and quantifies the resulting gases (Ehrhardt and Koeve, 1999); see Hurd and Spencer (1991) for a discussion of particulate N and C analyses and instruments.

4. DIRECT MEASUREMENT OF MAJOR DON COMPONENTS

4.1. Urea

Urea $((NH_2)_2CO)$ is excreted by larger organisms, can be a product of bacterial organic matter decomposition, and is a highly labile form of N for plankton nutrition (Bronk, 2002). Reports of concentrations in oceanic waters are relatively scarce, but are quite low ($<0.5\,\mu M$; Antia *et al.*, 1991). There are currently two methods commonly used to measure urea concentrations—the urease method (McCarthy, 1970) and the monoxime method (Mulvenna and Savidge, 1992; Price and Harrison, 1987).

In the urease method, urea is enzymatically hydrolyzed to CO_2 and NH_3 (McCarthy, 1970). The released NH_4^+ is then measured using the phenol hypochlorite method described above. The urease method commonly suffers from incomplete hydrolysis, resulting in an underestimation of urea concentrations.

The monoxime method is a colorimetric approach where urea is measured directly using diacetyl monoxime. Difficulties associated with the monoxime method include precipitate formation when the sample cools and rapid loss of color (Mulvaney and Bremer, 1979). The method also needs a higher reaction

temperature (85°C), thus requiring a heated water bath that can limit the number of samples run simultaneously. Goeyens *et al.* (1998) published a modified version of the monoxime method that allowed the reaction sequence to take place at room temperature albeit with a much longer reaction period—72 h versus 20 min for the high temperature method. The limit of detection was similar for the high temperature (85°C) method, 0.14 μM, and the room temperature (20°C) method, 0.10 μM (Goeyens *et al.*, 1998). A monoxime method has also been introduced for use with an autoanalyzer (Cozzi, 2004).

Comparisons of the two methods found that the urease method tends to underestimate concentrations in seawater and so we recommend the monoxime method for analysis of marine samples (Price and Harrison, 1987; Revilla *et al.*, 2005). With respect to estuarine samples, the monoxime method is more accurate and less affected by salinity than the urease method (Revilla *et al.*, 2005). There has been some concern that freezing samples may result in lower urea concentrations (Mulvenna and Savidge, 1992). Other researchers, however, have shown that freezing does not alter the urea concentration measured (Cochlan and Bronk, 2001; Price and Harrison, 1987).

4.2. Humic substances

Humic substances are a broad class of organic compounds operationally defined by their solubility at different pHs and retention on hydrophobic resins (Aiken, 1988; Thurman, 1985). There are three operational sub-categories of humic substances: humic acids, which are soluble at a higher pH but become insoluble at a pH < 2 (isolated using XAD-8 resin); fulvic acids, which are hydrophilic acids soluble under all pH conditions (isolated using XAD-4 resin), and humin, which is insoluble at any pH (Ishiwatari, 1992). For a review of humic substances in aquatic systems, see Hessen and Tranvik (1998), Benner (2002), and Chapter 3 by Aluwihare and Meador, this volume.

There are two common approaches used to quantify humic substances—measurement of fluorescence or extraction onto a resin. The use of fluorescence measurements is of limited use in N studies because the N fraction of the humic substances cannot be quantified directly. In resin methods, humic substances are extracted onto a macroporous acrylic ester resin. The most commonly used resin was formerly Amberlite XAD-8, but now Supelite DAX-8 is most often used. Both resins isolate similar bulk humic fractions from natural waters and produce solutions with similar chemical compositions (Peuravuori *et al.*, 2002). These resins also release small amounts of organic molecules (Aiken, 1988). Therefore, prior to the extraction of humics, the resin should be cleaned over several days via a Soxhlet extraction procedure (solvents include ether, acetonitrile, and methanol) followed by extensive rinses with hydrochloric acid (HCl), sodium hydroxide (NaOH), and DW (Aiken, 1985; Thurman, 1985). When isolating humics, the sample pH is reduced to <2 with 6N HCl, which protonates acid groups, prior to passage of the sample through the resin. After the sample passes through the resin, the column is rinsed with DW to remove residual salts. The humics are eluted from the column with 0.2N NaOH. Complete elution of all humic-N is difficult, however. Therefore, the most accurate

approach to measure humic-N concentrations is to measure the TDN concentration before and after the sample has passed through the column, with the concentration of humic-N taken to be the difference, rather than to measure the concentration of TDN in the eluted humic fraction. There is also a high temperature oxidation method for humic acid analysis that has not been commonly used (Fox, 1991). The relative solubility of humic and fulvic acids can be exploited to measure the concentration of these two fractions (e.g., Van Zomeren and Comans, 2007).

One should be cautious when quantifying humic-N based on the use of resin extraction. Recent work indicates that N associated with humics can dissociate when the pH is taken down to <2 prior to the passage through the resin column (See and Bronk, 2005). As a result, the C:N ratio of the isolated humic substances is likely higher than it is in natural waters.

4.3. Amino acids

Amino acids are the largest component of total DON identifiable at the molecular level, and a wealth of information is available from both molecular and enantiomeric analyses. The fact that amino acids are one of the few compound classes that can be readily quantified in unconcentrated seawater has resulted in a broad literature on marine amino acids evolving for nearly 30 years, using methods that have changed relatively little.

Dissolved amino acids are commonly divided into two pools that must be analyzed separately: DFAA exist as individual monomers in solution, while DCAA are defined operationally as additional amino acids liberated by acid hydrolysis. DCAA are thus presumably present mostly as polypeptides, a supposition supported in at least the high molecular weight (HMW) fraction by ^{15}N-nuclear magnetic resonance (NMR) spectroscopy data (discussed below). The operational nature of the DFAA versus DCAA definitions means that amino acids liberated from difficult matrixes (e.g., humic substances) also could make up a part of DCAA. Total hydrolysable amino acid (THAA) is another term commonly used to denote both pools together, when the sample is hydrolyzed but DFAA are not independently determined. Because the DFAA pool is typically much smaller than DCAA, THAA values are often assumed to be similar to DCAA.

Amino acids are polar and chemically diverse molecules, with side chains including a single proton, aliphatic chains, acids, bases, and aromatic rings. Methods used to determine the 15–20 most common protein and non-protein amino acids generally use derivatives to aid in separation, detection, or both. Because molecular-level amino acid analyses are routinely made in biomedical research, a variety of automated amino acid analyzers are commercially available. These typically consist of liquid chromatography coupled to automated-fluorescent derivatization, detector, and software optimized for common protein amino acids. Automated analyzers offer easy and rapid analysis of biological materials or purified proteins within a well known matrix, and have also been used successfully in ocean research (Ittekkot, 1982; Lee and Bada, 1977). Most recent investigators, however, have opted to set up their own amino acid methods using liquid or gas chromatographic systems tailored to their specific goals. In principle, the only analytical difference separating

DFAA and DCAA is the presence or absence of hydrolysis. In practice, however, the extremely low detection limits needed for DFAA limits the range of chromatography and detection options that can be used, while hydrolysis itself is problematic for some amino acids. For these reasons, DFAA and DCAA analysis are treated individually below.

4.3.1. Free amino acids

While DFAA make up a minor part of the total THAA pool (1–10%) they are nevertheless extremely important as substrates for microbial growth (e.g., Keil and Kirchman, 1991a) and important components of rapidly cycling DON (Bronk *et al.*, 2007). Because of extremely low concentrations, use of fluorescent derivatives that allow fmol to pmol detection levels are necessary in order to make measurements directly in seawater. Reverse-phase high pressure liquid chromatography (HPLC) separations after derivatization with OPA have remained the most widely used approach in ocean sciences since the early 1980s (e.g., Jones *et al.*, 1981; Lindroth and Mopper, 1979; Parsons *et al.*, 1984). Compound-specific DFAA measurements are commonly made directly in filtered seawater using automated pre-column OPA derivatization/injection coupled with reverse-phase chromatography and florescence detection (e.g., Cherrier and Bauer, 2004; Coffin, 1989; Keil and Kirchman, 1991a). For coastal samples, interference by NH_4^+ can be a problem, and corrections may be necessary (Tada *et al.*, 1998). As long as NH_4^+ levels are not high, total DFAA can also be estimated by a bulk fluorometric approach (Parsons *et al.*, 1984), because DFAA concentrations are often equal to the concentration of total dissolved primary amines (e.g., Delmas *et al.*, 1990; Kirchman *et al.*, 1989).

In practice, many difficulties of routine DFAA analysis in open ocean waters stem from the extremely low concentrations present. Great care must be taken to avoid contamination and to monitor procedural blanks. Assuming no special chromatographic problems exist, detection limits can also be affected by the sensitively of the florescence detector used. Simply replacing an old lamp may help substantially, and new generations of florescence detectors (e.g., offered by Shimadzu, Agilent, Varian, etc.) claim substantial improvement in power and optics. It is also possible to use column pre-concentration to increase sensitivity (Lee and Bada, 1975). This approach could be extremely useful for investigation of minor DFAA components, such as D-enantiomers of DFAA (Lee and Bada, 1975, 1977). However, because most of the common DFAA components can be measured directly, pre-concentration approaches have not been widely used.

4.3.2. Combined amino acids

4.3.2.1. Hydrolysis methods   Molecular-level analysis of THAA is dependent on hydrolytic cleavage of peptide bonds. Standard acid hydrolytic conditions developed for pure proteins remain in common use, typically employing 6N HCl at 100–110°C for 20–24 h, done under a N_2 atmosphere to minimize oxidative destruction of sensitive side-chains (e.g., Dauwe *et al.*, 1999; Henrichs *et al.*, 1984; Lee and Bada, 1977; Parsons *et al.*, 1984; Van Mooy *et al.*, 2002; Ziegler and Fogel, 2003). Several amino acids with acid-sensitive side-chains are, however, destroyed or severely degraded under these conditions. Asparagine and glutamine are

quantitatively converted to aspartic acid and glutamic acid, while the side-chains of tryptophan and cysteine are substantially degraded. In order to effectively measure acid-sensitive amino acids, base hydrolysis can be used (Wu and Tanoue, 2002). A number of ancillary approaches have also been devised to protect sensitive side chains from degradation, for example, by adding sacrificial compounds or organic acids (Liu and Chang, 1971). However, because the affected amino acids represent only a minor molar percent of the total amino acid pool, such measures have rarely been used for oceanic THAA analyses.

Alternatives to the standard acid hydrolytic conditions have also been examined for marine samples. Elevated temperatures can reduce reaction time, and addition of multiple internal standards can more effectively track losses related to specific side-chain functionality (Cowie and Hedges, 1992a). Gas phase hydrolysis (Keil and Kirchman, 1991b) is an alternative to standard aqueous hydrolysis that results in much reduced analysis time, and may have an important impact on THAA quantification in some samples. Keil and Kirchman (1991b) observed substantial increases in amino acid yield for a range of oceanic samples using a vapor-phase HCL/TFA mixture at 156°C. The highest increases were for open ocean DOM, suggesting that THAA in DON may have been underestimated by up to 300%. More recently, Jørgensen and Jensen (1997) demonstrated a vapor-phase microwave method using only HCl, which reduced hydrolysis time to ~20 min. while also increasing THAA yields relative to standard aqueous phase protocols. Because of the potential for shortened reaction times and increased recovery of some problem amino acids such as cysteine, vapor-phase has become common for protein hydrolysis in some biochemistry applications (e.g., Strydoma *et al.*, 1993) and has also been used to analyze marine samples (e.g., Cherrier and Bauer, 2004). One caveat, however, is that vapor-phase hydrolysis has been found to strongly affect the enantiomeric ratios of liberated amino acids (Kaiser and Benner, 2005).

4.3.2.2. Derivatization and chromatography   Once individual amino acids are liberated in dissolved form, they are typically separated by either HPLC or gas chromatography (GC) for molecular-level quantification. Both HPLC and GC have been used effectively, with each having strengths and limitations. As with DFAA, reverse-phase HPLC separations after derivatization with OPA (Lindroth and Mopper, 1979) has become perhaps the most widely used method (e.g., Cowie and Hedges, 1992b; Dauwe and Middelburg, 1998; Hubberten *et al.*, 1994; Ingalls *et al.*, 2003; Keil and Kirchman, 1993; Lee *et al.*, 2000; McCarthy *et al.*, 1996; Van Mooy *et al.*, 2002; Yamashita and Tanoue, 2003). Because hydrolyzed amino acids are relatively stable at room temperature, large sample sets can be derivatized and chromatographed automatically using HPLC auto-injectors equipped with sample pre-treatment capability. However, some amino acids are either not derivatized at all by OPA (e.g., proline, secondary amines in general), or typically have poor quantification and high variability (e.g., cysteine). 9-fluorenylmethyloxycarbonyl chloride (FMOC) is a common agent for derivatizing secondary amines, and can be used in combination with OPA to both detect proline as well as increase sensitivity for several other amino acids (Godel *et al.*, 1992). In practice, however, such enhancements to basic HPLC methods have rarely been used for ocean samples because these amino acids are minor

components of TDAA. One inherent drawback to HPLC methods is reduced chromatographic resolution relative to GC. Liquid chromatography–mass spectrometry (LC–MS) methods offer the sensitivity as well as molecular specificity to identify even unusual amino acids without needing to fully resolve components (Whitehead and Hedges, 2002); however, the necessity to desalt samples for electrospray MS applications diminishes its direct applicability for marine DON.

GC methods offer an attractive alternative to HPLC for applications where higher detection limits are acceptable (e.g., Harvey and Mannino, 2001; McCarthy *et al.*, 1998; Ziegler and Fogel, 2003). A wide variety of volatile amino acid derivatives can be made by sequential esterification and acylation of the carboxyl and amino terminal ends, respectively (see Knapp, 1979 for a long list). Additional derivatives, their chromatographic separation with different phases, and especially their applicability for compound-specific isotope analysis have also recently been compared (Corr *et al.*, 2007). These derivatives are readily separated by common GC phases, making GC a good method for quantification, isotopic, and enantiomeric amino acid analyses. Advantages of GC–based analysis include greatly increased chromatographic resolution, the relative ease and reproducibility of GC systems, as well the option for unambiguous peak and mixture identification using common bench–top gas chromatography–Mass spectrometry (GC–MS) instruments. Drawbacks are the relatively labor intensive wet chemistry used to make derivatives and the lower sensitivity of common GC detectors. The need to make derivatives by hand (and thus in small batches) greatly limits sample throughput, and larger samples are required relative to HPLC–florescent detection methods. However, using N and phosphorus selective detectors (NPD), or selective ion monitoring with GC–MS, can increase sensitivity about an order of magnitude versus standard flame ionization detectors (FID), while at the same time producing extremely clean and selective traces composed essentially only of the compounds of interest. Nevertheless, even with these approaches, sensitivity is generally too low for unconcentrated seawater analysis, and the enormous salt content (relative to organic N (ON)) can cause practical problems in performing derivatization reactions on dried seawater samples. These factors make GC techniques most useful for concentrated DON isolates, and in particular for investigation of enantiomeric (D/L), non–protein or minor amino acids where complex traces are anticipated, or MS compound verification is important.

4.3.3. Chiral (D/L) amino acids

Enantiomeric amino acid analysis has recently been of significant interest as an indicator of bacterial DON sources (Amon *et al.*, 2001; Fitznar, 1999; McCarthy *et al.*, 1998). Using GC, amino acid enantiomers can be easily resolved by substituting a chiral column for a non–chiral phase (e.g., McCarthy *et al.*, 1998). The same derivatives applicable for other GC techniques can be used, with the two most common being triflouro and pentaflouro anhydrydes (TFA, PFA). While one derivative is not necessarily preferred over others, the availability of different derivatization agents can be useful for resolving minor peaks in complex traces (such as often result from ocean DON). If a particular peak of interest is not well resolved or its identity is unclear, running the same sample derivatized with a different acylation agent may well resolve it.

For direct analysis of chiral amino acids in total seawater, a diasteriomeric-derivative HPLC approach has recently been described (Fitznar et al., 1999). Florescent diasteriomeric derivatives are made that can then be separated using a standard (and thus relatively inexpensive) reverse-phase HPLC column. This allows chiral amino acids to be measured in unconcentrated seawater samples with a protocol similar to standard OPA-based amino acid analysis (Dittmar et al., 2001). Obtaining good resolution of the doubled number of HPLC peaks can be somewhat challenging; however, the diasteriomeric method has an advantage in that since the derivatization reagent is chiral, two separate diasteriomers can be made for each compound. Each sample can thus be run using each reagent (D and L) in turn, resulting in unique separations that generally allow overlapping peaks in one trace to be resolved in the other (Fitznar et al., 1999). While an increase in effort, this approach also represents a powerful check on peak identification, allowing even minor D-AA peaks to be identified.

One important issue with D/L amino acid analysis is accounting for the racemization that occurs during hydrolysis. Chiral amino acids racemize during a hydrolysis reaction. The rates of racemization are amino acid specific and can be affected by a number of complex factors including position within a peptide, the size of the polymer structure, and the sample matrix (e.g., Bada, 1985). If this procedural racemization is not taken into account, the resulting D/L ratios (and any subsequent calculations, such as estimates of peptidoglycan derived from D–amino acid concentrations) will be in error to some degree. The extent of the problem will depend on the specific amino acid in question, being less severe with slower racemizing species (e.g., alanine), but especially important for the fast-racemizing amino acids like aspartic acid. Various approaches have been used to account for the extent of racemization during hydrolysis, including the use of deuterated HCl hydrolysis coupled with mass spectrometry (e.g., Amelung and Brodowski, 2002), and hydrolysis time series (Nagata et al., 1998). Kaiser and Benner (2005) have recently studied the extent of hydrolysis-related racemization in liquid versus vapor–phase protocols. These authors provide amino–acid specific racemization values, as well as equations that can be used to correct for hydrolysis–induced racemization (Kaiser and Benner, 2005).

4.4. Total protein/peptide

4.4.1. Colorimetric methods for bulk protein

As mentioned above, the large majority of recoverable amino acids are in combined form. This result indicates that peptides, and possibly proteins, are important DON constituents. Operational measurements of the amount of total protein in DON can be made by a wide variety of fluorometric and colorimetric assays. A partial list of those that have been applied to marine samples include coomassie blue (e.g., Mayer et al., 1986; Nunn et al., 2003; Setchell, 1981), Lowry's method (Clayton et al., 1988), the fluorescamine assay (Garfield et al., 1979), the bicinchoninic acid assay (Nguyen and Harvey, 1994), and the CBQCA (3–4–carboxybenzoyl-quinoline-2–carboxaldehyde) assay (Nunn et al., 2003). A number of the more common methods of protein analysis used in biochemical research, including a discussion of issues affecting their quantitative application, have recently been reviewed by Sapan et al. (1999).

Total protein assays have the advantage of being relatively straightforward compared to molecular-level analyses. Methods with fluorescence-based detection are also highly sensitive, and thus amenable directly to DON. Quantitative interpretation for environmental mixtures such as seawater, however, may be problematic for some samples. Most methods react with specific moieties (e.g., coomassie blue binds to lysine and arginine) and thus results obtained can depend on protein composition, size distribution, and even conformation (Sapan *et al.*, 1999), making the careful choice of calibration standards important. In addition, common components of natural samples, such as humic materials (e.g., Mayer *et al.*, 1986), carbohydrates (Sapan *et al.*, 1999), or NH_3 may interfere with quantification. Overall, colorimetric methods can be very useful as quick, likely semi–quantitative estimates of total protein or peptide. However, potential biases inherent in the mechanism of a specific method should be considered before one is chosen, and application of newer molecular assays (e.g., CBQCA) should be carefully examined in terms of natural sample matrix (Nunn *et al.*, 2003).

4.4.2. Specific protein analysis

Isolation and sequencing of specific proteins in the DON pool has recently begun to reveal a wealth of specific source information, as well as suggesting mechanisms for DON preservation (see Tanoue, 2000 for review of literature through 1990s). Proteins can be concentrated from large volumes of seawater using ultrafiltration with a nominal >10 kD size cutoff (discussed below, Powell and Timpermann, 2005). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has also been successfully used in both one and two dimensions to isolate discrete proteins from DON (e.g., Jones *et al.*, 2004; Tanoue, 1995; Yamada and Tanoue, 2006). This approach allows estimation of both the number and MW distribution of apparently intact proteins in DON. Total proteinaceous material isolated with this approach has been estimated to be up 10–30% of total DCAA, corresponding to 2–12% of total DON (Tanoue, 2000). Recovery, however, is strongly dependent on the exact protocol used for extracting proteins from seawater (Tanoue, 1995), and it is likely that significant work remains to be done to constrain quantitative estimates. The presence of humic materials and salts can also significantly limit gel quality (Jones *et al.*, 2004). Because of the difficulty of isolating entire proteins, as well as the fact that most proteinaceous material likely exists as smaller, partially degraded peptide units, approaches such as immunological techniques to probe smaller structures (e.g., Orellana *et al.*, 2003) may also be extremely valuable.

While methods for isolating discrete proteins are analytically challenging, when coupled with N-terminal amino acid sequencing (e.g., Tanoue *et al.*, 1995), immunochemical approaches (e.g., Suzuki *et al.*, 1997), or newer mass spectrometric techniques (Powell *et al.*, 2005), specific proteins can be identified, potentially offering a wealth of new information about DON sources. Recently, glycoproteins have also been detected in DON (Yamada and Tanoue, 2003), and 2-D electrophoresis suggests that highly varied glycosolated residues may even dominate the protein distribution of some ocean regions (Yamada and Tanoue, in review). These results suggest that a method for analyzing glyoproteins in DON may be an important future research target.

4.5. Amino sugars

Amino sugars are abundant compounds in plankton and bacteria, and thus constitute one of the largest potential DON sources. Biochemical "interrogation" studies have shown rapid turnover of common amino sugars, such as *N*-Acetyl glucoseamine (e.g., Kirchman and White, 1999; Riemann and Azam, 2002), indicating that amino sugars are key components of a rapidly cycling DON pool. Amino sugars are also major components of structural or cell-wall polymers in prokaryotes such as bacterial peptidoglycan, lipopolysaccharides, and archaeal pseudopeptidoglycan (Brock *et al.*, 1994). These structures are thought to be important sources for preserved DON (e.g., Boon *et al.*, 1998; McCarthy *et al.*, 1998; Tanoue *et al.*, 1995). NMR-based analyses of HMW-DON has also suggested that amino sugars could be a major component of this material (Aluwihare *et al.*, 2005; McCarthy *et al.*, 1997).

Common hexoseamines (glucoseamine and galactoseamine) can be quantified using modifications of standard amino acid HPLC protocols (Dauwe and Middelburg, 1998), or by HPLC after a mild sugar-specific hydrolysis and desalting (Ittekkot *et al.*, 1984a,b). In addition, a range of GC and GC-MS based methods are available that can quantify amino sugars after formation of volatile derivatives (Neeser and Schweizer, 1984; Whiton *et al.*, 1985). GC-MS methods have the substantial advantage of unambiguous peak identification, but as with amino acids making derivatives can be time consuming and result in losses. In addition, even in sediments and sinking particles the relatively small yields of amino sugars obtained by such methods (Dauwe and Middelburg, 1998; Ittekkot *et al.*, 1984a) suggest that they would not be suitable for direct DON analysis, although they can be used for concentrated samples (Aluwihare *et al.*, 2002). Overall, despite the potential importance of amino sugars to DON composition and cycling, relatively little research has directly targeted this compound class, most likely because of the lack of common methods for direct measurements.

The problem of sensitivity has recently been addressed with a high-performance anion-exchange chromatography—pulsed amperometric detection (HPLC-PAD) approach, specifically adapted for amino sugars in seawater (Kaiser and Benner, 2000). A similar method has also been published for amino sugars (as well as neutral sugars) in unconcentrated freshwaters (Cheng and Kaplan, 2003). While seawater samples must still be desalted via resin chromatography, the HPLC-PAD method of Kaiser and Benner (2000) allows fmol detection levels without derivatization, and it is readily coupled to automated sample injection. After optimizing hydrolysis conditions, near quantitative yields were reported for amino sugars spiked in natural DOM, and good recoveries were reported from a variety of natural materials (Kaiser and Benner, 2000). HPLC-PAD thus makes amino sugar molecular-level analysis possible on unconcentrated seawater samples, and also allows direct quantitative comparison with results from HMW DON and ocean particles. The first comprehensive sets of measurements using this technique on natural ocean water are providing a wealth of important new source information based on relative abundances of individual neutral sugar monomers (Benner and Kaiser, 2003; Davis and Benner, 2005). While the absolute contribution of identifiable common amino sugars has been found to be a relatively small fraction of the total DON pool (average

of 2–5% of HMW-DON, Benner and Kaiser, 2003; average of 0.7–1.5% of total DON; Davis and Benner, 2005), when compared to typical yields for amino acids (∼5–20% of total DON) it is nevertheless clear that amino sugars make up a major component of the DON that can be molecularly identified.

Recently several other innovative, if less specific and quantitative, analytical approaches have also been applied to concentrated DON samples, providing information about total amino sugar content and character in isolated DON. Direct temperature-resolved mass spectrometry (DT–MS) coupled with chemical ionization (NH_3) can be used to examine thermal sugar degradation products in solid materials (Boon et al., 1998). Though generally not considered quantitative, both DT–MS as well as Pyrolysis–MS (Lomax et al., 1991) provide information on relative abundance of types of amino sugars, while at the same time possibly accessing material protected from direct acid hydrolysis (Minor et al., 2001). These methods have indicated that both oceanic and estuarine HMW DOM has an important contribution from N-acetylated amino sugars (Boon et al., 1998; Simjouw et al., 2005). Another recent approach has been to apply differential (i.e., weaker vs. stronger) acid hydrolysis in concert with ^{15}N-NMR analysis (Aluwihare et al., 2005). This experiment exploits the relative facility of hydrolyzing acetyl groups from N-acetyl sugars, combined with the clear ^{15}N-NMR distinction between amides versus amines. An estimate of amino sugar in a sample can be obtained by comparing NMR spectra before and after weak hydrolysis. In HMW DON the results of this method suggest that amino sugars may comprise larger amounts, up to half of surface water HMW-DON, but substantially lower amounts in subsurface and deep waters (Aluwihare et al., 2005; see also Aluwihare and Meador, this volume).

4.6. Nucleic acids

DNA and RNA are major N-rich biochemical components of bacteria and viruses, and thus represent important potential sources to the DON pool. Recent methods have found oceanic nucleic acid concentrations in the low $\mu g\ L^{-1}$ range (Karl and Bailiff, 1989), suggesting that intact nucleic acids correspond to only a few percent of the total DON pool, despite the fact that rapid turnover suggests they are important N recycling compounds (e.g., Antia et al., 1991; Jørgensen et al., 1993).

Methods to directly measure concentrations of dissolved DNA (dDNA) and dissolved RNA (dRNA) typically involve an initial concentration or precipitation step, followed by detection and quantification by a florescent stain or dye. Common approaches have included ethanol precipitation followed by Hoechst 33258 staining (e.g., DeFlaun et al., 1986) that detects DNA only. Both DNA and RNA can be simultaneously measured using CTAB precipitation followed by reaction with 3–5 diaminobenzoic acid (Karl and Bailiff, 1989). Filtration and ultracentrifugation approaches for initial concentration steps have also been used (Paul et al., 1991), and have been combined with immunological detection (Jiang and Paul, 1995). Recent improvements have substantially decreased sample volumes and processing times required for dDNA analysis. Use of tetrasodium EDTA coupled with centrifugal concentration and SBYR Green-I florescent dye requires only ∼15 ml of

oligotrophic seawater and several hours processing time per sample (Brum *et al.*, 2004). A similar SBYR Green-I method has also been adapted for freshwater dDNA (Matsui *et al.*, 2004).

One complication for estimating the importance of nucleic acids as a source of *total* DON is, however, that common methods developed with biological processes in mind target relatively "intact" dDNA or dRNA, and may be less sensitive to partially degraded, bound, or otherwise altered material. The initial precipitation steps and dyes used for detection are commonly specific for molecule conformation or size. The SBYR Green-I dye (on which newer methods are based), for example, is a double stranded stain and is at least an order of magnitude less effective with single stranded molecules (Matsui *et al.*, 2004), and the EDTA step used for precipitation is most effective for material larger than 20 base pairs (Brum *et al.*, 2004). In addition, the operationally defined total "dissolved" DNA and RNA includes not only material truly in solution (and thus amenable to precipitation), but also material within viruses and tightly associated with colloids, humics or detritus (e.g., Brum *et al.*, 2004; Jiang and Paul, 1995). DNA present in an unknown "bound" form has been estimated in some marine studies at about half of that present in the "free" pool (Jiang and Paul, 1995). It is thus difficult to estimate what contribution degraded or altered nucleic acids may make to more refractory DON using current compound-specific methods, although NMR data has hinted that such material could be an important source of unsaturated N observed in deep ocean HMW material (McCarthy *et al.*, 1997).

5. BULK ORGANIC NITROGEN CHARACTERIZATION

The compounds that current methods can directly analyze likely make up the bulk of the rapidly cycling DON pool (see Chapter 3 by Aluwihare and Meador, this volume). However, the large majority of the total DON, especially in the subsurface ocean, still cannot be identified at the molecular-level. This presents us with a familiar analytical quandary. Without additional clues about the chemical nature of the majority of unidentified material, it is impossible to know where the methodological challenges lie: is it in the nature of the compounds themselves, i.e., novel or altered molecular structures for which new methods must be devised? In contrast, are there other limitations inherent in current methods related to the natural seawater/DON matrix? For example, are there physical associations or secondary reaction products that cause reduction in hydrolytic efficiency, which are the key barriers? To get beyond this circular problem, an enormous variety of spectroscopic and organic chemical methods exist that could be applied to organic mixtures and supply new information about the nature of bulk DON, including the distribution of compound classes, functional groups, isotopic ratios, etc. However, the devil is in the natural matrix—very few such techniques can be performed directly on aqueous samples. Low natural DON concentrations, coupled with vastly larger salt content, rule out many organic techniques that require a reasonably pure (non-diluted by mineral or salt) and fairly large (milligrams or more) sample. Finally, it is almost a

truism for detailed organic analysis that ''more is better''—the larger the sample, the greater the analytical possibilities become. For these reasons, a method that could quantitatively isolate a salt-free sample of the total dissolved material from seawater is needed (Bronk, 2002). To date no such method has been published, however, a great deal of information continues to be gained from a number of partial DON isolation techniques.

5.1. Organic nitrogen isolation techniques

5.1.1. Ultrafiltration

Tangential flow ultrafiltration (UF), also referred to as ''cross flow filtration,'' has become a widely used method since the early 1990s for isolating and desalting large quantities of DOM from seawater (e.g., Aluwihare *et al.*, 1997; Amon and Benner, 1994; Benner *et al.*, 1992, 1997; Guo, 2000; Guo *et al.*, 1996; Kolowith *et al.*, 2001; McCarthy *et al.*, 1996, 1998). Ultrafiltration is a size-based technique in which the sample is constantly recirculated across a membrane, progressively concentrating components that do not pass the membrane pores. Most seawater studies targeting total DOM have used polysulfone membranes with a nominal size cutoff of 1000 D, mainly because this is the smallest cutoff commonly manufactured that does not begin to retain larger seawater salts, thus allowing the filtration to proceed at a reasonable rate (e.g., Benner *et al.*, 1992, 1997). Dissolved material isolated by UF has been referred to in several different ways in the literature. In papers targeting the composition or cycling of the entire DOM pool, it has typically been abbreviated as UDOM (ultrafiltered DOM); this designation is functionally based, and thus avoids implicit assumptions about the nature of material isolated. Another body of literature uses ''colloidal'' OM to describe the same material, because the nominal >1000 D membrane cutoff also corresponds to a nominal size range for colloidal material, despite the fact that coupled UF and desalting likely destroys any natural non-covalent aggregates. It is also important to bear in mind that while in principle UF membranes retain only the HMW components exceeding the membrane pore size, in practice UF retains material based both on size as well as charge and other interactions with the membrane surface. One consequence of this more complicated mode of retention is that UF actually retains both high and low MW material during filtration (Guo, 2000). Final DOM recoveries thus depend on a concentration factor (i.e., total volume processed/final volume), and heavily on desalting, during which much of the originally retained DOM passes the membrane as ionic strength decreases (e.g., Benner *et al.*, 1997; Guo, 2000). After desalting, UF with 1000 D membranes typically recovers up to 30% of surface DOM and ∼20% from deeper waters. While relatively modest in terms of absolute recovery, because concentration takes place in a natural seawater matrix without chemical treatments or use of selective resins, UF isolates a more representative sample of total dissolved material than had been possible using previous chemically-selective methods (Benner *et al.*, 1997; Kaiser *et al.*, 2003). In addition, because UF systems can be operated in a continuous flow mode, extremely large volumes of water can be processed. This allows isolation of large amounts (grams are possible) of nearly pure DOM, making UF extremely useful for sample-intensive techniques.

An important issue with UF-based studies is how representative UDOM is of the total dissolved pool. For most bulk properties of DOM (e.g., total C:N ratio and stable and radiocarbon isotopes) material isolated by UF has been found to be fairly similar to the bulk pool (e.g., Benner *et al.*, 1997; McCarthy *et al.*, 1996). At the same time UDOM also displays offsets in some of its properties, generally consistent with size-reactivity continuum observations which suggest that HMW material is in general less degraded than LMW DOM (Amon and Benner, 1994). For example, ^{14}C content of UDOM in the subsurface Pacific indicates ages of 4000 to 5000 years (Loh *et al.*, 2004), which are similar to commonly reported values of 4000–6000 for total DOC, and clearly unrelated to modern ^{14}C values typical of POC. At the same time, comparison of measured UDOM ^{14}C values to literature ^{14}C values for total DOC also suggests that UDOM is somewhat younger than the total DOC at the any given depth horizon (Loh *et al.*, 2004).

For DON studies, ON mass balances for UF have not been directly determined. However, UF typically recovers material with C:N of ~14 in surface waters and 17–22 in the deep ocean (e.g., Benner *et al.*, 1997). Comparison to literature C:N ratios (DOC:DON data) from different locations can be problematic, in part because the large uncertainties that can be involved in subsurface DON estimates (discussed above) propagate into the DOC:DON ratio. Broad compilations (Bronk, 2002; Hopkinson and Vallino, 2004) suggest that C:N ratios of UDOM are slightly enriched in C, and thus UF may under sample total DON. In contrast, consistent with ideas that UDOM is enriched in more reactive material (e.g., Amon and Benner, 1994; Repeta and Aluwihare, 2006), as a general rule most identifiable ON compounds are somewhat enriched in the HMW fraction (i.e., "oversampled" by UF). Amino acids appear to be somewhat more representative of total DON in this respect, with THAA content and molar composition being similar between UDON and total DON in some studies (e.g., McCarthy *et al.*, 1996). Enantiomeric (D vs. L) ratios also very similar in some studies (Amon *et al.*, 2001; Dittmar *et al.*, 2001), while others have shown interesting site-specific differences in D/L ratios between high and low MW DON (Kaiser and Benner, 2008). In contrast, carbohydrates are substantially enriched in the higher MW DOM in upper ocean waters (e.g., Benner *et al.*, 1992; Pakulski and Benner, 1994; Skoog and Benner, 1997). Recent work has suggested that amino sugars are also more abundant in surface versus deep water UDOM (Aluwihare *et al.*, 2005). While amino sugar comparisons between LMW and HMW DOM have not been directly made on the same samples, comparison of amino sugars in HMW-DON from several ocean basins (Benner and Kaiser, 2003) versus amino sugars in total DON from the Chukchi/Beaufort Seas (Davis and Benner, 2005) supports the idea that UF also somewhat "over-samples" amino sugars relative to total DON pool, especially in surface ocean.

Overall, UF has numerous practical advantages for isolating large quantities of DON needed for detailed chemical study, and available data suggests its composition does not depart too greatly from the bulk oceanic DON pool. However, uncertainty remains, especially when departing from bulk composition and considering specific N-containing compounds that cannot be analyzed directly in seawater—caution should always be used in extrapolating results to the bulk

DON pool. Additional molecular-level and isotopic data comparing UDON with total DON will be extremely valuable, in particular comparison between $\delta^{15}N$ of UDON versus total DON as new methods for measuring $\delta^{15}N$ of total DON evolve.

5.1.2. Ion retardation resins

Perhaps the easiest method for isolating DON is through the use of ion retardation resin (e.g., BioRad AG 11 A8), which retards the flow of charged particles. The resin quantitatively removes salts including NH_4^+, NO_3^-, and NO_2^-, allowing DON to be isolated in the eluate (Bronk and Glibert, 1991, 1993a; Hu and Smith, 1998; Nagao and Miyazaki, 1999). Unfortunately, we know of no current distributor of ion retardation resin that is suitable for use with aquatic samples. DOW Chemical, the company that manufactured the resin marketed by BioRad and other distributors, changed the manufacturing process of the resin sometime in the mid 1990s such that the resin now retains variable amounts of DON. This DON retention is believed to be due to an accumulation of an organic film on the resin beads during manufacturing (BioRad, *personal communication*). To overcome this problem, AG 11 A8 resin can be manufactured and purified by buying another resin (Dowex anion exchange resin, BioRad AG1-X8) and then chemically altering it to produce AG 11 A8 as described in Hatch *et al.* (1957). The homemade resin, however, tends to breakdown rapidly, a problem not previously encountered with the resin produced by BioRad (Bronk, unpublished data).

5.1.3. Hydrophobic resin isolations

Another approach that could potentially be used for DON isolation is chemically selective resins. As discussed above, hydrophobic resin isolation has long been used in both terrestrial (Ertel *et al.*, 1986; Thurman and Malcolm, 1981) and marine waters (e.g., Stuermer and Harvey, 1974; Wilson *et al.*, 1983) to study dissolved humics. Thus, as a bulk isolation technique, resins are appropriate mainly for the humic component of DON, as described above for XAD-type isolations. A somewhat different spin on this theme is the use of SPE with C-18 columns, which has recently become widely used as a method to isolate hydrophobic DOM components for analyses that require desalting (e.g., Kaiser *et al.*, 2003; Kim *et al.*, 2003b; Koch *et al.*, 2005; Louchouarn *et al.*, 2000; Schwede-Thomas *et al.*, 2005). SPE has also been used in combination with UF in estuarine waters to increase the amount of total DOM recovered to near 70% (Simjouw *et al.*, 2005). Analysis of SPE-isolated material indicates, however, that SPE preferentially recovers the humic-like fraction (as would be expected), at the expense of polar biopolymers such as sugars and amino acids (Simjouw *et al.*, 2005). This is consistent with observations that SPE can strongly discriminate against total N, isolating material with significantly elevated C:N ratios (Koch *et al.*, 2005; See and Bronk, 2005). To overcome such resin-specific issues, it has been reported that a variety of different resins with a range of polarities and retention characteristics can be used in series to isolate nearly the entire DOM pool as discrete fractions, from both fresh and saline waters (Leenheer *et al.*, 2004). Such multi-resin experiments might be useful in the design of investigations targeting a specific fraction of the DON pool. However, their relatively labor-

intensive nature and concerns about blank contamination makes them less attractive for routine DON study.

5.1.4. Dialysis

Dialysis of discrete water samples is another approach that could potentially be used to isolate smaller samples of DON. Dialysis can be used effectively to isolate HMW DOM material (Heissenberger and Herndl, 1994), and with small membrane pore sizes could potentially be used to purify discrete DON samples. In freshwaters, dialysis has been used to remove inorganic N as a way to increase the accuracy of total DON measures, however, losses due to passage of LMW DON through the membrane and adsorption were concerns (Lee and Westerhoff, 2005). Dialysis has also been used in freshwaters for isolating DON for δ^{15}N measurement (Feuerstein et al., 1997). To date, however, dialysis has not been extensively investigated for seawater DON. Contamination and adsorption issues, as well as the relatively small amounts of material that can be collected, make dialysis most promising for compound-specific investigations. Other potential problems with the dialysis approach are the large amount of time needed to process samples (100 and 200+ h for fresh and salt water samples respectively), the lack of complete removal of salts and inorganic N, and the risk of bacterial contamination that could chemically alter the final dialyzed product.

5.2. NMR spectroscopy

Nuclear magnetic resonance spectroscopy has proven to be one of the most powerful techniques for characterizing complex natural geochemical samples because of its ability to non-destructively probe the chemical character of a range of organic nuclei that include most building blocks of organic matter (^{13}C, ^{15}N, ^{1}H, and ^{31}P). Basic techniques, theory and applications have been reviewed in a variety of articles and texts (e.g., Preston, 1996; Sanders and Hunter, 1988; Wilson, 1987). NMR spectra for geochemical samples such as DOM primarily differ from those of pure compounds in having broad and overlapping spectral lines, reflecting the fact that natural mixtures contain multiple variations within structural families. While specific compounds can thus rarely be identified, integration of spectral regions provides a broad view of the composition of the entire sample, in contrast to very narrow but detailed information available from molecular-level analyses. Coupled with sample isolation methods, both solid and liquid-state NMR techniques have been used to study oceanic DOM (e.g., Aluwihare et al., 1997; Benner et al., 1992; Clark et al., 1998; Hedges et al., 1992; McCarthy et al., 1996; Repeta et al., 2002; Stuermer and Payne, 1976), as well as to target nitrogenous material (Aluwihare et al., 2005; McCarthy et al., 1997).

Solid-state experiments use a dry sample that is packed into a rotor and spun at high frequency inside the spectrometer's magnetic field. This approach is termed cross-polarization magic angle spinning (CPMAS), and is the standard protocol for solids. It relies on transfer of magnetization from protons to C (or other nuclei) in order to achieve rapid analyses with reasonably narrow spectral lines. Cody et al.

(2002) contains a recent compact overview of solid-state NMR techniques in geochemical samples. With CPMAS essentially the full range of material in a mixture can be examined, including very HMW or insoluble components, making it particularly valuable for materials that have undergone major diagenetic alteration. CPMAS also has the advantage (especially significant for N studies) of concentrating the maximum amount of sample inside the magnetic field. The most common solid-state experiments on DOM isolates have been ^{13}C-CPMAS (e.g., Benner *et al.*, 1992; Hedges *et al.*, 1992; McCarthy *et al.*, 1993); [15]N (McCarthy *et al.*, 1997) and [31]P (Clark *et al.*, 1998) experiments can also be readily performed.

Liquid-state experiments are the more familiar variety used for organic structural elucidation. The sample is dissolved in a specialized solvent that is as free as possible of the nucleus of interest (e.g., if a ^{1}H-NMR liquid experiment is to be performed using an aqueous solvent, D_2O is used). Liquid state experiments inherently produce much sharper spectral lines, and thus have potential to provide more detailed structural information. In practice, however, very complex mixtures such as natural DOM produce complex overlapping resonances resulting in spectra that can closely resemble the broad lines of their solid state counterparts (e.g., Repeta *et al.*, 2002). ^{1}H-NMR experiments can be conducted rapidly on marine DOM, revealing the major distribution of those structural types that are soluble (e.g., Aluwihare *et al.*, 1997, 2002). In addition, liquid-state NMR offers the possibility of a wider variety of elaborate experimental possibilities, including multi-dimensional experiments targeting linkages between specific nuclei (e.g., Hertkorn *et al.*, 2006; Kaiser *et al.*, 2003; Kim *et al.*, 2003a).

For the nitrogenous component of DOM, as indicated above, ^{15}N nuclei can be examined directly (e.g., Aluwihare *et al.*, 2005; McCarthy *et al.*, 1997). Even with sample concentration, however, the relatively low natural abundance of ^{15}N generally makes solid-state experiments the method of choice. Experiments with ^{15}N-CPMAS have been valuable in revealing the major functional distribution of N in HMW DON (McCarthy *et al.*, 1997), and when combined with variable hydrolysis methods have been used to probe the relative amounts of amino-sugar and peptide amide bonds (Aluwihare *et al.*, 2005; Aluwihare and Meador, this volume). However, the fairly limited range of common N biochemical functionality makes the information potential of ^{15}N-NMR more limited than that for either C or H. [15]N spectra for DON study are thus most valuable when used in combination with other approaches mentioned above. Multi-dimension experiments to directly examine the linkages between N and C are possible, however, the low abundance of ^{15}N precludes their use on natural samples with current technology. Such techniques might, however, be employed in the future in mesocosms with DON production and degradation studies. Solid-state double cross-polarization experiments between C and N have been conducted with degrading plant remains, but these required isotopically enriched samples in both ^{13}C and [15]N (Knicker, 2002). A great deal more solid-state ^{15}N work has been described in the terrestrial and sedimentary literature (e.g., see Knicker, 2004 for a review), which might be applied to future work with isolated DON material, and particularly the more humic fraction from the deep ocean.

> ## 6. Basic Principles for the Measurement of Nitrogen Fluxes

6.1. Bioassays

There are two main approaches used to determine uptake rates—bioassays and tracers. In bioassays, the concentration of a given substrate is monitored over time (e.g., Seitzinger *et al.*, 2002). The advantage of bioassays is that they are relatively easy, and require only that suitable incubation conditions be maintained and the ability to measure the concentration of the substrate of interest. There are, however, drawbacks and limitations to their use. First, they only provide net uptake (or production) rates. If there is substantial substrate regeneration, such as one would expect in the case of NH_4^+, the uptake rate will be underestimated or even unmeasurable if the rate of regeneration is greater than the rate of uptake. Second, there is a fine line between incubating a sample long enough so that there is a detectable change in concentration and incubating it so long that bottle effects skew the results. Care must also be taken to maintain the sample under as close to *in situ* conditions as possible.

6.2. Stable isotopes

6.2.1. Basic principles

The other approach used to measure flux rates is the application of isotopic tracers and this approach is reviewed in detail in Glibert and Capone (1993) and Chapter 31 by Lipschultz, this volume. In the case of N, there are two isotopes that have been used—radioactive ^{13}N and stable ^{15}N. Unfortunately, ^{13}N has a very short half–life ($\sim$10 min) such that easy access to a nuclear reactor is required (e.g., Fuhrman *et al.*, 1988; Suttle *et al.*, 1990). The isotope ^{15}N, however, is widely used to trace N flow in the water column and sediment both in short-term, small volume incubations (e.g., Dugdale and Goering, 1967) and long-term, whole-system enrichments (e.g., Tobias *et al.*, 2003).

   The use of stable isotopic tracers is based on following a labeled compound from one pool (the source pool) to another (the target pool) (Sheppard, 1962). The principles described below are the same regardless of whether one is measuring rates in the water column or sediment. What changes are the protocols used to isolate the different fractions prior to isotopic analysis. The basic equation to calculate the flux rate from one pool to another is:

$$\text{Rate} = \frac{\text{Atom \% (R) of the Target Pool}}{\text{Atom \% (R) of the Source Pool} \times \text{Time}} \times [\text{Target Pool}] \qquad (28.4)$$

where atom %, often referred to as R in the literature, is the ratio of $^{15}N:^{15}N + ^{14}N$ in a pool, time is the length of incubation, and the brackets denote concentrations (see Chapter 31 by Lipschultz, this volume, for nuances in the use of this equation).

Relative to bioassays, advantages of the isotope tracer method include higher sensitivity, shorter incubation times, ability to measure gross uptake (e.g., total uptake corrected for any release of N during the incubation), and the ability to measure uptake in the presence of high rates of regeneration. One of the disadvantages of the approach is that the addition of a tracer may perturb the system if the addition is substantial, relative to the ambient concentration of the added substrate. This is particularly problematic in open ocean studies where ambient NH_4^+ and NO_3^- are at or near the limit of detection. Under these conditions, even a small "trace" addition (e.g., 0.03 μM) can be a serious perturbation.

6.2.2. Isolation of pools

Prior to isotopic analysis, either for natural abundance measurements or at the end of tracer incubations, the relevant N pools must be isolated. Here we review the isolation of the most important N fractions: PN, NH_4^+, NO_3^-, NO_2, DON, and urea.

6.2.2.1. NH_4^+ There are several methods available to isolate NH_4^+, including distillation, SPE, wet chemical, and direct diffusion. In distillation, the pH of the sample is raised to convert soluble NH_4^+ to volatile NH_3, generally with NaOH or MgO (e.g., Gilbert *et al.*, 1982). The sample is then heated, preferably under vacuum, condensed in a cool condenser, with the condensate ultimately trapped in a mildly acidic solution that converts the isolated NH_3 back to soluble NH_4^+. The acidified NH_4^+ is then evaporated and spotted on a filter (e.g., Harrison, 1978) or captured on a molecular sieve (e.g., Hoch *et al.*, 1992; Velinsky, 1989). Lipschultz (1984, cited in Glibert and Capone, 1993) used the molecular sieve to directly isolate NH_4^+ by placing it on a filter and then pouring the sample through the filter. While this is a quick and efficient method for freshwater, it is not usable with brackish or seawaters.

In SPE, the NH_4^+ in the sample is converted to indophenol following a variation of the phenol/hypochlorite method described above for measuring NH_4^+ concentrations. The indophenol solution is passed through an octadecyl C18 SPE column that binds the indophenol to the resin. The indophenol is then eluted with methanol. The recovery of NH_4^+ with this method is generally low, less than 30% (Selmer and Sorensson, 1986). Alternately, the indophenol can be extracted into dichloromethane (Dudek *et al.*, 1986); recovery is not explicitly stated but the blank calculations appear to assume it is quantitative. Recently the SPE approach has been modified to allow the analysis of low N seawater (0.01–0.1 μM) samples to be analyzed using a GC–MS (Clark *et al.*, 2006).

There are also a number of wet chemical approaches used to isolate NH_4 including precipitation with mercuric chloride (Fisher and Morrisey, 1985). This method, however, is problematic with marine samples and generates particularly toxic waste. A newer method converts NH_4^+ in natural waters to NO_2^-, via hypobromite, and then to N_2O using a sodium azide/acetic acid buffer solution; the N_2O produced is than analyzed using a mass spectrometer (Zhang *et al.* 2007).

Ammonium has also been isolated using direct diffusion (Brooks *et al.*, 1989; Kelley *et al.*, 1991; Sigman *et al.*, 1997; Slawyk and Raimbault, 1995). In this method, the pH is raised and an acidified glass fiber filter is suspended above the sample and the bottle is sealed. The sample is then placed in a heated oven or placed on a shaker table. The increase in pH will produce volatile NH_3, which is then captured on the acidified filter (Brooks *et al.*, 1989); another variation of this approach places the acidified filter into a Teflon envelope (Sigman *et al.*, 1997). Any method that requires a change in pH, such as diffusion or distillation, should be used with care because of the risk of hydrolyzing amino groups from organic compounds (Bronk and Ward, 2000; Sigman *et al.*, 1997). Hydrolysis will result in transfer of label from the DON pool into the NH_4^+ pool and this transfer would be difficult to quantify. Another potential artifact to consider is that incomplete isolation can lead to isotopic fractionation. This is especially problematic with distillation and diffusion methods. Holmes *et al.* (1998) published a diffusion method that used large sample volumes (up to 4 L) but that accounts for the isotope fractionation. Finally, whatever method is selected it is important to quantify the method blank and to correct the final atom % enrichment accordingly (see section below).

6.2.2.2. NO_3^- and NO_2^-   Isolation procedures for NO_3^- generally involve converting the NO_3^- to some other form. Nitrate can be converted to NH_4^+ with DeVarda's Alloy (e.g., Bronk and Ward, 1999), zinc dust (e.g., Gardner *et al.*, 1995) or titanium(III) chloride ($TiCl_3$, Cresser, 1977), and then the NH_4^+ is isolated using any of the suite of NH_4^+ isolation methods described above.

Nitrate can also be converted to NO_2^- using cadmium reduction (columns or spongy cadmium) as described above. Once the NO_3^- is in the form of NO_2^-, the NO_2^- can be isolated via organic extraction (e.g., Olson, 1981) or with SPE after the NO_2^- is converted to an azo dye (Kator *et al.*, 1992). Nitrate can be isolated by conversion to N_2O via sodium azide in an acetic acid buffer solution (McIlvin and Altabet, 2005). Another approaches uses a genetically engineered denitrifier to convert NO_3^- to N_2O (Sigman *et al.*, 2001); the bacteria will denitrify NO_3^- in a sample to N_2O, but lacking nitrous oxide reductase the bacteria cannot take the reaction to completion and form N_2. The N_2O produced by either approach can then be analyzed on a mass spectrometer. A more detailed discussion of these methods is presented in Chapter 31 by Lipschultz, this volume.

6.2.2.3. DON   At present, there are three basic approaches used to isolate DON for tracer experiments: wet chemical isolation, ion retardation or dialysis, described above. The suitability of a method is judged by its ability to remove all of the DIN forms (NH_4^+, NO_3^-, and NO_2^-) and to isolate the DON pool with high efficiency. Ultrafiltration is not a suitable isolation method for use in determining DON release rates because it only isolates the higher MW fraction and thus allows the LMW moieties, which are likely important short-term release products, to be lost.

There are several variations of the wet chemical approach, first introduced by Axler and Reuter (1986), but all involve removing inorganic N species from

solution so that the remaining DON can be analyzed. In this approach, NH_4^+ is removed by raising the pH slightly, thus effecting a change from soluble protonated NH_4^+, to the more volatile NH_3 via diffusion in a heated oven (Slawyk and Raimbault, 1995) or vacuum distillation (Bronk and Ward, 1999). A high-speed concentrator (e.g., SpeedVac) is particularly useful for NH_4^+ removal because the process can take place at room temperature or lower, thus decreasing the possibility of acid hydrolysis (Bronk, unpublished data). Nitrate in the sample can then be converted to NH_3 with DeVarda's alloy, with the NH_3 again removed through volatilization (Bronk and Ward, 1999; Miyajima $et\ al.$, 2005; Slawyk and Raimbault, 1995) or any of the other approaches outlined above. Both of these techniques can suffer from the artifact of losing labile DON as a result of base hydrolysis (Bronk and Ward, 2000). The problem in the isolation is likely the lengthy diffusion step undertaken to remove NH_4^+, NO_3^-, and NO_2^- from solution. A number of other protocols have been used with varying success to remove NO_3^- and NO_2^-, including vanadium (VIII, Cox, 1980; Garside, 1982), titanium (TiIII, Cresser, 1977; Cox, 1980; Bronk $et\ al.$, unpublished data), and other DeVarda's alloy approaches (Page $et\ al.$, 1982). Breakdown and loss of DON is always a danger due to the rigorous reducing conditions needed to remove NO_3^-. Some researchers even use heated diffusion as a mechanism to remove labile DON before isolation of NO_3^- (Sigman $et\ al.$, 1997). For a review of potential artifacts during wet chemical DON isolation and the different types of DON release rates and their calculation protocols see Bronk and Ward (2000) or Chapter 31 by Lipschultz, this volume.

6.2.2.4. Urea
Urea can be isolated for isotopic analyses by using urease to break down urea to NH_4^+ (e.g., Lomas $et\ al.$, 2002; Slawyk $et\ al.$, 1990). Before urease is added to the sample, however, the preexisting NH_4^+ must be removed, ideally with distillation. Rysgaard and Risgaard-Peterson (1997) removed the preexisting NH_4^+ with high efficiency using cation exchange.

6.2.2.5. Particulate nitrogen
Particulate N is isolated by filtration with a filter capable of withstanding the combustion needed to convert the N on the filter to N_2 gas so that the sample can be analyzed isotopically. The most commonly used filter is composed of glass fibers; these include the GF/F or GF/C filters with nominal pore sizes of 0.7 and 1.2 µm, respectively. Many other commonly used filters, such as polycarbonate or cellulose filters, produce a large amount of CO_2 upon combustion, which will rupture ampoules or combustion tubes in mass spectrometers. One problem with using glass fiber filters, however, is that they retain a variable amount of detritus and bacteria, in addition to phytoplankton. Some separation of bacteria and phytoplankton can be achieved using size fractionation, but any filter used must withstand the combustion required with mass spectrometric analysis. Suitable filters include those made of silver or alumina silicate (e.g., Anopore®). Silver filters are available in a range of pore sizes (0.2–5.0 µm), but they are expensive with notoriously slow filtration rates. The alumina silicate filters come in 0.2 µm pore sizes, suitable for isolating the bacterial fraction, however, they too have very low flow rates as well as being very brittle. If one wants to collect the bacterial fraction, it is

often quicker to preconcentrate over a 0.2 μm filter with a higher flow rate, such as a Supor$^®$ membrane, and then filter the concentrated sample onto a silver or Anopore$^®$ filter as a final step. Regardless of the filter type used, all should be baked (450°C for a minimum of 2 h) to remove contaminant N; be sure to remove the plastic ring on Anopores$^®$ prior to backing.

Flow cytometry can also be used to separate phytoplankton from bacteria and detritus (e.g., Lipshultz, 1995; Casey *et al.*, 2007). Another technique being used to remove detritus from particulate samples is centrifugation with colloidal silica (Hamilton *et al.*, 2005). With this technique the sample is mixed with the colloidal silica and then centrifuged. Detritus and living cells are separated based on density differences; detritus tends to be heavier.

6.2.3. Isotopic analysis

Isotopic analysis separates isotopes on the basis of small but significant differences in mass (see Chapter 31 by Lipschultz, this volume). This can be done using an emission spectrometer or a mass spectrometer. With both types of instruments, the sample must be converted to a gas prior to analysis. Emission spectrometers operate based on the principle that a N_2 gas molecule comprised of a ^{15}N and ^{14}N will fluoresce at a different frequency then a molecular comprised of two ^{14}Ns or two ^{15}Ns (reviewed in Preston, 1993). Isotope ratio mass spectrometers separate and quantify the different N_2 gas molecules based on their behavior when they are accelerated through a magnetic field (reviewed in Mulvaney, 1993). Emission spectrometers seem to have fallen out of favor in the quest for bigger and better instruments. They do have advantages, however. Emission spectrometers are much cheaper to purchase and maintain, they are portable, they require smaller masses of N for analysis than many mass spectrometers, and there is no possibility for carry over from sample to sample because each sample is sealed in an ampoule prior to analysis.

7. Measurement of Nitrogen Uptake and Release Rates

7.1. Uptake rates

7.1.1. Substrate additions

A large suite of substrates are now available with ^{15}N label (NH_4^+, NO_3, NO_2, urea, amino acid mixtures, and individual DFAA). Many of the organic forms are also available with dual labels (^{15}N and ^{13}C) such that the flux of both N and C can be followed simultaneously. Similarly, ^{13}C–labeled sodium bicarbonate can be added to ^{15}N incubations such that an estimate of primary production and N uptake can be derived from the same sample (Dauchez *et al.*, 1995; Legendre and Gosselin, 1997; Parker, 2005; Slawyk *et al.*, 1977).

In addition to commercially available tracers, methods exist for making a number of other labeled substrates. In the case of DON uptake studies, one option is to have the resident plankton community make the DON tracer by adding ^{15}N–labeled

NH_4^+ or NO_3^- and then incubating (Bronk and Glibert, 1993b; Veuger *et al.*, 2004). During the course of the incubation the labeled substrate is incorporated and some fraction is released as $DO^{15}N$. This $DO^{15}N$ can then be isolated and added back to a new water sample to quantify DON uptake (Bronk and Glibert, 1993b; Bronk *et al.*, 2004). Labeled humic substrates can be made by growing *Spartina alterniflora*, a common marsh plant, with ^{15}N-labeled NH_4^+ in the sediment for several months (See and Bronk, 2005). The *Spartina* is then cut, dried, ground in a mill, and then incubated in the dark while stirring in recently collected coastal seawater. The resident bacteria in the seawater ''humify'' the *Spartina* particles generating humic substances. These labeled humics can then be isolated from solution using macroporous resins, as described above, and then used to quantify humic-N uptake (See, 2003; See *et al.*, 2006). The humics can also be dually labeled with ^{13}C by enclosing the *Spartina* plant in a bag and volatizing ^{13}C-labeled sodium bicarbonate with acid (See *et al.*, 2006).

7.1.2. Incubation considerations

Three things must be considered when incubating samples—light, temperature, and time. To measure *in situ* (in place) rates, ideally, all conditions during the incubation would the same as those where the sample was collected—or as close to it as possible. With respect to light, the light field should mimic the location and depth where the samples were collected. This can be accomplished by neutral density screens or, better yet, neutral density film (e.g., Cochlan and Bronk, 2003). Temperature is also important to consider, particularly when measuring rates (think Q_{10}). If vertical profiles are done, cells deeper in the water column are generally much cooler than those at the surface and must be incubated accordingly. Temperature controlled water baths are ideal. In a pinch a cooler and ice can do the job but care must be taken to keep the water well mixed. One way to avoid issues of light and temperature is to do *in situ* incubations where samples are tethered on a line at the depths where they were collected (e.g., Smith *et al.*, 2000). With respect to length of incubation time, generally speaking, the shorter the better. The shorter the incubation time, the lower the likelihood and severity of bottle effects (e.g., Venrick *et al.*, 1977). The desire for short incubations must be balanced, however, with the need for incubations to be long enough to see measurable changes in the parameter of interest. One common exception to the shorter is better rule occurs when daily integrated rates are needed, in which case, 24 h incubations are used.

7.1.3. Uptake rate calculations

Traditionally, N uptake rates have been based on the accumulation of ^{15}N-label in cells, defined as a net uptake rake (Bronk *et al.*, 1994), and are calculated based on the tracer principles described above with the extracellular substrate pool (e.g., NH_4^+) as the source pool and PN as the target pool:

$$\text{Uptake Rate} = \frac{\text{Atom \% (R) PN}}{\text{Atom \% (R) } NH_4^+ \times \text{Time}} \qquad (28.5)$$

Uptake rates can be presented as specific uptake (often designated as V), with units of per time, or absolute uptake (often designated as rho, ρ), with units of mass N per volume per time. Specific uptake rates are useful when comparing size fractions or systems that may differ substantially in biomass as they are more a measure of the physiological process of uptake. The absolute uptake rate is calculated by multiplying the specific uptake rate by the PN concentration (see Chapter 31 by Lipschultz, this volume).

During the course of the incubation, two processes can occur that impact uptake rates: isotope dilution and DON release. In the case of isotope dilution, unlabeled substrate is released during the course of the incubation, diluting the atom % enrichment of the source pool. In the case of NH_4^+ this occurs as a result of bacterial remineralization and zooplankton excretion (e.g., Dugdale and Wilkerson, 1986; Glibert et al., 1982). Failure to account for isotope dilution in the case of NH_4^+ uptake can result in large underestimates in NH_4^+ uptake rates. In the case of NO_3^- and NO_2^- uptake, isotope dilution can occur as a result of nitrification (e.g., Ward et al., 1989). Most studies do not take into account isotope dilution of the NO_3^- pool, with some notable exceptions (e.g., Lipschultz, 2001). Evidence suggests, however, that nitrification can be an important process at the base of the euphotic zone and so NO_3^- isotope dilution should be quantified (Ward et al., 1989). Methods to isolate NH_4^+ and NO_3^- for isotopic analysis are described above (also see Chapter 31 by Lipschultz, this volume). Isotope dilution could also affect uptake rates of organic substrates such as urea, which can have high rates of regeneration (Bronk et al., 1998), though this correction is not commonly done due to the labor intensive methods involved. To correct uptake rates for isotope dilution an exponential average is calculated based on the initial and final atom % enrichment of the source pool (e.g., Glibert et al., 1982).

In addition to isotope dilution of the DIN pools, DON can be released during the course of the experiment. The DON pool can be isolated so that the amount of ^{15}N released to the pool can be quantified. This recently released N was taken up by the cells and so should be included in the uptake calculation. This rate is referred to as a gross uptake rate, ρ_G, and is the sum of measured uptake plus any N that was taken up and released as DON. The difference between gross and the traditionally measured net uptake rates results from the release of ^{15}N-label to the DON pool. When ^{15}N-labeled DON is released to the extra cellular DON pool, it is no longer in the pool and so is not included in the traditional calculation of N uptake (Bronk and Ward, 2000; Bronk et al., 1994). The following equation is used to calculate a gross N uptake rate p(ρ_G):

$$\rho_G = \frac{(PN \times PN\ at\%xs) + (DON \times DON\ at\%xs)}{DIN\ at\%xs \times Time} \quad (28.6)$$

where PN and DON are the mass of N in the PN and DON pools, PN and DON at%xs are the ^{15}N atom % enrichments of the PN and DON pools minus the atom % of an atmospheric N standard, DIN at%xs is the atom % excess enrichment of the DIN pool, and time is the period of incubation (Bronk et al., 1998).

7.2. Release rates

7.2.1. NH_4^+ regeneration

Here we describe methods to measure three release rates: NH_4^+ regeneration, urea regeneration and DON release. Rates of NH_4^+ regeneration can be measured using the principle of isotope dilution. To measure regeneration, a tracer addition (generally at <10% of the ambient concentration) is added to a water sample. At the end of the incubation the atom % enrichment of the NH_4^+ pool is either measured directly, using HPLC (Gardner and John, 1991; Gardner et al. 1991, 1993), or using a mass spectrometer after the pool is isolated using one of the methods outlined above. The assumption is made that any NH_4^+ released during the incubation is $^{14}NH_4^+$. This will dilute the $^{14}NH_4^+$ and $^{15}NH_4^+$ present at the start of the experiment. The regeneration rate is then measured by following the flow of the unlabeled NH_4^+ into the labeled NH_4^+ pool (Blackburn, 1979; Caperon et al., 1979). To measure rates using the principles of isotope dilution one must measure the change in isotopic composition of a pool over time and then solve the following equations:

$$P_t = P_0 + (d - u)t \qquad (28.7)$$

$$\ln(R_t - R_a) = \ln(R_0 - R_a) - [d/(d - u)][\ln P_t/P_0] \qquad (28.8)$$

where P_t and P_0 are the ambient concentrations of NH_4^+ at the end and beginning of the incubation, respectively, u is the absolute uptake rate, d is the regeneration rate, t is the incubation time, R_t and R_0 are the atom % enrichments of PN at the end and beginning of the incubation and R_a is the atom % of ^{15}N in the atmospheric standard.

7.2.2. Urea regeneration

Rates of urea regeneration can be calculated in an analogous fashion to that used for NH_4^+. The urea pool can be isolated using the urease method described above. The atom % of the isolated urea pool is measured at the start and end of the experiment and the regeneration rate is calculated using the equations above after replacing NH_4^+ with urea. Urea uptake and regeneration can also be measured simultaneously by performing parallel incubations with comparable concentrations of ^{14}C and ^{15}N-labeled urea. The ^{14}C-labeled samples are used for the determination of isotope dilution and the rate of urea regeneration (Hansell and Goering, 1989).

7.2.3. DON release

There are two general ways to calculate a DON release rate. The first is to measure the passage of ^{15}N from an intracellular ON pool to the extracellular DON pool (Bronk and Glibert, 1991, 1993a). This rate is specified "the intracellular pool (IP) DON release rate" to indicate that intracellular pools are used in its calculation. The isolation of the intracellular DON pool also allows the calculation of a transformation rate, which is a measure of the transformation of NH_4^+ to organic N intracellularly (Bronk, 1999). There are a number of different methods used to isolate the intracellular N pool including bursting cells with boiling distilled water and rupturing the cells with trichloroacetic acid (e.g., Clayton et al., 1988, Lanvin and Lourenço, 2005).

An easier, less labor intensive means of measuring DON release rates was later introduced by Bronk *et al.* (1994) that was based solely on the change in the ^{15}N atom % enrichment of the extracellular DON pool and did not require isolation and measurement of the intracellular ON pool (Bronk *et al.*, 1994). This DON release rate is calculated as the difference between the gross N uptake rate (ρ_G) and the traditionally determined net N uptake rate (ρ) defined above:

$$\text{DON release rate} = \rho_G - \rho = \frac{\text{DON at\%xs} \times [\text{DON}]}{\text{DIN at\%xs} \times \text{Time}} \qquad (28.9)$$

where DIN and DON at%xs are the ^{15}N atom % enrichments of the DIN and DON pools respectively, minus the atom % enrichment of the atmospheric N standard, [DON] is the concentration of DON, and time is the incubation time (Bronk and Ward, 2005).

This DON release rate, referred to as the extracellular pool (EP) release rate, is equivalent to the rate termed ρDIN by Slawyk *et al.* (1998). We suggest that the more common EP DON release rate should be taken as "the DON release rate" (Bronk and Ward, 2000; Bronk *et al.*, 1994). When the transfer of N from the intracellular to the extracellular DON pool is being measured, that rate should be specified an IP DON release rate as originally described (Bronk, 1999).

Though in general the IP and EP DON release rates are similar, short-term variations in the atom % enrichment of the intracellular ON pool can cause some variations between them (Bronk, 1999; Mulholland *et al.*, 2004). The IP DON release rate is also very labor intensive and requires a host of assumptions, and therefore is less robust than the EP DON release method. The IP and EP distinctions are only necessary when both types of rates are being discussed (Bronk and Ward, 2000).

There are three potential artifacts that must be considered when measuring DON release. First, is the breakage of cells during filtration (e.g., Goldman and Dennett, 1985). If cells break releasing ^{15}N into the DON pool the rate of DON release will be overestimated. Second, is the potential for stressing cells during collection or incubation. Every effort should be made to maintain samples at *in situ* light and temperature conditions at all times and to avoid any additional environmental stresses. Third, underestimation of DON release can also occur due to loss of DON during the isolation process. Any method that includes heating of the sample under basic conditions has the potential to cause base hydrolysis and loss of recently released DON, and thus should be carefully evaluated before use on a given set of samples (Bronk and Ward, 1999; Slawyk and Raimbault, 1995). Loss of DON would be expected to vary depending on the composition of the DON pool, which could explain the different DON recovery efficiencies reported using various methods. These include 91.2% to 98.9% recovery for waters off the coast of France (Slawyk and Raimbault, 1995), generally less than 80% in Japanese coastal waters (Hasegawa *et al.*, 2000), and 42.7%±8.8% in waters from two rivers in Georgia and the South Atlantic Bight (Bronk, unpublished data).

8. Natural Abundance Measurements

The stable isotope ^{15}N is useful as tracer because it has the chemical characteristics of ^{14}N—almost! There is a kinetic isotope effect that causes the rate of reaction of molecules containing ^{15}N to be slightly different than those containing ^{14}N (reviewed in Shearer and Kohl, 1993). In chemical reactions that involve phase changes (i.e., evaporation), or that are catalyzed by enzymes, this kinetic isotope effect will result in a slight discrimination against the heavier isotope, a process known as fractionation. For example, if one were to boil a solution containing NH_3, of a known enrichment, the NH_3 that evaporated initially would have a slightly higher proportion of ^{14}N and the pool of NH_3 remaining in solution would become progressively more enriched with ^{15}N. These slight differences in ^{15}N natural abundance can be exploited to determine nutrient sources (e.g., Peterson, 1999; Tobias *et al.*, 2001), define trophic relationships, or to decipher large-scale biogeochemical changes (e.g., Altabet *et al.*, 2002). For a thorough coverage of natural abundance methods and applications see Lajtha and Michener (1994), Fry (2006) and Chapter 29 by Montoya (this volume).

Natural abundance measurements are made against a standard, atmospheric N_2 in the case of ^{15}N measurements, and are expressed in delta (δ) units:

$$\delta^{15}N = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000\% \qquad (28.10)$$

R is defined in equation 4 above; data are described as the per mil ^{15}N excess. Prior to isotopic analysis, the various inorganic and organic N fractions must be isolated as described in the sections above.

9. Measurement of Other Nitrogen Transformation Rates

9.1. Nitrification

Nitrification is the process that converts NH_4^+ to NO_2^- and then NO_3^-. There are four main methods for measuring nitrification—bioassays, incubation with $^{14}CO_2$ with N-serve inhibition, incubation with $^{15}NH_4^+$ or $^{15}NO_2^-$, and ^{15}N isotope dilution (see method comparison by Enoksson, 1986). We offer a brief review here but direct the reader to Chapter 5 by Ward (this volume) for a more detailed discussion of the nitrification process and its measurement.

The bioassay approach involves incubating a sample in the dark and then measuring the change in NO_2^- and NO_3^- concentrations over time after the addition of NH_4^+. This is the simplest method but it suffers from all the problems of bioassays noted in earlier sections, most notably the requirement of long incubation times and the simultaneous uptake of the recently produced NO_2^- and NO_3^- resulting in an underestimated or unmeasurable rate. Some studies have

supplemented this approach with the application of inhibitors. For example, acetylene and N-serve have been used to inhibit NH_4^+ oxidation and chlorate has been used to inhibit NO_2^- oxidation (Bianchi *et al.*, 1997; Billen, 1976). If NO_2^- oxidation is inhibited by the addition of chlorate, for example, any change in NO_2^- concentration can be attributed to NH_4^+ oxidation.

One of the most sensitive ways to measure nitrification is to combine the inhibitor N-serve with ^{14}C incubation (Enoksson, 1986). This approach exploits the chemolithoautotrophic nature of nitrifiers whereby they incorporate $^{14}CO_2^-$ while oxidizing N. Fixation of $^{14}CO_2^-$ is measured in the presence and absence of the N-serve (see Chapter 5 by Ward, this volume for nuances of N-serve use) with the difference being attributed to nitrifiers. To convert the $^{14}CO_2^-$ fixation rate to a nitrification rate requires the application of a conversion factor, which can be problematic (e.g., Glover, 1985).

In the ^{15}N method, $^{15}NH_4^+$ or $^{15}NO_2^-$ is added to a sample and then the appearance of ^{15}N label in the NO_2^- or NO_3^- pools is measured over time (e.g., Olson, 1981). The advantage of this technique is that it is a direct, unambiguous measure of the process. One disadvantage is that the increase in NH_4^+ or NO_2^- concentrations caused by the tracer addition can result in enhanced NH_4^+ or NO_2^- oxidation rates (e.g., Helder and De Vries, 1983; Ward *et al.*, 1984). When small additions of ^{15}N are used, the isotope dilution of the substrate pool must be monitored over time.

Another tracer approach that has been used is to quantify nitrification is isotope dilution; this is an analogous approach to the much more commonly applied NH_4^+ isotope dilution approach discussed earlier (Glibert *et al.*, 1982). In this method the isotope dilution of the NO_2^- pool (in the case of NH_4^+ oxidation) or the NO_3^- pool (in the case of NO_2^- oxidation) is monitored over time (e.g., Capone *et al.*, 1992; Clark *et al.*, 2007).

9.2. Denitrification

Denitrification is the reduction of $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ gas that is mediated by bacteria under anaerobic conditions, most generally in microbial mats and sediments. There are a number of methods to measure denitrification: acetylene inhibition, isotope pairing, changes in N_2 fluxes, and changes in the N_2 to argon (Ar) ratio. Each of the techniques has their pros and cons and none is clearly superior under all conditions (see reviews by Cornwell *et al.*, 1999; Chapter 6 by Devol, this volume).

In the acetylene inhibition technique, acetylene is added to a water sample, which inhibits the reduction of N_2O to N_2 (Sorensen, 1978). The accumulation of N_2O is then measured using gas chromatography and an electron capture detector and the denitrification rate is taken to be equal to the total N_2O flux. One potential problem is incomplete inhibition of N_2O reduction to N_2, particularly in the presence of hydrogen sulfide, a compound commonly found under anaerobic conditions. Another potential problem with the technique is that acetylene also inhibits nitrification, a process that often supplies the NO_3^- and NO_2^- substrates for denitrification. To inhibit nitrification is to inhibit denitrification if it is at all substrate limited (Hynes and Knowles, 1978).

The isotope pairing approach was developed to quantify what fraction of measured denitrification was fueled by substrates in the water column, versus coupled nitrification–denitrification (Nielsen, 1992). In this approach, $^{15}NO_3^-$ is added to water overlying sediments. The denitrification rate is calculated from the rate of formation of $^{29}N_2$ and $^{30}N_2$ (Rysgaard et al., 1993). N_2 gas formed from denitrification in the water column will incorporate $^{15}NO_3^-$ while denitrified N_2 from coupled nitrification–denitrification will incorporate $^{14}NO_3^-$.

The most direct approach to estimate denitrification is the direct measurement of N_2 fluxes. Concentrations of dissolved N_2 gas are very high in natural waters, however, such that any direct measurement of N_2 gas flux must be done against a huge background. One way to get around this is to incubate cores with N_2-free water. Seitzinger (1987) used this approach in long-term incubations where N_2-free water overlying the sediment was changed daily for 10 days. After the tenth day, the sediment was incubated for 24 h, and the change in N_2 gas concentration in the overlying water (measured with gas chromatography) was taken to be the denitrification rate. One potential problem with this technique is the long incubation time needed and resulting depletion of labile organic substrates (e.g., Boynton et al., 1995). Multiple intercomparisons of the various methods have been done. In general the direct measure of N_2 gas fluxes gives higher denitrification rates than the acetylene inhibition or isotope pairing techniques (Cornwell et al., 1999).

The newest approach to measuring denitrification uses a mass spectrometer that can rapidly measure the N_2/Ar ratio in water using membrane inlet mass spectrometry (MIMS, Kana et al., 1994, 1998). In this approach, sediment cores are incubated under continuous flow conditions. The N_2/Ar ratio is then measured at the inlet and outlet of the core over time. Advantages of the technique are the short incubation times needed (usually less than 12 h), and the high throughput and small sample size ($\sim$5 ml) of MIMS analysis.

Anammox (i.e., anaerobic ammonium oxidation) is a recently discovered process that also results in N_2 gas production and so is another form of denitrification (see Chapter 6 by Devol, this volume). The overall anammox reaction is $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$. To date anammox rates have been quantified using parallel incubations with either ^{15}N-labeled NH_4^+ or ^{15}N-labeled NO_3^- and then analyzing the mass of the N_2 gas formed in the two incubations (e.g., Thamdrup et al., 2006). The formation of $^{29}N_2$ in the $^{15}NH_4^+$ treatment indicates anammox. The formation of $^{30}N_2$ in the $^{15}NO_3^-$ treatment indicates classic denitrification (see Chapter 6 by Devol, this volume for a more detailed discussion).

9.3. N_2 fixation

In the process of N_2 fixation, dissolved N_2 gas is taken up and converted to NH_4^+ and ultimately biomass (see Chapter 4 by Capone and Carpenter, this volume). Nitrogenase, the enzyme that catalyzes the fixation of N_2, is deactivated by oxygen so care must be taken not to introduce oxygen during the measurement. There are two commonly used methods to measure N_2 fixation—$^{15}N_2$ incorporation and acetylene reduction. Using the tracer approach, $^{15}N_2$ is injected into a gas tight bottle containing the water sample (e.g., Montoya et al., 1996; Mulholland et al., 2004). At the end

of the incubation (generally 1–12 h) the sample is filtered and analyzed mass spectro-metrically in a fashion analogous to the ^{15}N uptake samples described above. During the course of the incubation $DO^{15}N$ release can occur such that the rate measured using the tracer approach is a net uptake rate (Mulholland *et al.*, 2006).

The acetylene reduction approach is based on the ability of nitrogenase to reduce substrates with triple N bonds. During the measurement, acetylene gas ($HC\equiv CH$) is reduced to ethylene ($H_2C=CH_2$) in a theoretical molar ratio of 3:1 relative to N_2 gas ($N\equiv N$). To estimate N_2 fixation with this approach, a water sample is sealed in a gas-tight container and acetylene is added (Capone, 1993; Montoya *et al.*, 1996). At the end of the incubation, the concentration of acetylene and ethylene is measured using flame ionization gas chromatography. The rate of N_2 fixation is then calculated using a conversion factor to convert acetylene reduction to N_2 gas fixation. Release processes do not affect the acetylene reduction method such that the rate measured approximates a gross N_2 fixation rate.

Of the two approaches, the acetylene reduction technique is much simpler and less labor intensive. It is also considerably easier to take a gas chromatograph to sea so that rate measurements can be made at a field site. The acetylene reduction method is also more sensitive and reproducible than the tracer approach, and requires smaller sample volumes. The advantage of the tracer approach, however, is that no conversion factor is needed. The use of the 3:1 conversion factor has been debated for some time. A recent study compared rates of N_2 fixation in the Gulf of Mexico over a three-year period and found that the ratio of acetylene reduction to $^{15}N_2$ fixation was seldom 3:1 (Mulholland *et al.*, 2006). A review of the literature found that it varied from 0.9 (Capone *et al.*, 2005) to a high of 22 (Mulholland and Bernhardt, 2005; Mulholland *et al.*, 2004) suggesting that the difference between N_2 fixation rates measured with acetylene reduction (gross uptake) versus those measured with $^{15}N_2$ gas (net uptake) could be exploited to estimate N release during the incubation (i.e., gross − net, Mulholland *et al.*, 2004).

10. RECOMMENDATIONS FOR FUTURE RESEARCH

In this review we have tried to cover a broad range of topics relating to the analysis of both organic and inorganic N in the sea. Necessarily, the treatment of each has had to be relatively brief, but we hope as a whole it will provide the reader a useful reference to the current state of the art at the beginning of the twenty first century. We close below with a series of notes regarding the limitations of current methods, promising ideas for ongoing research, and new areas that are rapidly evolving.

10.1. Reference materials

The increased focus on building large global datasets of nutrient and organic concentrations has highlighted the wide disparity that can exist in results measured in different labs (Hopkinson *et al.*, 1993). These discrepancies were especially problematic for the measurement of DOC (Sharp *et al.*, 1993, 2002a) and DON (Sharp *et al.*, 2002b). Two

of the most important steps in achieving consistency between laboratories have been the development of suitable reference material (Sharp *et al.*, 2002a) and agreement on standard procedures (e.g., Knap *et al.*, 1997). Community standards for DOC analysis are now widely used (Hansell, 2005) and standard humic substances are available from the International Humic Substances Society (http://www.ihss.gatech.edu), which can be used for chemical characterization and NMR studies. There are, however, currently no corresponding community-wide sets of reference materials commonly used for most of the other analyses discussed here. A widely available set of different sample types (including sediments, plankton, or different waters) for which values would be commonly reported would be an invaluable asset for standardizing results from specific organic and other analyses. A push to identify, produce, and promote the standard use of such materials could be one of the most important of analytical efforts.

10.2. Improving DON isolation methods

A ''holy grail'' for DON (and DOM in general) remains a rapid and portable method to quantitatively isolate and desalt a large dissolved sample (Bronk, 2002). Such a method would not only allow a wealth of diverse techniques to be brought to bear on the largest unknown fraction of DON, but would allow direct isotopic measurements and compound-specific mass balances. Examples of some approaches currently being explored to improve DON recovery on both small and large scales include 'homemade' ion-retardation resins (Bronk, unpublished data), electrodialysis (Vetter *et al.*, 2007), and use of nano-filtration membranes coupled to standard ultrafiltration approaches (McCarthy *et al.* unpublished data).

Until new methods are perfected, however, improvements might be made by altering existing isolations, and by paying closer attention to the relationship between isolated material and the total DON pool. For example, as discussed above, UF isolates $\sim$20–30% of DOM after desalting, but the material retained by UF membranes before desalting can be much larger (Guo, 2000, McCarthy, unpublished data). For analyses that do not require desalting (e.g., wet hydrolysis protocols) substantial increases in the fraction of total DON recovered might be achieved by simply eliminating desalting steps, while carefully monitoring losses associated with increasing concentration factors. Confidence in any DON isolation method could also be increased by routinely examining the relationship between isolated material and the bulk DON. Careful DON mass balance should be measured for any isolated fraction, and direct comparisons should be made where possible with specific compound classes examined.

10.3. Hobbled by hydrolysis? Chipping away at the black box

The initial step in molecular-level analysis of DON's most abundant organic compound classes is typically acid hydrolysis, necessary to break polymeric biomolecules into monomers for separation and detection. While the subsequent derivatization and chromatography steps can be shown to be quantitative (it is only on the basis of these post-hydrolysis steps that molecular-level methods are usually termed ''quantitative'' in the first place), the efficiency of the key initial hydrolysis remains largely a black box—overall efficiency is not known, extent of side reactions are not well

understood, and compound-specific losses are difficult to quantify. Data from the HMW-DOM literature suggest that this hydrolysis step itself may be problematic, and could account for an important part of the gap between total DON and the fraction we can identify at the molecular-level. Comparisons between ^{15}N–NMR data for HMW-DOM, and molecular-level recoveries discussed above just do not add up. They suggest either that very large amounts of unknown amide-compounds (or small variants of known structures such as common amino acids and amino sugars) dominate detrital ON composition, or alternately that despite the efficiency of hydrolysis for pure compounds, efficiency in detrital natural mixtures is far lower. While it is almost certain that no single explanation accounts for the low recoveries of identifiable DON components (i.e., multiple structural variants certainly are likely, and have been demonstrated for some newly discovered carbon DOC components, e.g., Hertkorn *et al.*, 2006), potential problems with the key hydrolysis step are typically ignored, but may be a key issue.

Some possible hydrolysis issues include secondary condensation reactions (e.g., Allard *et al.*, 1998) or the physical environment of biopolymers (e.g., Knicker and Hatcher, 1997; Nagata and Kirchman, 1997; Nguyen and Harvey, 2001), which might act to limit efficiency of standard aqueous methods. Since hydrolytic cleavage will likely remain central for molecular-level analyses, investigating hydrolysis methodology may be a very important, if highly un-glamorous, analytical endeavor. A series of extraction and hydrolysis method comparisons has recently been published for sugars in marine samples (Panagiotopoulos and Sempere, 2005). A similar systematic study specifically aimed at DON components has not been done, but would be extremely valuable. In particular, approaches designed to minimize melanoidan formation during hydrolysis (e.g., Allard *et al.*, 1998) should be investigated, and vapor-phase hydrolysis conditions might also be re-examined. The reasons for the substantial increases in vapor-phase amino acid yields noted in some samples, but not others (e.g., Jørgensen and Jensen, 1997; Keil and Kirchman, 1991b), have not been resolved, but might be consistent with increased penetration of shielded or encapsulated OM. Finally, overall extent of formation of secondary products and fate of major biochemicals might be examined by a combination of solid-state NMR and isotopic tracers. Overall, while improvements in hydrolysis conditions may be made, devising a perfect hydrolysis is not likely. This makes understanding and quantifying the extent of losses (i.e., is hydrolysis 80%, 50%, or 25% efficient for major N compounds?) and especially the selectivity of any hydrolytic losses extremely important to closing DON compositional budgets. As rapidly advancing mass-spectral approaches give us new tools to examine complicated ranges of structures (see below), a parallel effort to define the limitations of commonly used hydrolyses could help to significantly narrow the mystery of the majority "unidentified" DON.

10.4. DON Geomolecules? Exploring the nature of "humic" DON

New methods in ultra-high resolution mass-spectrometry are making progress in understanding complex distributions of similar structural variants, providing the promise of new tools to understand how wide the distribution of ON structures

in DON really is, as well as at last getting at nitrogenous humic materials. Despite the fact that the majority of DON appears to be composed of familiar biomolecules (like amino acids and amino sugars), solid state NMR of HMW-DON also suggests that increasing levels of unsaturated N compounds are present in the mid-water and deep ocean (McCarthy *et al.*, 1996). Electrospray ionization (EI) coupled to Fourier transform cyclotron resonance mass spectrometry (FTCR-MS) is a rapidly evolving ultra-high resolution MS approach that can potentially resolve the entire suite of individual compounds in dissolved humic materials (see Kujawinski *et al.*, 2002 for a good overall introduction, and Marshall *et al.*, 1998 for a full review). It has been shown that every individual compound can be resolved for natural humic and fulvic samples with mass accuracy up to 0.001 D (Kujawinski *et al.*, 2002; Stenson *et al.*, 2003), suggesting it is possible to generate molecular formulas for all components across the range of MW falling within ocean DOM (Koch *et al.*, 2005), and thus focus on compounds containing N. Drawbacks include the fact that ESI is not a quantitative technique (i.e., ionization is selective), and for ocean DOM it is necessary to obtain both concentrated and desalted samples, which introduces an additional level of selectivity.

Despite these limitations, the detailed molecular information of EI-FTIR-MS promises an important increase in our knowledge of the most murky of DOM components. The few studies that have so far been done on ocean waters use some form of SPE with acidified samples to simultaneously achieve both ends (e.g., Koch *et al.*, 2005; Kujawinski *et al.*, 2004), while at the same time likely removing much of the DON (Koch *et al.*, 2005). For investigating the humic fraction of DON, this selectivity may actually be an advantage. SPE isolates the smaller MW components that make up majority of ocean DOM, and also likely account for the most refractory components (Amon and Benner, 1994).

10.5. Higher order of information: Proteomics in DON

At the other end of the molecular size spectrum, rapidly advancing mass spectrometry techniques for determining protein sequence are being coupled with advances in genomics, creating new tools to decipher information locked in DON's largest surviving biomolecules. Proteomics employs the rapidly evolving field of protein mass spectrometry to examine the range of proteins expressed by organisms (e.g., Aebersold and Mann, 2003; Tyson *et al.*, 2004; Venter *et al.*, 2004). For study of DON these constitute new tools for understanding both the phylogeny of major groups contributing to the DON pool, as well identifying active enzymes that may be important in DON cycling. Using ultrafiltration to isolate the protein size fraction, proteomics has just begun to be applied to DON (Powell *et al.*, 2005) and has already suggested that a broader range of dissolved protein types may exist than had been previously identified. Proteomics approaches should be highly complimentary to the growing literature using N-terminal sequence approaches discussed above (Tanoue, 2000). Finally, expanding projects undertaking broad surveys of ocean genomes (''Metagenomics,'' e.g., Rusch *et al.*, 2007) may provide key background databases needed to interpret newly acquired proteomic information.

10.6. Compound-specific isotopes: New molecular level tools for cycling and origin

The instruments to make rapid, compound–specific isotope measurements have provided one of the most important new set of organic geochemical tools for tracing OM source and process. The large majority of this work has been done with non-polar compound classes using GC coupled to isotope ratio mass spectrometry (GC-IRMS). Compound-specific isotopic analysis of amino acids, and to a lesser extent sugars, are becoming common and represent a powerful new approach to understanding source and transformation of ON in the environment. Differences between diagnostic amino acids can reveal a new level of information about ON including original autotrophic δ^{15}N and number of subsequent trophic transfers (McClelland and Montoya, 2002), food webs of origin (Corr *et al.*, 2005), and potentially extent of microbial heterotrophic processing (McCarthy *et al.*, 2004, McCarthy *et al.*, 2007; Ziegler and Fogel, 2003).

As continuous flow IRMS instruments become common, it is likely that these measurements will greatly extend information from standard molecular % data of N biopolymers. While most early work has been done on either non–marine samples or biota, compound specific δ^{13}C– (McCarthy *et al.*, 2004) and δ^{15}N–amino acid (McCarthy *et al.*, 2007) have been made in HMW-DOM. Similar GC-IRMS techniques have also been developed for sugars (e.g., Derrien *et al.*, 2003; Macko *et al.*, 1998; Teece and Fogel, 2007), although they have not yet been explored for amino sugars in DON. Obtaining enough sample would be a challenge here, however, compound-specific analyses of amino sugars might be capable of addressing source issues as well as provide an independent means to assess the relative contribution of these compounds to the total DON.

In addition to stable isotopes, since the mid-1990s molecular-level radiocarbon (^{14}C) measurement has become a frontier in compound-specific isotopic measurement (Eglinton *et al.*, 1996). Compound-specific ^{14}C provides another independent dimension of source information, and in many cases a way to directly investigate cycling rates of compound-classes of interest (Aluwihare *et al.*, 2002; Eglinton *et al.*, 1997; Pearson and Eglinton, 2000; Repeta and Aluwihare, 2006). Compound-class or compound-specific ^{14}C offers, for the first time, the ability to directly estimate relative cycling rates of major DON components, independent of bulk DOC. Compound class-level isolations and ^{14}C measurement of total amino acids can be made using modifications of standard hydrolysis and cleanup protocols (Wang *et al.*, 1998; Loh *et al.*, 2004). Evolving compound-specific ^{14}C methods for collecting and purifying individual major nitrogenous compounds will in the future significantly improve resolution and accuracy of these initial efforts.

10.7. Rates—Who is doing what?

Currently the bulk of all N uptake rates ever measured have used some form of glass fiber filter. As a result, the rates represent uptake by autotrophs as well as a variable fraction of the bacterial community. This has broad implications when one considers the use of such commonly calculated parameters as the f-ratio, the ratio of new to

total (new plus regenerated) production (Eppley and Peterson, 1979). The f-ratio is estimated using ^{15}N-based uptake rates of NO_3^- uptake (new production) to NO_3^- plus NH_4^+ uptake (regenerated production). Now consider that a significant fraction of the NH_4^+ uptake measured is not fueling primary producers at all but is instead being taken up by heterotrophic bacteria caught on the GF/F filter. Flow cytometry, when combined with other techniques, has a great potential for expanding our understanding of competitive interactions because microbial groups can be distinguished optically, sorted, and then collected for specific analyses (e.g., Pel *et al.*, 2004a,b). Consequently, incubations can include intact communities, maintaining ecological integrity, while rate processes can be evaluated on a group-specific basis. Another advantage of using flow cytometry is that detritus, an often large but inactive component of the PC and PN pools, can be removed or sorted out of samples so as not to bias estimates of cellular C and N in the active pools.

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Nitrogen Stable Isotopes in Marine Environments

Joseph P. Montoya

Contents

1. Introduction

Nitrogen is a critical constituent of organic matter and is in short supply in many oceanic environments. The marine nitrogen cycle is complex, involving multiple oxidation states of nitrogen and multiple pathways interconnecting most of the biologically active pools. These processes occur on a broad range of spatial and temporal scales; some of which are not readily accessible using traditional biological measurements such as the ^{15}N-tracer studies described elsewhere in this volume (see Lipschultz, Chapter 31, this volume). The natural abundance of the rare stable isotope of nitrogen, ^{15}N, can provide critical insights

into processes acting on a variety of scales with minimal alteration and manipulation of the system being studied and with spatial and temporal integration that complements experimental studies using ^{15}N-labeled substrates, which generally involve short-term incubations of small, isolated samples of water under *in situ* or simulated *in situ* conditions.

The distribution of nitrogen isotopes within marine ecosystems can provide a record of the sources of N supporting biological production and the major pathways and mechanisms moving nitrogen through the biota. In general terms, the isotopic composition of a pool of nitrogen can be used in identifying the relative importance of sources that are isotopically distinct, or in identifying processes that add or remove nitrogen with a characteristic pattern of isotopic discrimination. The primary challenge in using this biogeochemical approach is the complexity of the marine nitrogen cycle and the potential influence of multiple processes on the isotopic composition of many of the biologically active pools of nitrogen. Successful use of nitrogen isotopes in resolving N cycle fluxes and processes requires an understanding of the general distribution of nitrogen isotopes in marine systems, the nature of isotopic fractionation, and a careful consideration of the processes at work in the system of interest. Here I provide an overview of the general isotopic systematics of nitrogen in marine systems and the ecosystem-level processes that lead to variations in isotopic composition. I will use examples drawn from marine systems with a focus on processes occurring in the water column of the modern ocean. Paleoceanographic applications of nitrogen isotopes are discussed elsewhere in this volume (see Galbraith *et al.,* Chapter 34, this volume), as are experimental approaches using substrates artificially enriched in ^{15}N (see Lipschultz, Chapter 31, this volume).

2. BACKGROUND

Substantial variations in the natural abundance of ^{15}N in marine systems were first documented in the 1950s (Hoering, 1955; Parwel *et al.,* 1957), and the earliest focused studies of nitrogen isotopic abundance in marine biota were carried out beginning about a decade later (Miyake and Wada, 1967; Wada *et al.,* 1975; Wada and Hattori, 1976; Minagawa and Wada, 1984). These and subsequent studies of the distribution of nitrogen isotopes in marine systems suggested that the natural abundance of ^{15}N in marine systems could be used as an indicator of the sources of nitrogen entering an ecosystem and supporting biological productivity, and as an index to the trophic position of marine animals. These general themes continue to motivate much of the ongoing work on the stable isotopes of nitrogen in marine systems.

2.1. Basic definitions

^{15}N is a minor stable isotope, constituting 0.365 at% of the global pool of nitrogen (Nier, 1950). As a practical matter, it is much easier to quantify the difference in isotope abundance or in isotope ratio (e.g., ^{15}N:^{14}N) between two samples than to

measure the absolute isotope ratio in either one. This analytical constraint leads to the common use of the "δ" convention for expressing the natural abundance of ^{15}N in a sample relative to the isotopic composition of a standard reference material:

$$\delta^{15}N = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000 \qquad (29.1)$$

where R_{sample} and R_{standard} represent the isotope ratio $^{15}N{:}^{14}N$ in a sample and in atmospheric N_2, respectively. $\delta^{15}N$ values therefore represent the "per mil" (‰) difference between the isotope ratio of a sample and that of atmospheric N_2. For natural samples in marine environments, $\delta^{15}N$ varies between about -2‰ and 30‰. Extreme values tend to be associated with regional dominance of either N_2-fixation or denitrification, providing one of the broadest scale nitrogen isotope contrasts in the ocean (see later text). Note that the $\delta^{15}N$ of a sample is a simple linear transform of its isotope ratio ($^{15}N{:}^{14}N$). For natural abundance level samples, the isotope ratio is effectively equal to the mole-fraction of ^{15}N (^{15}X) in the sample:

$$^{15}X = \frac{^{15}N}{^{15}N + {}^{14}N} = \frac{R}{1 + R} \approx R \qquad (29.2)$$

when R is small relative to 1. This equation makes it simple to convert between $\delta^{15}N$ values and the at-% units commonly used in tracer-level studies of the nitrogen cycle (e.g., Lipschultz, Chapter 31, this volume) since

$$\text{at\%}^{15}N = 100 \times \frac{R_{\text{sample}}}{1 + R_{\text{sample}}} \qquad (29.3)$$

and

$$R_{\text{sample}} = \left(\frac{\delta^{15}N}{1000} + 1\right) \times R_{\text{standard}} = \left(\frac{\delta^{15}N}{1000} + 1\right) \times 0.00366 \qquad (29.4)$$

2.2. Kinetic fractionation

Most biologically mediated reactions in the marine nitrogen cycle involve measurable discrimination against the heavy isotope, ^{15}N, which is critical in producing the ecosystem-level patterns we observe in $\delta^{15}N$ of marine nitrogen. Kinetic isotope fractionation reflects the difference in reaction rate for molecules containing the two isotopes of nitrogen, which in turn reflects the different bond strengths and mobilities of these isotopic species. The degree of isotopic fractionation associated with a reaction is commonly expressed as a dimensionless

fractionation factor, α, which is simply the ratio of rate constants for molecules containing the different isotopes:

$$\alpha = {}^{14}k/{}^{15}k \qquad (29.5)$$

In this case, ${}^{14}k$ and ${}^{15}k$ are the rate constants for molecules containing the light and heavy isotopes, respectively, and most reactions discriminate against the heavier isotope, yielding $\alpha > 1$. The inverse convention ($\alpha' = {}^{15}k/{}^{14}k$) is also used by some authors (e.g., Mariotti *et al.,* 1981), potentially leading to confusion when fractionation factors reported by different authors are compared. For most reactions, α falls between 1.000 and 1.030, making it convenient to define an isotopic discrimination factor that more clearly highlights this range of variation:

$$\epsilon = (\alpha - 1) \times 1000 \qquad (29.6)$$

For systems not artificially enriched in ${}^{15}N$ (e.g., tracer experiments), the discrimination factor is essentially equal to the difference between the $\delta^{15}N$ of the substrate and the $\delta^{15}N$ of the product of a fractionating reaction:

$$\epsilon \approx \delta^{15}N_{substrate} - \delta^{15}N_{product} \qquad (29.7)$$

This relationship holds for the substrate pool undergoing reaction and the product being formed from that pool, i.e., for the instantaneous state of the reaction system. The preferential removal of ${}^{14}N$ in the course of a reaction will naturally lead to a progressive increase in the $\delta^{15}N$ of the remaining substrate pool. For example, phytoplankton assimilation of nitrate (NO_3^-) typically shows a discrimination factor (ϵ) of 1–10‰ (Montoya and McCarthy, 1995; Waser *et al.,* 1998a,b; Needoba *et al.,* 2003, 2004). Figure 29.1 shows the trajectory of $\delta^{15}N$ of NO_3^- and phytoplankton biomass in a closed system (e.g., a culture flask) initially containing 20 μmol L^{-1} NO_3^- and a very small inoculum of phytoplankton that discriminate against ${}^{15}N$ with $\epsilon = 5$‰. Note that both the NO_3^- and the phytoplankton biomass become progressively enriched in ${}^{15}N$ (i.e., $\delta^{15}N$ increases), but that the mean $\delta^{15}N$ of the combination of NO_3^- and phytoplankton must remain constant because of the conservation of mass and isotopes within a closed system. When the reaction has gone to completion and all the NO_3^- has been converted to phytoplankton biomass, the phytoplankton $\delta^{15}N$ is equal to the $\delta^{15}N$ of the original pool of substrate (Fig. 29.1). In other words, there is no record of the isotopic fractionation once the reaction has gone to completion. Isotopic fractionation is observable only under conditions of partial consumption of the substrate pool, or in an open system with continuous addition of substrate or removal of product as the reaction proceeds. The openness of a system, i.e., the degree to which it exchanges nitrogen with the rest of the world, has important implications for its isotopic systematics (Wu *et al.,* 1997; Sigman *et al.,* 1999) and is a critical consideration in the design and interpretation of isotopic studies of ecosystems.
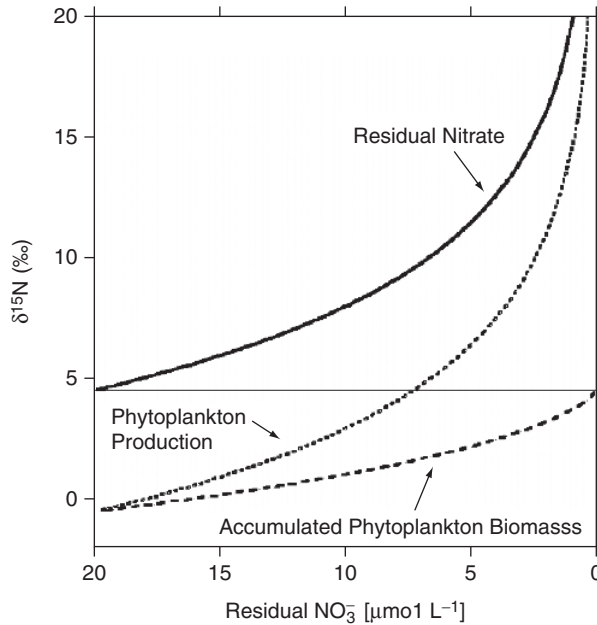
Figure 29.1 Changes in $\delta^{15}N$ of nitrate and phytoplankton biomass in a closed system during phytoplankton growth and consumption of nitrate with an isotope discrimination facter (ϵ) of 5‰. As phytoplankton grow, the $\delta^{15}N$ of the residual nitrate and phytoplankton biomass pool both increase, as does the $\delta^{15}N$ of the biomass formed at any instant ("phytoplankton production").

2.3. Isotopic fractionation and oceanic nitrogen

As an isotopic system, the nitrogen cycle is quite complex, with multiple pathways, many with significant isotopic fractionation, connecting many of the pools of interest (Fig. 29.2). From a process point of view, primary producers clearly play a central role by assimilating inorganic nitrogen and injecting it into the food web, where it may be passed from one organism to another through trophic interactions before being ultimately remineralized (Fig. 29.2A). The isotopic point of view is somewhat different since the strongest fractionations are associated with dissimilatory processes that do not move nitrogen directly into or through the food web (Fig. 29.2B), but rather from one inorganic pool to another. For example, denitrification (dissimilatory nitrate reduction) converts NO_3^- to N_2, which has the net effect of depleting the ocean's supply of combined nitrogen and doing so with strong discrimination against ^{15}N. This results in an increase in the $\delta^{15}N$ of the major pool of oceanic combined nitrogen (NO_3^-) concomitant with a reduction in its overall size. In contrast, N_2-fixation adds combined nitrogen with a low $\delta^{15}N$ to the ocean, counteracting both the mass and isotopic effects of denitrification (Brandes and Devol, 2002).
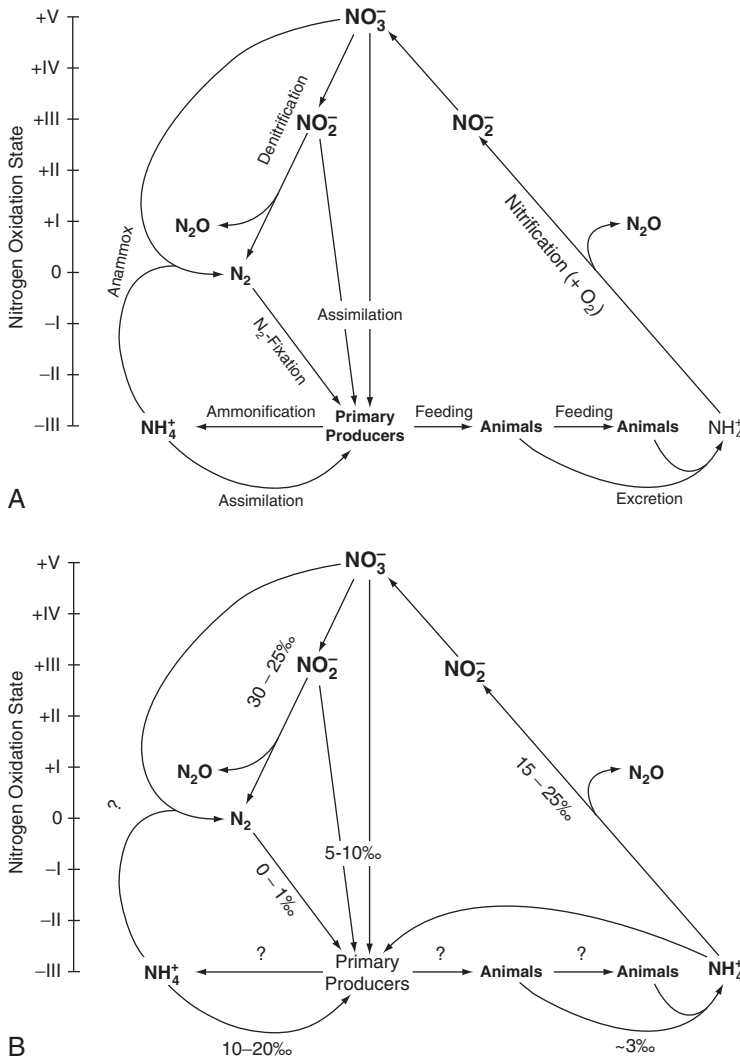
Figure 29.2 Schematic overview of the marine nitrogen cycle. A: Important species, their oxidation state (vertical axis), and major biological transformations of nitrogen (arrows). B: Typical values of the isotopic enrichment factor (ϵ) are shown for reactions that have been characterized isotopically. Estimates of ϵ were drawn from the available literature on N_2–fixation and the $\delta^{15}N$ of diazotrophs (Carpenter *et al.*, 1997; Delwiche and Steyn, 1970; Hoering and Ford, 1960; Macko *et al.*, 1987; Montoya *et al.*, 2002), denitrification (Barford *et al.*, 1999; Cline and Kaplan, 1975; Delwiche and Steyn, 1970; Mariotti *et al.*, 1981, 1982; McCready *et al.*, 1983; Miyake and Wada, 1971; Voss *et al.*, 2001; Wada, 1980;), nitrification (Delwiche and Steyn, 1970; Mariotti *et al.*, 1981; Miyake and Wada, 1971; Yoshida, 1988), NO_3^- uptake (Montoya and McCarthy, 1995; Needoba *et al.*, 2003; Needoba and Harrison, 2004; Pennock *et al.*, 1996, 1998; Wada and Hattori, 1978; Waser *et al.*, 1998a, 1998b;), NO_2^- uptake (Wada and Hattori, 1978; Wada, 1980), NH_4^+ uptake (Cifuentes *et al.*, 1989; Montoya *et al.*, 1991; Pennock *et al.*, 1988; Wada, 1980; Wada and Hattori, 1978), and zooplankton excretion (Checkley and Miller, 1989).

2.3.1. Nitrate

Nitrate is the largest pool of combined nitrogen in the ocean, with deep water concentrations around 20 to 30 μmol L^{-1} in the Atlantic and up to 45 μmol L^{-1} in the Pacific. The isotopic composition of the NO_3^- pool is affected by a variety of processes that move N in and out of the ocean or its biota (Fig. 29.3), and subsurface NO_3^- acts as a critical isotopic end member for biological production in the upper water column. Of the processes shown in Fig. 29.3, pelagic denitrification and N_2–fixation are generally viewed as the major, long-term controls on the size and isotopic composition of the oceanic pool of NO_3^- (Brandes and Devol, 2002).

Under suboxic conditions, microbial nitrate respiration is an important energetic pathway that ultimately leads to removal of combined N from the ocean. This process is accompanied by strong isotopic discrimination (Barford *et al.,* 1999; Brandes *et al.,* 1998; Cline and Richards, 1972; Mariotti *et al.,* 1981; McCready *et al.,* 1983; Voss *et al.,* 2001), so the partial consumption of NO_3^- that typically occurs during pelagic denitrification in the large oceanic Oxygen Minimum Zones (OMZs) has a strong impact on the $\delta^{15}N$ of the residual NO_3^- pool (Fig. 29.3). In contrast, sedimentary denitrification generally results in complete consumption of NO_3^-, whether it diffuses in from the overlying seawater or is generated *in situ*
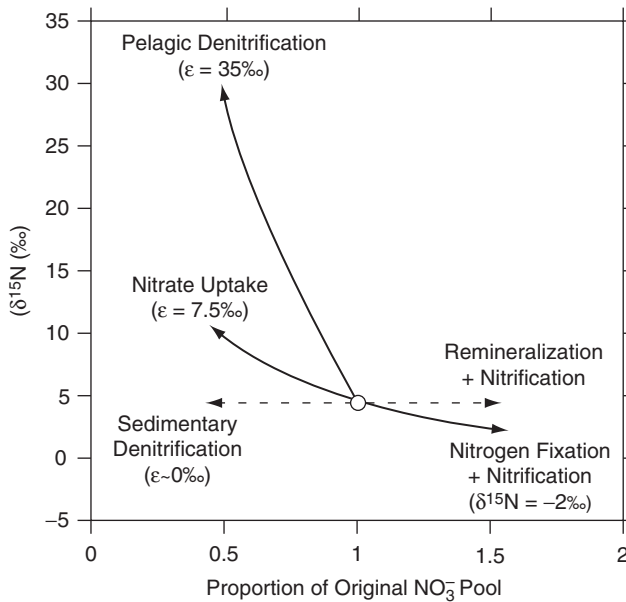
Figure 29.3 Schematic showing the impact of different processes on the $\delta^{15}N$ of oceanic NO_3^-. The trajectories for denitrification, nitrate uptake, and sedimentary denitrification were each calculated using a closed-system Rayleigh model with an isotope discrimination factor (ϵ) in the middle of the range given in Fig. 29.2. The two nitrification trajectories shown were calculated with a mass balance model for remineralization and nitrification of organic matter produced from average deep water NO_3^-, or diazotrophic organic matter with a $\delta^{15}N$ of -2‰ (Montoya *et al.,* 2002).

through nitrification of ammonium produced in the sediments (Brandes and Devol, 1997, 2002). As a result, sedimentary denitrification results in no expression of the strong isotopic fractionation associated with the denitrification process itself (Fig. 29.1), making this loss term isotopically invisible (Fig. 29.3).

Nitrification is another process that discriminates strongly between the nitrogen isotopes (Delwiche and Steyn, 1970; Miyake and Wada, 1971; Mariotti $et\ al.,$ 1981; Yoshida, 1988) as microbes oxidize NH_4^+ to NO_3^- under oxic conditions. Nitrification is a critical process in the remineralization of organic matter and its conversion to NO_3^-, so this isotopic fractionation could potentially be a source of ^{15}N–depleted NO_3^- in the water column (Ostrom $et\ al.,$ 1997; Sutka $et\ al.,$ 2004). In the upper and mid water column of oligotrophic waters, however, a situation similar to the case of sedimentary denitrification applies: nitrification typically goes to completion, leaving behind little or no residual substrate (NH_4^+) in solution. As a result, nitrification simply transfers the nitrogen initially present in NH_4^+ into the NO_3^- pool along with its isotopic signature. When the organic matter remineralized is itself derived from deep water NO_3^-, then there is no net impact on the $\delta^{15}N$ of NO_3^- when remineralization and nitrification occur (Fig. 29.3). In contrast, when the organic matter is derived from an isotopically distinct pool of nitrogen (e.g., diazotroph biomass), remineralization and nitrification can lead to a significant change in the $\delta^{15}N$ of the ambient NO_3^- pool (Fig. 29.3).

In summary, the $\delta^{15}N$ of NO_3^- acts as an integrator of the major biological sinks and sources of nitrogen within a water column. As shown in Fig. 29.3, pelagic denitrification and N_2-fixation are the major processes that strongly alter the $\delta^{15}N$ of oceanic NO_3^-. Denitrification reduces the size of the oceanic pool of NO_3^- while increasing its $\delta^{15}N$. In contrast, N_2-fixation lowers the $\delta^{15}N$ of NO_3^- while increasing the overall size of the pool of oceanic combined nitrogen. Both processes generate isotopic signatures that can propagate into other components of the ecosystem, including the near surface biota (Voss $et\ al.,$ 2001; McClelland $et\ al.,$ 2003; Montoya $et\ al.,$ 2002;) and benthic sediments (Altabet $et\ al.,$ 1995; Ganeshram $et\ al.,$ 1995). In effect, the isotopic discrimination associated with these ecosystem-level processes creates isotopic signals that can potentially be exploited to study the nitrogen cycle on a broad variety of spatial and temporal scales.

2.3.2. Particulate organic matter

Most marine autotrophs require combined nitrogen as a substrate for growth, making the oceanic pool of NO_3^- a critical determinant of the isotopic composition of the biota. Phytoplankton express significant isotopic discrimination during uptake of NO_3^- (Montoya and McCarthy, 1995; Waser $et\ al.,$ 1998a,b; Needoba $et\ al.,$ 2003; Needoba and Harrison, 2004), leading to production of biomass depleted in ^{15}N relative to the available substrate and progressive enrichment of the residual NO_3^- as uptake proceeds (Fig. 29.1). In most waters, however, the complete consumption of nutrients entering the upper water column results in production of biomass with a $\delta^{15}N$ that reflects that of the NO_3^- entering the mixed layer, though a gradient in NO_3^- concentration and $\delta^{15}N$ is generated at the base of the mixed layer (Altabet $et\ al.,$ 1986).

Not all marine producers are dependent on NO_3^- uptake to sustain growth. A variety of prokaryotes are able to reduce and assimilate N_2 into biomass, making them potentially independent of existing pools of combined nitrogen in the ocean. These N_2-fixers, or diazotrophs, are taxonomically diverse and include a variety of cyanobacteria and bacteria (Zehr et al., 1998, 2000, 2001; Langlois et al., 2005). N_2-fixation is accompanied by only modest isotopic discrimination (Hoering and Ford, 1960; Delwiche and Steyn, 1970; Macko et al., 1987), so diazotroph organic matter is slightly depleted relative to atmospheric N_2, which is used as the standard reference material for nitrogen isotope measurements and therefore has a $\delta^{15}N$ of 0‰ by definition. Field measurements show that marine diazotrophs, including the colonial cyanobacterium Trichodesmium as well as diatom–diazotroph assemblages all have a $\delta^{15}N$ of -1 to -2‰ (Carpenter et al., 1997; Montoya et al., 2002).

Finally, the role of remineralization and recycling of nitrogen within the upper water column deserves consideration. This recycling pathway is quite efficient in the sense that NH_4^+ does not accumulate in significant quantity in the mixed layer of oceanic systems. As a result, remineralization simply redistributes nitrogen among the different components of the bulk field of particulate organic matter and does not appear to have a significant impact on the distribution of isotopes in the upper water column.

2.3.3. Animals and food webs

Animals consistently show a "trophic" enrichment in ^{15}N abundance in which the tissues of a consumer have a $\delta^{15}N$ that is 2–4‰ higher than the $\delta^{15}N$ of the animal's food (DeNiro and Epstein, 1981; Fry, 1988; Minagawa and Wada, 1984; Montoya et al., 1990, 1992, 2002; Mullin et al., 1984; Wada et al., 1987;). The physiological and biochemical processes that generate this trophic effect are not well understood, though excretion of isotopically depleted NH_4^+ appears to play a primary role in enriching animals tissues in ^{15}N (Bada et al., 1989; Gannes et al., 1997).

An animal at isotopic steady state must consume and release nitrogen with the same average $\delta^{15}N$. These isotopic fluxes are not well characterized for any animal (Gannes et al., 1997, 1998), let alone marine zooplankton, but a generally consistent picture has emerged from two independent lines of evidence summarized in Fig. 29.4. First, little isotopic fractionation occurs during ingestion and assimilation of food, which results in production of fecal pellets with a $\delta^{15}N$ typically similar to that of the food (Altabet and Small, 1990; Checkley and Entzeroth, 1985; Montoya et al., 1992). Second, because marine animals typically produce NH_4^+ as their major nitrogenous waste product, the primary driver of the trophic effect in marine ecosystems appears to be the isotopic fractionation associated with production of NH_4^+ through deamination of proteins and amino acids (Bada et al., 1989; Macko et al., 1986). Loss of the isotopically depleted NH_4^+ generated through deamination will tend to increase the $\delta^{15}N$ of the remaining organic nitrogen within an animal's tissues (Fig. 29.4).

One interesting implication of the qualitative model shown in Fig. 29.4 is that the size of the trophic effect is a function of the partitioning of assimilated nitrogen between new biomass and excreta. The greater the excretory losses, the greater the difference in $\delta^{15}N$ between an animal and its food. This implies that the "trophic
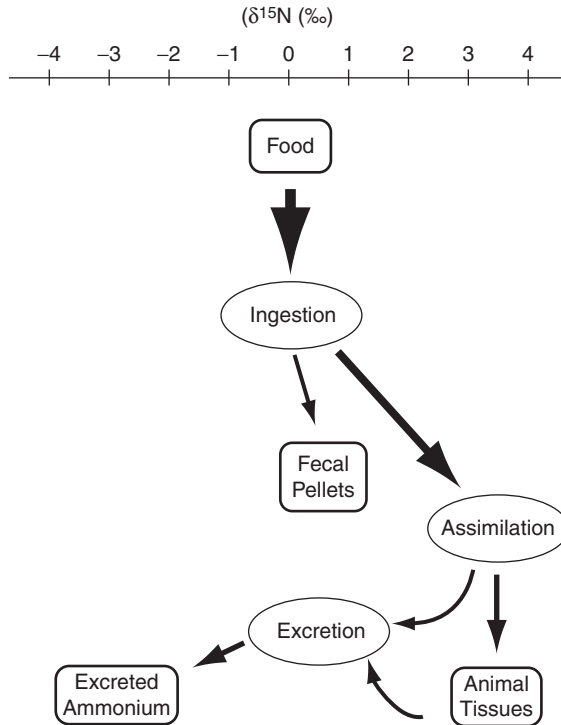
Figure 29.4 Schematic showing the major steps in the processing of nitrogen by an animal. Arrows represent fluxes of nitrogen, ovals label processes, and rectangles show important pools of nitrogen. Limited or no isotopic fractionation appears to occur in connection with the assimilation of food (Altabet and Small, 1990; Checkley and Entzeroth, 1985; Montoya *et al.*, 1992). In contrast, deamination reactions, which play a central role in excretion by marine animals, fractionate significantly (Bada *et al.*, 1989; Macko *et al.*, 1986). The net result of this process is to partition the ingested nitrogen between animal tissues that are enriched in ^{15}N relative to the food and excreted NH_4^+ with a $\delta^{15}N$ lower than that of the food.

effect'' is more a measure of nitrogen transfer efficiency from food to tissues than a measure of the animal's average trophic position. Viewed in slightly different terms, the increase in $\delta^{15}N$ between an animal's food and its tissues is really a measure of the animal's net growth efficiency rather than trophic position as such.

3. ANALYTIC CONSIDERATIONS

The increasing availability of mass spectrometers and on-line interfaces for sample preparation has made ^{15}N natural abundance measurements accessible to a broad community of biological oceanographers, and stable isotope measurements are becoming a routine tool in studies of marine ecosystems. In general, ^{15}N measurements are now carried out with continuous flow systems that integrate a

preparatory system (e.g., an elemental analyzer) with a mass spectrometer (Barrie *et al.,* 1990; Owens and Rees, 1989). The mass spectrometer typically measures the isotopic composition of N_2 generated by combustion and carried in a stream of He gas to an open split interface that introduces a small fraction of the gas stream into the ion source for measurement. This analytical approach generally requires little or no manipulation of organic samples, though rigorous care must be taken to avoid contamination with exogenous nitrogen, particularly when tracer-level ^{15}N experiments are being carried out in the vicinity (see Lipschultz, Chapter 31, this volume). Since N_2 is typically the analyte, the reduction of atmospheric contamination in the preparatory system is also quite important.

Samples of oceanographic interest often pose additional analytical challenges simply because they are difficult to obtain in large quantities and most mass spectrometry systems require fine-tuning and careful attention to leaks in order to process samples containing less than a few micromoles of nitrogen. In practice, open ocean samples including suspended particles, small zooplankters, and sinking organic matter are often available only in μmole or sub-μmole quantities. This creates a strong incentive to minimize the mass requirements of the analytical systems used for marine samples, and to develop methods for correcting for the influence of any analytical blank, which will disproportionately affect small samples. Even with substantial care to reduce this source of contamination, a nitrogen blank on the order of 0.05–0.15 μmol N is typical for systems in current use. For analysis of the smallest samples, the influence of this blank can be removed numerically provided that an appropriate range of mass and isotopic standards is run, allowing estimation of the size and $\delta^{15}N$ of the blank.

3.1. Blank corrections: Theory

Operationally, isotope ratio mass spectrometers measure the difference in ^{15}N abundance between a sample and a reference gas calibrated to atmospheric N_2. This reference gas calibration may be carried out directly by comparison to atmosphere scrubbed of O_2 (Mariotti, 1983), or indirectly using any of a number of organic and inorganic secondary standards (e.g., nitrate and ammonium salts, acetanilide, glutamic acid) available from NIST or IAEA. For precise system calibration and correction of blank effects, it is also important to analyze a size series of a working standard that is chemically similar to the samples of interest. Although many samples are now analyzed by fee-for-service facilities that carry out these corrections for individual investigators, an understanding of the blank correction methodology is helpful in the interpretation of isotopic data and provides a framework for carrying out any isotope mass balance calculation (e.g., mixing models to resolve isotopically distinct source contributions).

Correcting for an analytical blank is a multi-step process involving separate regression analyses to evaluate the size of the blank and the dependence of $\delta^{15}N$ values on size. This information can then be used in a mass balance model to remove the isotopic influence of the blank. In general, the nitrogen analytical blank is small enough that blank corrections are minor for samples containing more than 1.5–2 μmol of nitrogen.

Any real sample can be treated as a mixture of sample material and contaminants of various origins. If the blank contribution is constant in composition and magnitude across analyses, then a simple mass balance can be constructed for the mixture analyzed:

$$M_{\text{mix}}\delta^{15}N_{\text{mix}} = M_{\text{sample}}\delta^{15}N_{\text{sample}} + M_{\text{blank}}\delta^{15}N_{\text{blank}} \tag{29.8}$$

where M_X is the size of an individual pool X (sample, blank, or the mixture). Since M_{mix} is just the sum of the other masses, Eq. (29.8) can be easily rearranged to produce an expression for $\delta^{15}N_{\text{sample}}$:

$$\delta^{15}N_{\text{sample}} = \left(\frac{M_{\text{mix}}\delta^{15}N_{\text{mix}} - M_{\text{blank}}\delta^{15}N_{\text{blank}}}{M_{\text{mix}} - M_{\text{blank}}} \right) \tag{29.9}$$

which can be used to extract the $\delta^{15}N$ of the sample independent of the analytical blank. To do so, the size of the blank and its isotopic composition must be measured independently, which is straightforward provided that size series of standards of known elemental and isotopic composition are included in each analytical run.

3.2. Measurement of sample and blank size

We use separate standards, methionine and peptone, interspersed in each analytical run to estimate the mass and isotopic composition of the blank. The separate standards provide some redundancy since both blank parameters can be estimated independently with each standard. In practice, calibrating the mass response of the mass spectrometer and the size of the blank require pure standards of known elemental composition and mass, while the isotopic composition of the blank can be readily measured by analyzing an isotopic standard whose elemental composition is less well constrained (e.g., peptone).

We use precisely weighed samples of methionine or another pure nitrogenous compound to calibrate the mass response of the analytical system. Sample size is typically measured as the integrated response (beam area) of the mass spectrometer ion collectors, and a regression of beam area as a function of the known nitrogen content of the sample will yield a good estimate of both the system sensitivity (slope of the regression) and the size of the blank (intercept of the regression), M_{blank}. These quantities in turn allow calculation of the size of any sample or standard analyzed.

Once the size of the blank is known, its isotopic composition ($\delta^{15}N_{\text{blank}}$) can be calculated from analysis of a range of sizes of a standard of uniform isotopic composition (e.g., peptone). A regression of the $\delta^{15}N$ measured as a function of the inverse of the size of the analytes will have the form:

$$\delta^{15}N_{\text{mix}} = \delta^{15}N_{\text{sample}} + \left(\frac{1}{M_{\text{mix}}} \right) \times M_{\text{blank}}(\delta^{15}N_{\text{blank}} - \delta^{15}N_{\text{sample}})$$

$$\tag{29.10}$$

which is simply a rearrangement of Eq. (29.8). This regression will have an intercept equal to the true $\delta^{15}N$ of the standard and a slope equal to the term:

$$\text{Slope}(\text{Eq.}(29.10)) = M_{\text{blank}}(\delta^{15}N_{\text{blank}} - \delta^{15}N_{\text{standard}}) \qquad (29.11)$$

which can be rearranged to give an expression for $\delta^{15}N_{\text{blank}}$ in terms of known or measured quantities:

$$\delta^{15}N_{\text{blank}} = \delta^{15}N_{\text{standard}} + \left(\frac{\text{slope}(\text{Eq.}(29.10))}{M_{\text{blank}}}\right) \qquad (29.12)$$

Finally, the size and $\delta^{15}N$ of the blank can be used in Eq. (29.9) to correct for the influence of any analytical blank. This approach allows measurement of the $\delta^{15}N$ of small samples with high precision and accuracy. We estimate that the overall error in our measurement is less than 0.15‰ for samples containing more than 0.6 μmol N.

4. ECOSYSTEM LEVEL PATTERNS IN δ^{15}N

The $\delta^{15}N$ of oceanic organic matter shows a number of broad-scale patterns that reflect the impact of nitrogen cycle processes on both a regional and a global level. The simplest application of $\delta^{15}N$ measurements is in resolving the relative contributions of different, isotopically distinct sources of N to biological production. Simple isotopic mixing models can be used to calculate these contributions provided that no more than two end members are important in the system of interest. In general, for a system that depends on two such sources (1 and 2), the $\delta^{15}N$ of any target pool that integrates these two sources will be the weighted average of the source terms:

$$\delta^{15}N_{\text{target}} = X_1 \times \delta^{15}N_1 + X_2 \times \delta^{15}N_2 \qquad (29.13)$$

where X_1 and X_2 represent the fractional contributions of Sources 1 and 2, respectively, to the target pool. Since $X_1 + X_2 = 1$, this equation is easily solved for the contribution of either source pool to the target pool:

$$X_1 = \frac{\delta^{15}N_{\text{target}} - \delta^{15}N_2}{\delta^{15}N_1 - \delta^{15}N_2} \qquad (29.14)$$

$$X_2 = \frac{\delta^{15}N_{\text{target}} - \delta^{15}N_1}{\delta^{15}N_2 - \delta^{15}N_1} \qquad (29.15)$$

Note that Eq. (29.13) is simply a general case of the isotope mixing model used in blank corrections above (Eq. (29.8)) and is applicable to any situation in which a

mixture is derived from two isotopically distinct pools. This approach can be extended to food web studies with the inclusion of a trophic effect to account for the increase in $\delta^{15}N$ with trophic position (Montoya *et al.*, 2002).

Next, I will examine several examples in which isotopic contrasts provide insight into nitrogen cycle dynamics in different marine environments. Since the choice of system is important in ensuring that isotope budgets are appropriate and tractable, my goal is to provide examples of systems in which $\delta^{15}N$ measurements can be used profitably rather than attempt exhaustive coverage of the diverse and growing literature on nitrogen isotopes in the ocean.

4.1. Terrigenous inputs

On average, the $\delta^{15}N$ of terrestrial organic matter is lower than that of marine nitrogen, an isotopic contrast that can be exploited to trace the impact of terrigenous nitrogen in coastal marine systems (De Brabandere *et al.*, 2002; Heaton, 1986; Rau *et al.*, 1981; Sweeney *et al.*, 1980; Tucker *et al.*, 1999; Van Dover *et al.*, 1992;). This is a particularly interesting area of research given the increasing impact of anthropogenic nitrogen in coastal and nearshore systems. As in any ecosystem, stable isotope studies of nearshore waters require careful consideration of the potential inputs of nitrogen, the possibly complex pathways of nitrogen movement through the system, and the frequent involvement of both sedimentary and water column processes in nitrogen cycling. Nonetheless, isotopic approaches often provide the best tool for linking changes in coastal production or biomass to exogenous nutrient inputs. Rather than relying simply on correlative studies of loading and ecosystem response, ^{15}N budgeting approaches allow tracing of anthropogenic nitrogen as it moves into the biota and through the food web.

The generally low $\delta^{15}N$ of terrestrial systems is typically retained in sludge and particulate matter produced by sewage plants. Nitrogen isotopic studies in a number of coastal systems have demonstrated that this low $\delta^{15}N$ propagates into the benthic food web and can be used to quantify the impact of solid materials in sewage on the nearshore biota (Rau *et al.*, 1981; Spies *et al.*, 1989; Tucker *et al.*, 1999; Van Dover *et al.*, 1992).

When dissolved pools of nitrogen are considered, the isotopic scenario becomes substantially more complicated, often involving multiple sources and pathways to/ from each of the major dissolved pools of nitrogen. The major dissolved forms of nitrogen in sewage, terrestrial runoff, and groundwater are often enriched in ^{15}N because of isotopic fractionation during nitrification and volatilization in the case of NH_4^+, or denitrification in the case of NO_3^- (Heaton, 1986; McClelland and Valiela, 1998b; Schlacher *et al.*, 2005; Sheats, 2000; Voss and Struck, 1997). The degree of isotopic enrichment depends strongly on the details of the system, but terrestrial inorganic nitrogen entering coastal waters often has a $\delta^{15}N$ significantly higher than the $\delta^{15}N$ of marine pools of inorganic nitrogen. This isotopic contrast in turn can be exploited to trace the incorporation of terrigenous nitrogen into various components of nearshore systems including sediments, primary producers, plankton, and fish (Cole *et al.*, 2006; Lapointe *et al.*, 2004, 2005; McClelland *et al.*, 1997; McClelland and Valiela, 1998a,b; Schlacher *et al.*, 2005; Sheats, 2000; Voss and Struck, 1997).

4.2. Nitrogen sources for pelagic primary production

As noted earlier, deep water NO_3^- plays a central role in setting the $\delta^{15}N$ of oceanic biota through its large contribution to primary production. The $\delta^{15}N$ of NO_3^- shows significant regional variation, particularly in oxygen minimum zones where denitrification consumes NO_3^- and raises the $\delta^{15}N$ of the residual NO_3^- (Fig. 29.3 and below). Deep water NO_3^- typically has a $\delta^{15}N$ between 3 and 6‰, with a global average of about 4.8‰ (Liu and Kaplan, 1989; Sigman *et al.*, 2000).

In high latitude systems, the $\delta^{15}N$ of NO_3^- is closely linked to the $\delta^{15}N$ of mixed layer organic matter and spatial variations in the $\delta^{15}N$ of both NO_3^- and particulate organic matter reflect the isotopic fractionation associated with phytoplankton uptake of NO_3^- (Altabet and François, 1994, 2001; Sigman *et al.*, 1999; Wu *et al.*, 1997). Similar variations have been found in the equatorial upwelling system, where consumption of NO_3^- creates a latitudinal gradient in the $\delta^{15}N$ of organic matter (Altabet, 2001). In general, the $\delta^{15}N$ of surface and mixed layer particles varies inversely with NO_3^- concentration in these systems, reflecting the impact of isotopic fractionation during NO_3^- uptake (Fig. 29.1) by primary producers.

On a basin scale, data from the Atlantic Meriodional Transect (AMT) program show clear regional and temporal variations in the $\delta^{15}N$ of particles collected at the surface. Data from two cruises, AMT-3 in September–October 1996 and AMT-10 in April–May 2000, show similar patterns for the North Atlantic but differ markedly south of the equator (Mahaffey *et al.*, 2003, 2004; Mino *et al.*, 2002). During the autumn cruise (AMT-3) the distribution of $\delta^{15}N$ values was similar in both the North and the South Atlantic, with low values in the oligotrophic gyres, near the Equator, and at high southern latitude (Mino *et al.*, 2002). For the transect as a whole, Mino *et al.* (2002) found a strong negative correlation between the $\delta^{15}N$ of surface particles and the depth of the nitracline. This relationship was even stronger when data from stations in the tropical North Atlantic with significant populations of *Trichodesmium* were excluded from the analysis. At these tropical stations, Mino *et al.* (2002) used a mass balance approach to estimate that N_2-fixation contributed at least 38% of the standing stock of nitrogen at the surface. Interestingly, $\delta^{15}N$ values from the tropical South Atlantic were even lower than those from the North Atlantic, but Mino *et al.* did not attempt to estimate the importance of N_2-fixation in that region, presumably because *Trichodesmium* was not observed at those stations (2002). This may deserve reexamination in view of recent molecular and biogeochemical studies highlighting the importance of diazotrophs other than *Trichodesmium* in oligotrophic waters (Montoya *et al.*, 2004; Langlois *et al.*, 2005; Zehr *et al.*, 1998, 2001, 2007; Montoya *et al.*, 2007).

The lowest $\delta^{15}N$ values from the spring cruise (AMT-10) occurred at the northern end of the transect where a phytoplankton bloom was under way and the low values reflected isotopic fractionation during NO_3^- uptake (Mahaffey *et al.*, 2003, 2004). In the oligotrophic gyre of the North Atlantic, low values similar to those reported by Mino et al. (2002) were found and attributed to N_2–fixation (Mahaffey *et al.*, 2003, 2004). In contrast to the general similarity between cruises in the North Atlantic, the data from cruise AMT-10 did not show low $\delta^{15}N$ values in the oligotrophic gyre of the South Atlantic. Mahaffey *et al.* (2004) argued that the uniform $\delta^{15}N$ of particles between the Equator and 40°S implied production based

on NO_3^-. As noted above, the isotopic data from AMT–3 are consistent with a significant input of nitrogen through N_2-fixation in the South Atlantic, which suggests a seasonal or interannual shift between diazotrophy and production based on NO_3^- in that basin. In any case, the difference between hemispheres in the spring is quite striking and deserves additional attention.

The AMT data set provides clear evidence for the regional importance of N_2-fixation, at least in the North Atlantic. This general picture is consistent with a number of other isotopic studies of N_2-fixation in the subtropical North Atlantic (Capone *et al.*, 2005; McClelland *et al.*, 2003; Montoya *et al.*, 2002). In this region, isotope mass balance calculations indicate that N_2-fixation is the dominant source of nitrogen supporting production of mixed layer biomass throughout the western half of the basin (Montoya *et al.*, 2002). The N_2-fixation contribution appears to be particularly high in the southwestern region affected by the plume of the Amazon River, where dense populations of both *Tricho-desmium* and diatom–diazotroph associations occurred (Carpenter *et al.*, 1999) and a mass-balance calculation indicated that N_2-fixation accounted for an average of 68% of the standing stock of particulate nitrogen in the upper 100 m of the water column (Montoya *et al.*, 2002). For a zonal survey across the subtropical North Atlantic, Montoya *et al.* (2002) estimated that 36% of the standing stock of particulate nitrogen was derived from N_2-fixation. This esti-mate agrees very well with that of Mino *et al.* (2002) and implies a regional rate of N_2-fixation of 850 µmol N m^{-2} day^{-1} (Capone *et al.*, 2005). This general approach has also been extended to the zooplankton, though the calculations are complicated by the trophic effect within foodwebs. Nonetheless, Montoya *et al.* (2002) estimated that as much as 60% of the zooplankton standing stock in the southwestern North Atlantic, and 47% of zooplankton biomass in the central part of the basin, was derived from N_2-fixation. Although the movement of diazotroph nitrogen into the food web is not surprising, the trophic linkages and pathways involved are not yet well understood.

The AMT data set provides clear evidence for both spatial and temporal changes in $\delta^{15}N$ on a broad scale in the Atlantic. In this context, it's worth noting that a recent study carried out at the Bermuda Atlantic Time Series (BATS) station has shown a limited role for N_2-fixation in the annual nitrogen budget there (Knap *et al.*, 2005), suggesting that this process may be less important in the northern portions of the Subtropical Atlantic. This is in sharp contrast to findings at the Hawaii Ocean Timeseries (HOT) station, which has produced the longest and most detailed time series of upper ocean $\delta^{15}N$ available to date. At the HOT station, Dore *et al.* (2002) have measured the $\delta^{15}N$ of sinking particles for a period extending from 1989 to 2001. Over this interval, the $\delta^{15}N$ of sinking organic matter at HOT varied seasonally and the twelve year record shows a clear secular trend of decreasing $\delta^{15}N$ with time (Dore *et al.*, 2002). A mass balance calculation allowed Dore *et al.* (2002) to estimate that N_2-fixation accounted for about half of the total export production at Station ALOHA over this time period, with the remainder supported by NO_3^- injected into the mixed layer from below (see Karl *et al.*, Chapter 16, this volume).

4.3. Impact of denitrification on water column nitrogen

Denitrification is the largest loss term for oceanic nitrogen and plays a key role in regulating the availability of combined nitrogen in the ocean on long time scales (Codispoti *et al.,* 2001; Codispoti, 2007) (see Devol, Chapter 6, this volume). While sedimentary denitrification is unlikely to alter the $\delta^{15}N$ of oceanic NO_3^- (Fig. 29.3), pelagic denitrification plays a central role in the isotopic balance of the ocean since it does not typically go to completion and therefore produces residual NO_3^- with an elevated $\delta^{15}N$ (Fig. 29.3 and Section 2.3.1).

Both NO_3^- and particles in and near the major oceanic denitrifying regions have a $\delta^{15}N$ that is high relative to typical open ocean values (Brandes *et al.,* 1998; Cline and Richards, 1972, 1975; Liu and Kaplan, 1989; Sutka *et al.,* 2004; Voss *et al.,* 2001). Within the oxygen minimum zones (OMZs) proper, the $\delta^{15}N$ of NO_3^- varies with the NO_3^- deficit, frequently approaching 20‰ in the core of the OMZ (Brandes *et al.,* 1998; Cline and Kaplan, 1975; Voss *et al.,* 2001). This elevated $\delta^{15}N$ signature can propagate into suspended particles in the water column, presumably through vertical mixing and injection of ^{15}N-enriched NO_3^- into the surface mixed layer, where it can in turn be consumed by primary producers (Voss *et al.,* 2001; Montoya and Voss, 2006). Although the further movement of this isotopic signature into animals has not been investigated in detail, this strong isotopic signal may provide a useful tool for investigating food web dynamics in and near the productive waters above the major OMZs in the world ocean.

The high $\delta^{15}N$ generated within the major oceanic OMZs can also propagate substantial distances horizontally through advective processes. For example, the high $\delta^{15}N$ generated in the Eastern Tropical North Pacific may affect ecosystems hundreds or thousands of kilometres away from the OMZ proper (Liu and Kaplan, 1989; Montoya and Voss, 2006; Saino and Hattori, 1987; Sutka *et al.,* 2004). The location of the major OMZs either adjacent to (Eastern Tropical Pacific) or within (Arabian Sea) oligotrophic regions where N_2-fixation can play a large role in supporting primary production creates a very strong regional isotopic contrast. This is particularly notable in the Arabian Sea, where dense blooms of *Trichodesmium* occur directly above a major OMZ (Capone *et al.,* 1998), creating very strong isotopic contrasts between the surface layer and the waters below (Montoya and Voss, 2006). These isotopic gradients provide what are in effect large scale tracer experiments that can be exploited in studies of the nitrogen cycle on a basin scale.

5. CURRENT CHALLENGES AND OPPORTUNITIES

Nitrogen isotope measurements are becoming increasingly accessible to the oceanographic community and large field programs (e.g., HOT and AMT) now routinely include the $\delta^{15}N$ of particulate organic matter as a core measurement. The two examples discussed above have generated the broadest spatial (AMT program) and temporal (HOT program) coverage of nitrogen isotopic variation in the ocean.

Extending these studies to other regions and other components of marine ecosystems, including the pelagic food web and microbial community, is an ongoing challenge. Several specific examples of open questions and opportunities for research in nitrogen isotope biogeochemistry follow.

5.1. Isotope budgets

Animals show a consistent enrichment in ^{15}N relative to their food, but the factors and processes that generate this trophic effect are not yet well understood. I argue above (Section 2.3.3) that the magnitude of the trophic effect should depend on the partitioning of nitrogen between growth and catabolism in individual animals. Stable isotope measurements may thus provide a novel tool for quantifying the net growth efficiency of animals in the field, but this application will require a more detailed understanding of the ways that animals fractionate the nitrogen isotopes. A complete isotope budget for a representative zooplankter would provide the theoretical framework for understanding and interpreting the trophic effect quantitatively. In addition, a mechanistic understanding of isotope fractionation in animals will greatly facilitate the interpretation of temporal variations in δ^{15}N associated with animal growth. Both of these applications would allow use of stable isotope measurements in studies of trophic structure and energy flow in marine ecosystems.

On a broader scale, ecosystem–level isotope budgets can provide insight into the major fluxes of nitrogen in and out of the ocean. For the ocean as a whole, the overall δ^{15}N of combined nitrogen must be strongly affected by the balance between N_2-fixation and pelagic denitrification since these two processes have opposing effects on both the nitrogen content and isotopic composition of oceanic nitrogen. Brandes and Devol (2002) have used an oceanic nitrogen isotope budget to estimate a global rate of sedimentary denitrification on the order of 280 Tg N year^{-1}. For the oceans to be at or near steady state, this high a loss rate would require an oceanic N_2-fixation rate several times greater than even the highest among current estimates (Capone *et al.,* 2005; Lee *et al.,* 2002; Montoya *et al.,* 2004). While the ocean may indeed be out of steady state today, a flux imbalance of the magnitude implied by this isotope budget could not be sustained for long without significantly reducing the overall oceanic inventory of combined nitrogen (Brandes and Devol, 2002). The isotope budgeting approach is sensitive to the δ^{15}N of both NO_3^- in the ocean and the N_2 produced through denitrification. Increased spatial coverage of δ^{15}N measurements, particularly in and near the major denitrifying zones will be important in refining oceanic isotope budgets and improving the flux estimates they produce. Similarly, direct experimental measurement of the δ^{15}N of nitrogen lost through denitrification will be very important in resolving the current state of the oceanic nitrogen budget, particularly the magnitude of denitrification removing nitrogen from the ocean.

5.2. Analytical advances

Automation and reduced sample size requirements are both important factors in making stable isotope measurements practical in oceanographic studies. New generation instrumentation is compact, often modular in design, and contains relatively

few mechanical parts (e.g., the Isoprime). In addition to the increased availability and flexibility of isotope ratio mass spectrometers, the variety of specialized, automated systems for sample preparation is expanding. Although isotope ratio mass spectrometers still require too much fine-tuning and care to be treated as turnkey systems, it is becoming increasingly feasible for an individual investigator to acquire a dedicated system for his/her lab group. Stable nitrogen isotope measurements are well on their way to being a routine, if not always straightforward, analytic tool in ocean science.

A number of recent developments have extended the range of environments where stable isotope budgets can be applied to studies of the nitrogen cycle. For example, the ''denitrifier'' method for extracting nitrate for isotopic analysis has greatly reduced the quantity of nitrate required for analysis (Sigman *et al.,* 2001) and has allowed measurement of the $\delta^{15}N$ of nitrate in profiles extending into the upper part of the nitracline (e.g., Knapp *et al.,* 2005). By combining this approach with a persulfate oxidation step, Knapp *et al.* (2005) have been able to measure the $\delta^{15}N$ of both nitrate and dissolved organic nitrogen in the upper water column at the Bermuda Atlantic Time Series site. Taken together, these methodological developments hold great promise for ongoing efforts to use nitrogen isotope budgets in constraining the magnitude of processes such as N_2-fixation and denitrification.

Our current knowledge of the isotopic systematics of marine systems is largely based on analysis of bulk samples of organic and inorganic nitrogen. Measurements of the carbon isotopic composition ($\delta^{13}C$) of specific biomarker molecules has been very important in paleoceanographic and carbon cycle studies, but the relatively low abundance of nitrogen relative to carbon and the analytical challenges inherent in isolating and analyzing nitrogenous compounds have both slowed the use of this biomarker approach in nitrogen cycle studies. In recent years, methodological advances have made it feasible to measure the $\delta^{15}N$ of a spectrum of amino acids isolated from relatively small samples of organic matter (Macko and Uhle, 1997; Metges *et al.,* 1996; Metges and Petzke, 1997). Lab and field studies with marine zooplankton have shown that the $\delta^{15}N$ of individual amino acids varies systematically within animals with a much larger dynamic range than is typically seen for bulk organic mattter (McClelland *et al.,* 2003; McClelland and Montoya, 2002). Interestingly, some amino acids (e.g., phenylalanine) provide a record of the $\delta^{15}N$ of the primary producers at the base of the food web, while others (e.g., glutamic acid) show a strong trophic effect (McClelland and Montoya, 2002). This means that the tissues of an animal contain isotopic information on both the sources of nitrogen supporting biological production and the overall trophic distance (or nitrogen transfer efficiency) between the primary producers and the animal sampled. McCarthy *et al.* have extended this approach to sedimenting organic matter and have found that the variance in $\delta^{15}N$ among different amino acids appears to provide additional information on the type of heterotroph responsible for consuming and reworking sinking organic matter (McCarthy *et al.,* 2007). Sample preparation for compound-specific isotope analysis is still quite complex, as is the gas chromatography/mass spectrometry analysis itself, but this biomarker approach shows great promise for extending the range of processes and rates that can be assessed with stable isotope measurements.

5.3. Conclusions

Stable isotope natural abundance measurements have the great advantage of providing information on nitrogen cycle processes with little or no manipulation of the system, in contrast to tracer-based rate measurements (see Lipschultz, Chapter 31, this volume). The natural abundance and tracer approaches are strongly complementary, providing insight into the nitrogen cycle on very different spatial and temporal scales. Nonetheless, a number of important caveats must be considered in any application of stable isotope natural abundance measurements:

1. Stable isotope measurements are useful in resolving the contribution of isotopically distinct sources of nitrogen entering a system, but both spatial and temporal variation in the isotopic composition of those sources must be considered and factored into the study design.
2. The magnitude of processes that have distinctive isotopic fractionation patterns can potentially be quantified if the fractionations are known accurately. There is a significant ongoing need for careful measurement of isotopic fractionation patterns under controlled laboratory conditions as well as under field conditions.
3. The openness of the system and the nature of its exchanges with the outside world must be known in order to interpret isotopic distributions and trends and to construct meaningful isotopic budgets.

With these caveats in mind, natural abundance measurements can provide an inherently integrative measure of nitrogen cycle processes while effectively sampling the ocean on a time scale defined by the turnover time of the pool of interest. The increased availability of the necessary instrumentation coupled with reductions in the size of sample required for analysis now make it feasible to include nitrogen stable isotope measurements in virtually any field program. Recent advances in compound specific nitrogen isotope analyses show great promise for adding an additional level of detail to our knowledge of the nitrogen cycle.

At this point, we have a reasonably good general idea of the characteristic spatial and temporal patterns of variation in the $\delta^{15}N$ of the major oceanic pools of nitrogen, but we still lack a complete theoretical framework for interpreting stable isotope measurements. There is a fundamental need for basic laboratory studies focused on the pathways and processes that generate the clear patterns we see in the $\delta^{15}N$ of marine nitrogen.

The growing set of $\delta^{15}N$ measurements make it increasingly feasible and interesting to incorporate isotopic constraints into ecosystem and biogeochemical models of nitrogen in the ocean. This is both a sign of maturity in the field and a reason to take stock of our sampling and analytical practices. Potential differences among labs clearly need to be considered when combining data sets for inclusion in basin or global scale models, but I am not aware of any systematic effort to intercalibrate measurements made by different groups working on the isotope biogeochemistry of oceanic nitrogen. In a similar vein, our current knowledge of the $\delta^{15}N$ of marine nitrogen is strongly biased toward bulk particulate organic matter collected in the North Atlantic, at HOT, and in a few other regions of the ocean (e.g., the Eastern

Tropical North Pacific and the Arabian Sea). A robust isotope budget for the ocean will require extension of these sampling efforts to other waters.

Time series of measurements from a variety of locations will also greatly enhance the utility of nitrogen isotope measurements, particularly in quantifying the flux of nitrogen through the biota during blooms and other transient events. These are effectively natural tracer experiments created by isotopic fractionation during phytoplankton consumption of inorganic nitrogen and provide an ecosystem-level analog to the short-term tracer experiments typically carried out in small volumes aboard ship. Complementing nitrogen stable isotope measurements with focused rate measurements may ultimately provide the best strategy for studying the dynamics of the nitrogen cycle on both short and long temporal and spatial scales.

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Molecular Approaches to the Nitrogen Cycle

Bethany D. Jenkins *and* Jonathan P. Zehr

Contents

1. Introduction

Nitrogen (N) is a key element whose availability regulates the productivity of marine and terrestrial ecosystems. Nitrogen availability is controlled by biogeochemical transformations, many of which are catalyzed only by microorganisms. Although the concentrations of nitrogenous compounds and rates of transformations can be measured using various methods, the key to understanding nitrogen cycling in the marine environment is linking the abundance and activity of specific microorganisms to observed patterns and rates of individual processes. In the past 20 years, the application and development of molecular, genetic, and genomic approaches

have provided the means to examine microorganisms involved in the nitrogen cycle, and to investigate the factors that control the rates of transformations, through mechanisms from the level of gene expression to enzyme activity (Zehr and Ward, 2002).

There are many ways molecular biological approaches contribute to our understanding of the nitrogen cycle:

- Molecular methods have proved powerful for delineating the remarkable diversity of uncharacterized organisms that contribute to various aspects of nitrogen cycling.
- Molecular biology provides a means for elucidating the ecological function of microorganisms, i.e. by relating temporal and spatial patterns in microbial distribution to N cycling processes.
- Molecular tools can be used to interrogate the physiological status of microorganisms that perform specific biogeochemical reactions, to evaluate whether those organisms are active, and to determine factors that control their growth and distribution. Ultimately it may be possible to link activity to models of nitrogen fluxes.

This chapter aims to provide an overview of studies of key protein coding genes, enzymes, and other functional markers, "functional gene" approaches, that have been used to dissect the contributions of uncultivated organisms to various nitrogen transformations. Since other chapters in this book contain detailed discussions of specific processes in the nitrogen cycle, we will focus our discussion at the beginning of this chapter to describe recent progress in the application of molecular methods to study the function of microbial organisms in the marine environment. We will then conclude by discussing how these methods are applied to studies of major components of the marine N cycle. The field of molecular biology is advancing so rapidly that there will likely be technological breakthroughs in the near future that are not discussed in this chapter. Therefore, this chapter is not meant to be exhaustive, but aims to highlight how molecular biological methods have illuminated our understanding of the marine nitrogen cycle to date.

2. RECENT ADVANCES IN MOLECULAR METHODS FOR STUDYING UNCULTIVATED MARINE MICROORGANISMS ACTIVE IN NITROGEN CYCLING

Advances in molecular biological techniques in the past two decades have demonstrated unequivocally that the diversity of organisms in the marine environment is poorly represented by extant organisms in culture collections. Molecular approaches are now essentially an integral part of many, if not most, studies of microbially mediated processes, as evidenced by other chapters in this book. Initial efforts applying molecular biology to studies of organisms in the marine environment used the polymerase chain reaction (PCR) to amplify ribosomal RNA (rRNA) genes from DNA extracted from natural samples (DeLong, 1992;

Fuhrman *et al.*, 1993; Giovannoni *et al.*, 1990). rRNA genes are widely used for sequence detection and establishing the relationship between organisms with phylogenetic analysis for several reasons: universal PCR primers have been developed that target all the rRNA genes in broad taxonomic groups (i.e. eubacteria, archaea, eukaryotes), the rRNA gene has evolved at a fairly constant rate and has not been subject to lateral gene transfer (Woese, 1987), and rRNA genes are often present in multiple copies, increasing detection limits. Phylogenetic information based on rRNA sequences sheds light on the physiological role of microorganisms in cases where biochemical potential is restricted to specific phylogenetic lineages. In many instances, biochemical capacity is dispersed in distantly related taxonomic groups and cannot be detected on the basis of rRNA gene sequences. In the case of the nitrogen cycle, this is true particularly for denitrifiers and nitrogen fixers. To better understand the diversity of biochemical potential in the environment, approaches have been developed to target genes with specific enzymatic or regulatory function, "functional genes" (Ward, 1996; Zehr and Capone, 1996; Zehr and McReynolds, 1989). Studies directed at functional genes also allow for detection of gene expression directly in the environment and identification of those microorganisms that may be active in N cycling processes. It has been argued that gene expression data can be correlated with a rate determining process such as rates of enzymatic activity (Corredor *et al.*, 2004). Although this may be the case in some instances, the link between gene expression and rates of a reaction catalyzed by an enzyme are governed by many processes, including the relationship between transcription and translation, and post-translational protein regulation. The expression of many genes is also subject to control by the activity of regulatory factors, nutrient availability, and time of day. For example, some genes are transcribed in a daily cycle, such as those encoding nitrogenase in cyanobacteria, where gene transcription often starts prior to the function of the protein (Chen *et al.*, 1997). A temporally changing magnitude of gene expression makes the correlation between enzyme rates and gene expression sensitive to the time of day that the RNA sample is collected and processed. Thus, measurements of gene expression are probably best used as a first order approach to identify microorganisms that are active in a process, or determine factors that regulate activity. Studies with cultivated organisms are critical for interpreting patterns of gene expression in different organisms and for identifying genes whose expression patterns are useful to target in the environment.

Most approaches analyzing the diversity of functional genes in the environment rely on the same basic techniques: the design and optimization of PCR primers that target the gene of interest followed by cloning and sequencing of the PCR product amplified from the environmental sample. Since the PCR product can represent a heterogeneous group of organisms, it is often necessary to sequence many clones to detect all the sequence types represented in any single sample. A very similar approach can be used to study the diversity of expressed genes in the environment by reverse transcribing RNA prior to PCR amplification (RT-PCR). Studies assaying the diversity of nitrogen cycling genes continue to demonstrate a remarkably high diversity in natural populations, and that the bulk of microorganisms involved in the nitrogen cycle have yet to be cultivated. Although libraries of

sequence data from the environment are informative for diversity studies, they can be time consuming and costly to generate, limiting throughput.

In the last decade, new tools have been developed to assess species diversity and distribution without extensive cloning and sequencing. Denaturing Gradient Gel Electrophoresis (DGGE) (Piceno and Lovell, 2000) resolves differences in bulk PCR products amplified from environmental samples based on melting temperature differences between individual sequences in the PCR products. The complexity of a DGGE pattern can be used to infer diversity and profiles can be compared between samples. Although it can be difficult to assign a taxonomic relationship between DGGE patterns, it is possible to excise bands from a DGGE gel, clone and sequence them to identify their origin. rRNA sequence diversity in mixed-community DNA can be analyzed with automated rRNA intergenic spacer analysis (ARISA), which amplifies 16S–23S ribosomal DNA internally transcribed spacer regions and detects the separated products on a fragment analyzer (Fisher and Triplett, 1999; Hewson and Fuhrman, 2004). Restriction fragment length polymorphism (RFLP) is another way to compare sequence heterogeneity in PCR products from natural communities on the basis of differences in restriction enzyme digestion patterns and their resolution by size using gel electrophoresis. Terminal restriction length polymorphism (T-RFLP) (Horz et al., 2000; Moeseneder et al., 1999; Scala and Kerkhof, 2000) is a similar method whereby one of the PCR primers is labeled with a fluorophore and the resulting fragment pattern is resolved by using a DNA sequencer. Similar to DGGE, these fragment analysis methods work well for comparing relative sequence complexity and patterns of complexity between samples. These methods can also be used to assign a phylogenetic relationship between patterns of fragments in instances where different restriction enzymes discriminate well between different taxonomic groups of sequences. This is not always the case, and it can sometimes be difficult to interpret T-RFLP patterns in terms of sequence phylogeny. A database of sequence information is invaluable in interpreting whether or not T-RFLP patterns are phylogenetically coherent.

More recently, methods using DNA array technology have been developed to profile gene sequence diversity and "fingerprint" the taxonomic relationships between genes sequences derived from the environment (Jenkins et al., 2004; Moisander et al., 2006, 2007; Steward et al., 2004; Taroncher-Oldenburg et al., 2003; Tiquia et al., 2004, 2006; Wu et al., 2001). DNA arrays, either macroarrays (membranes) or microarrays (glass slides) are spotted with oligonucleotides or amplified gene sequences. The arrays are hybridized with labeled DNA, commonly an amplified PCR product from an environmental sample. Each array spot will bind to DNA in the hybridization pool with varying degrees of sequence similarity depending on the stringency of hybridization (Fig. 30.1). The signal at each spot not only contains sequences that are an identical match to the array probe but also other sequences that are a close match to the array probe (Moisander et al., 2006, 2007; Steward et al., 2004; Taroncher-Oldenburg et al., 2003). Therefore, relative abundances of different sequence types may not necessarily be inferred from the hybridization intensity of individual spots but the hybridization patterns are a useful phylogenetic comparison between samples (Jenkins et al., 2004; Moisander et al., 2006). A limitation of hybridization-based methods, such as gene arrays, is that probe
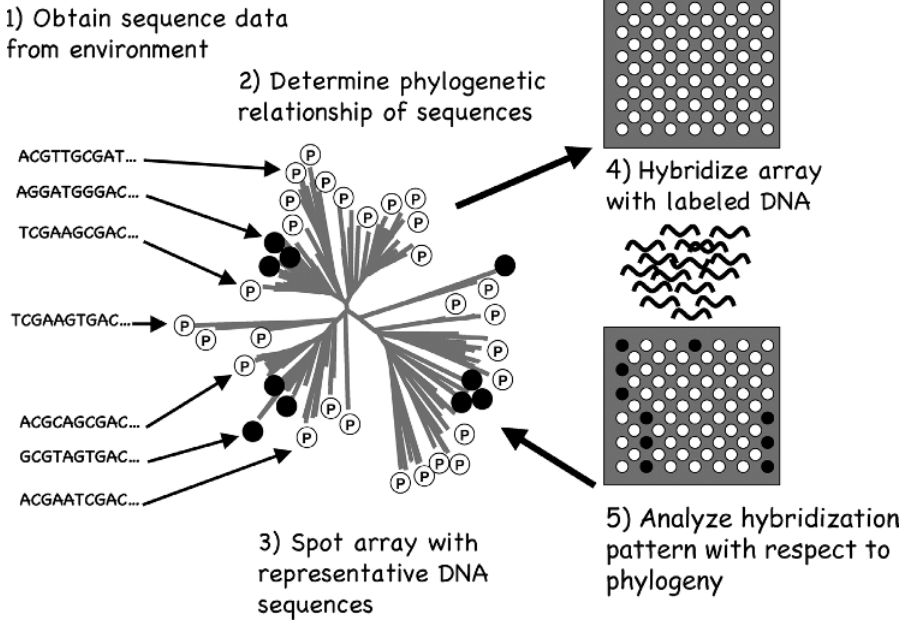
Figure 30.1 DNA array methodology to study the presence of sequence types in the environment. These experiments are based on previous knowledge of the sequence types in the area of interest (1) and their phylogenetic relationships (2). Arrays are spotted with DNA or oligonucleotide sequences of interest, indicated with a P (3) and hybridized with labeled DNA or cDNA (RNA copy) (4). The hybridization pattern is interpreted in terms of the phylogenetic relationship of the array probes with signal (5).

development depends upon *a priori* knowledge of sequence types likely to be present in the environment being assayed. This can require a large sequence database prior to array construction, since diversity can be high in many environments. Probes can also cross-hybridize to varying extents, depending upon sequence relatedness and binding specificities. Thus, all the array approaches require "ground-truth" sequence data in order to be appropriately interpreted.

To date, all of the methods discussed above rely on the amplification of DNA or RNA from an environmental sample, typically by PCR. PCR amplification can be biased (Becker *et al.*, 2000; Lueders and Friedrich, 2003; Speksnijder *et al.*, 2001; Suzuki *et al.*, 1998; Webster *et al.*, 2003). It is possible to eliminate an amplification step with array technology (Peplies *et al.*, 2003; Taroncher-Oldenburg *et al.*, 2003; Tiquia *et al.*, 2004), but sensitivity can be an issue when assessing functional gene sequences in the environment because they are often less abundant than rRNA sequences and specific taxonomic groups are only a fraction of the total biomass present.

The advent of quantitative PCR techniques makes it possible to quantify the abundance and expression of a specific gene and closely related sequences, or the abundances of taxonomic groups depending on the specificity of quantitative PCR

primer design (Short *et al.*, 2004; Wawrik *et al.*, 2002). A variety of quantitative PCR methods have been used to determine the abundance of sequence types in different aquatic environments including SYBR green dye binding (Lopez-Gutierrez *et al.*, 2004; Wawrik *et al.*, 2002), and the use of fluorogenic Taqman® probes (Church *et al.*, 2005a; Foster *et al.*, 2007; Harms *et al.*, 2003; Short *et al.*, 2004) and molecular beacons (Harms *et al.*, 2003). Design and optimization of quantitative PCR techniques also requires a database of sequence information to determine the specificity of primer design. For example, if a gene is highly conserved, it can be difficult to design primers specific to an individual sequence type because primers may amplify other sequences, sometimes even those that are distantly related. Quantitative PCR can be used to estimate abundances of organisms (Harms *et al.*, 2003) in the environment when copy number of a target gene is known and primers specific to the organism of interest are used. Quantitative PCR is also a very powerful technique to compare gene expression between samples (Wawrik *et al.*, 2002), and correlate gene expression with gene copy number (Church *et al.*, 2005a).

Numerous techniques have been used to visualize cells on the basis of recognition of specific molecular sequences. To date, most of these approaches target 16S rRNA since it is abundant in cells and there are large sequence databases of rRNA sequences from cultivated organisms and the environment. Oligonucleotides corresponding to rRNA sequences obtained from the environment can be used for visualizing and enumerating cells *in situ* (Amann *et al.*, 1995). Most of these detection techniques rely on fluorescently labeled DNA probes that are hybridized directly to cell preparations (fluorescent *in situ* hybridization, or FISH). The fluorescing cells are identified by recognition of the hybridizing probe DNA sequence and visualized by microscopy (Amann *et al.*, 1995; Pernthaler *et al.*, 2003) or flow cytometry (Sekar *et al.*, 2004). Therefore, these approaches work well to enumerate microorganisms that function in the nitrogen cycle for which the ribosomal RNA sequences are known, but do not work for uncultivated microorganisms where the rRNA phylogeny is not known. This is the case with N_2 fixing microorganisms, for example, since nitrogen fixation genes are dispersed amongst closely related phyla. In instances where rRNA phylogeny is not a useful indicator of biological function, visualization procedures are dependent upon hybridization to genes or transcripts of functional genes. Functional genes are constrained in this approach by the fact that mRNA transcript targets are lower in copy number than ribosomal RNA. Recent advances in FISH technology indicate that it is possible to detect mRNA of functional genes (Pernthaler and Amann, 2004), but this technique has yet to be applied to genes in the nitrogen cycle.

Molecular microbial ecology methods are being developed that link species identification with activity, typically combining molecular identification methods with isotope labeling. For a comprehensive review of these methods, see Neufeld *et al.* (2007). One of these approaches combines isotope labeling followed by microautoradiography and hybridization techniques such as FISH (FISH–MAR) and microarrays (isotope arrays). FISH–MAR has been done with ^{3}H labeled substrates (Cottrell and Kirchman, 2000; Lee *et al.*, 1999; Ouverney and Fuhrman, 1999), ^{14}C labeled substrates (Lee *et al.*, 1999), and [33]Pi labeled substrates (Lee *et al.*, 1999). Cottrell and Kirchman incubated estuarine water with ^{3}H labeled amino acids, proteins,

N-acetylglucosamine, and chitin and used MICRO-FISH to study bacterial community members actively using these dissolved organic nitrogen (DON) sources. Adamczyk et al. (2003) extracted RNA from nitrifying sludge samples labeled with ^{14}C bicarbonate and hybridized the RNA to a 16S oligonucleotide microarray to identify ammonia-oxidizers that were actively fixing CO_2. Unfortunately, the only usable radioactive isotope of N is ^{13}N, whose 9.96-min half-life precludes use in these applications.

Stable isotope probing (SIP) combines isotope labeling with density gradient centrifugation to separate nucleic acids corresponding to organisms by actively incorporating the label into biomass (recently reviewed in Friedrich, 2006 and Neufeld et al., 2007). Most SIP methods involve incubation of a microbial community with a ^{13}C labeled substrate followed by isolation of the ^{13}C labeled DNA (Radajewski et al., 2003). The labeled DNA can then be assayed by normal methods (PCR, cloning, sequencing) to determine the phylogenetic affiliation of organisms that used the ^{13}C labeled substrate. For example, this method has been used to identify autotrophic ammonia-oxidizers in freshwater sediments (Whitby et al., 2001). Successful separation of DNA following a stable isotope pulse is dependent on at least 15–20% incorporation of ^{13}C before it can be isolated by buoyant density (Radajewski et al., 2000). Therefore, the success of this technique depends on a high replication rate in the community being studied so that de novo DNA synthesis occurs and label is incorporated. To circumvent this problem, SIP methods have also been developed for RNA which has a higher turnover rate compared with DNA. As in DNA-SIP, RNA is separated by gradient centrifugation followed by RT-PCR of the isotopically enriched RNA (Manefield et al., 2002a,b). Techniques such as FISH, FISH-MAR, and even genome sequencing can then be used to identify microorganisms in each RNA fraction. For example, Ginige et al. (2004) used ^{13}C methanol as a substrate in a batch reactor and combined SIP, FISH, and microautoradiography to identify active anaerobic denitrifiers. The most recent SIP applications have been developed for use with ^{15}N labeled compounds to study identify active diazotrophs in soils (Buckley et al., 2007) and hold promise for the study of N cycling microorganisms in marine environments. SIP methods using ^{15}N incorporation are challenged by the need for a high amount of isotope incorporation and good nucleic acid separation by density gradient centrifugation (Buckley et al., 2007). Other new means to link metabolic activity and phylogenetic identification of individual cells in complex microbial communities combine rRNA based in situ hybridization methods with stable isotope imaging techniques. Orphan et al. (2001) pioneered the use of FISH with secondary-ion mass spectrometry (SIMS) to identify methane-consuming Archaea in anoxic marine sediments. The resolution of SIMS and sensitivity of micro-autoradiography has been improved by the use of nanometer-scale SIMS (NanoSIMS) to study the fate of ^{13}C and ^{15}N labeled substrates in microbial communities. Behrens et al. (2008) evaluated NanoSIMS for studying a simple consortium consisting of a filamentous cyanobacterium and a heterotrophic α-Proteobacterium in complex microbial aggregates obtained from human oral biofilms. Raman microscopy can also be used to follow stable isotope incorporation into cells and was coupled with FISH by Huang et al. (2007) to identify Pseudomonas populations active in degrading ^{13}C-napthalene in groundwater microcosm experiments. In a similar experiment, Neufeld et al. (2008) identified methylotrophic communities in surface water samples by cloning and sequencing high

molecular weight DNA generated from a genomic DNA amplification step after isolation via gradient centrifugation. Continued development of these exciting new methods will help us not only identify the metabolically active consortia in marine systems but also indicate preferred substrates for their activity.

Methods have also been developed to use oligonucleotides to capture RNA of specific phylogenetic groups and measure natural abundances of carbon isotopes of the captured RNA. MacGregor *et al.* (2006) used streptavidin–coated magnetic beads and biotinylated oligonucleotides to capture rRNA from North Sea sediment cores incubated with ^{13}C–labeled acetate, proionate, amino acids or glucose and measured the natural abundances of the carbon isotopes of the captured RNA. Pearson *et al.* (2008) used a similar method to investigate bacterial incorporation of petroleum hydrocarbons from a simulated oil spill on a salt marsh ecosystem to identify bacteria active in the remineralization of hydrocarbons.

A ''full cycle'' molecular approach directed at functional genes in the nitrogen cycle can be devised that combines many of the methods described above. It begins with the extraction of nucleic acids from the environment (or isotopically labeled nucleic acids), followed by the identification of specific sequences types, the use of sequence data to design amplification (e.g. PCR, quantitative PCR) or hybridization (e.g. FISH, DNA arrays) approaches for probing species diversity in the environment, and the use of these methods in the environment to determine the autecology and gene regulation of individual genotypes, species, or strains (Fig. 30.2).

3. The Power of Genomics to Reveal Metabolic Potential

Genome sequencing provides us with the ability to ascertain the genetic capacity of organisms to transport and metabolize various nitrogen sources and understand nitrogen metabolism in the context of other metabolic pathways present in the organism. Genome sequencing can also explain different niches inhabited by different organisms in the marine environment. For example, the recently completed genome sequencing of a number of marine unicellular cyanobacteria allows us to compare these closely related (96% identical rRNA sequences) phytoplankton in terms of their potential to compete for and utilize different nitrogen sources. In marine *Synechococcus* strains, genes are present for transporting and assimilating nitrate and nitrite (Palenik *et al.*, 2003). Genome sequences from *Prochlorococcus* strains (MIT9303, MIT9312, MIT9313, MIT9515, NATL1A, AS9601, CCMP1986, CCMP1375, MED4, SS120) show an absence of nitrate reductase (*narB*) genes. The genomic data confirms studies showing that cultivated *Prochlorococcus* strains lack the ability to assimilate nitrate (Dufresne *et al.*, 2003; Rocap *et al.*, 2003a). Some Prochlorophytes cannot assimilate nitrite, although several strains adapted to low–light regimes can grow on nitrite (Moore *et al.*, 2002). Genome sequencing shows that several low–light adapted *Prochlorococcus* strains (MIT9313, MIT9211, NATL2A) have retained the assimilatory nitrite reductase gene (*nirA*), which suggests that some strains are capable of exploiting the nitrite maximum found at the base of the photic zone (Rocap *et al.*, 2003a).
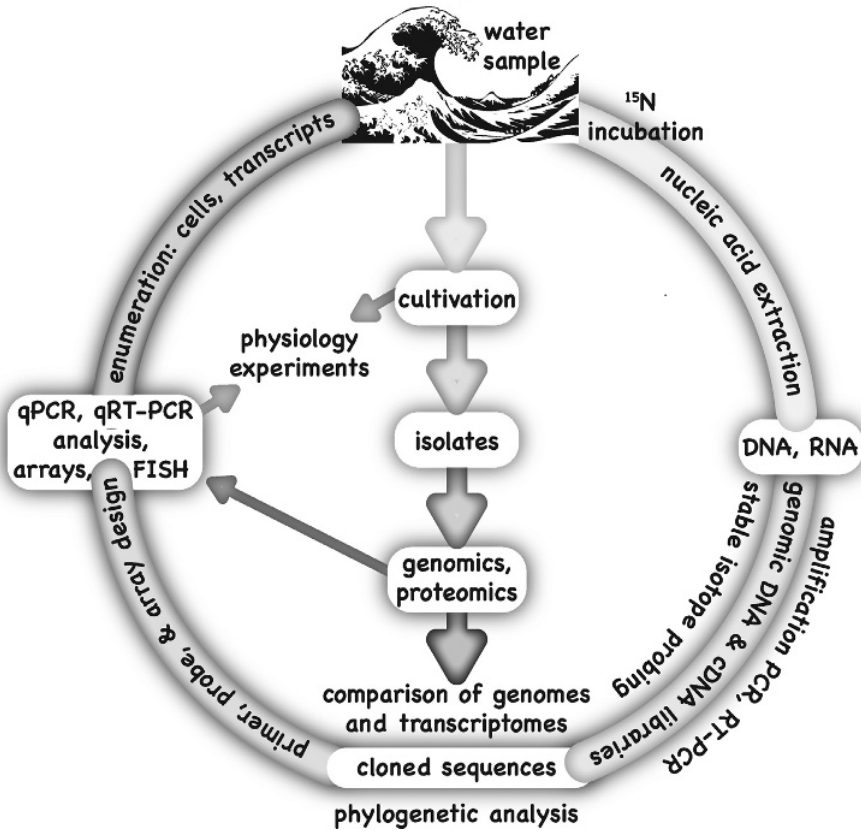
Figure 30.2 Molecular strategies based on nucleic acid analysis to study microbes involved in the nitrogen cycle. Grey arrows show a "full cycle" analysis where DNA sequences extracted from the environment are used to construct probes for detection of organisms directly by hybridization-based assays (FISH; arrays) or PCR based methodology. The "closure" of the circle is represented by arrows pointing back to the water sample to indicate the probes can be used again to enumerate organisms or gene expression in the environment. Other arrows indicate methods such as cultivation and laboratory experiments that are useful for interpreting gene expression data in environmental samples.

Tolonen *et al.* (2006) used whole genome microarrays to examine the response of the high-light adapted *Prochlorococcus* strain MED4 and the low-light adapted strain MIT9313 to nitrogen starvation. Although both strains had elevated expression levels of many genes involved in nitrogen sensing and transport, the strains differ in expression patterns of carbon metabolism genes (Tolonen *et al.*, 2006). These findings in *Prochlorococcus* strains, linking the ability to use different nitrogen sources to adaptation to the light field, suggest an evolution of genomes in response to the habitats found in the vertical structure of the surface waters of the ocean, and more importantly suggest a genetic basis for variability in the use of "regenerated" and "new" nitrogen.

Genome sequencing also reveals other differences in utilization of nitrogen sources between cyanobacteria (see also Chapter 24, Scanlan and Post, this volume).

Some *Prochlorococcus* strains can assimilate urea and some can assimilate cyanate, whereas some strains (e.g. SS120) only appear to utilize ammonium. Genome sequencing also shows that *Synechococcus* sp. WH8102 is a transport generalist relative to *Prochlorococcus* strains and can utilize organic N sources (Palenik *et al*., 2003). The recently completed sequencing of the N_2 fixing cyanobacteria *Crocosphaera watsonii* and *Trichodesmium erythraeum* should reveal information regarding the different strategies these cyanobacteria employ to fix nitrogen. *Crocosphaera* is a unicellular diazotroph that separates oxygenic photosynthesis and N_2 fixation by fixing N at night. *Trichodesmium* fixes N_2 during the day when it is photosynthetically active.

The genome sequence of heterotrophic *Silicibacter pomeroyi*, a member of the marine *Roseobacter* clade that is abundant in surface waters, contains high numbers of peptide and amino acid transporters as well as the transporters and assimilatory genes for ammonium and urea (Moran *et al*., 2004). The genome is lacking genes for the transport and assimilation of other inorganic nitrogen sources (Moran *et al*., 2004). Therefore, the nutrients *Silicibacter* can utilize are consistent with a lifestyle in the surface ocean where other N sources are not abundant.

Genomics can also reveal unexpected biochemical potential. For example, the genome sequencing of the diatom *Thalassiosira pseudonana* revealed genes encoding all the enzymes in a complete urea cycle, which is unprecedented in other eukaryotic photoautotrophs. It is hypothesized that intermediates from the urea cycle may be used to synthesize polyamines including those required for frustule formation (Armbrust *et al*., 2004).

Genomic technology has also been employed to obtain sequence information from large fragments of DNA from uncultivated marine microbes. High molecular weight DNA from seawater can be cloned as large fragments into bacterial artificial chromosomes (BACS) (Zeidner *et al*., 2003) and fosmids (Beja *et al*., 2000; Stein *et al*., 1996). Fosmid and BAC libraries can be screened for genes of interest (either for 16S or functional genes). Complete sequencing of BACs and fosmids can reveal novel biochemical potential present in marine bacterial populations such as a light-driven proton pumps encoded by proteorhodopsin (Beja *et al*., 2001) and the presence of enzymatic pathways consistent with reverse methanogenesis in bacteria from anoxic sediments (Hallam *et al*., 2004). Screening BAC and fosmid libraries should prove fruitful for identifying genomic regions associated with functional genes involved in nitrogen transformations or discovering more information about uncultivated microbes such as planctomycetes that have recently been shown to perform novel nitrogen transformations (e.g. Annamox). BAC and fosmid library data can be used to compare genomic organization of individual genes between closely related organisms (gene synteny) and to compare the diversity within populations (Fig. 30.2). Improvements in sequencing throughput and sequence assembly algorithms allow for whole genomes to be assembled from consortia of complex microbial populations, "metagenomics". Venter *et al*. (2004) pioneered the use metagenomics to analyze microbial communities in Sargasso Sea surface water (Venter *et al*., 2004). The staggering size of the database from the Venter *et al*. (2004) project (over one billion base pairs of DNA sequence) and the identification of over a million new genes in 2000 L of sea water highlights the genetic diversity in marine ecosystems. This work has been extended as part of the J. Craig Venter Institute's

Global Ocean Sampling Expedition (Rusch *et al.*, 2007). The resulting database is available to the public (Seshadri *et al.*, 2007) and has identified 6.12 million predicted proteins from mainly surface water samples; doubling the number of known proteins and shows that we have yet to identify most metabolic potential in the environment (Yooseph *et al.*, 2007). Metagenomic methods have also been used for a global survey of marine viruses (Bench *et al.*, 2007; Angly *et al.*, 2006) and for studies of picoeukaryotic diversity (Massana *et al.*, 2008). Once caveat to direct shotgun cloning from the environment (Venter *et al.*, 2004) is that important microbes in the N cycle can escape detection. For example, diazotrophs may not be detected, since they are often present in low abundances relative to other prokaryotes. Libraries of expressed genes from the environment, ''metatranscriptomes'' are useful for identifying active genes and can show pathways of nitrogen acquisition (Frias-Lopez *et al.*, 2008; Poretsky *et al.*, 2005) and also provide exciting information regarding where and when genes are active in the environment.

Metagenomic methods are also powerful ways to assemble genomes of organisms that dominate enrichment cultures, but have yet to be isolated in pure culture. The genome of the uncultured anammox bacterium *Kuenenia stuttgartiensis* was assembled this way from bioreactor consortia and many new genes likely involved in the anammox pathway were identified (Strous *et al.*, 2006). Metagenomic data can also be analyzed to look at evolutionary relationships between organisms in different marine environments. Zehr *et al.* (2007) analyzed metagenomic data from the unicellular nitrogen fixing cyanobacteria, *Crocospheara watsonii*, and found extremely low genomic diversity in widely distributed populations.

In the coming years, we are likely to see an explosion of genome sequence data and genome sequencing methods coupled with other methods to assist in identification of target organisms. New sequencing technology will improve the throughput of whole genome shotgun sequencing projects (Goldberg *et al.*, 2006; Sogin *et al.*, 2006). Methods such as flow cytometry and whole genome amplification have been coupled in order to sequence genomes from single cells of marine microorganisms (Stepanauskas and Sieracki, 2007; Zhang *et al.*, 2006). Various gene expression profiling methods are being used to study the dynamics of organisms such as dinoflagellates and coccolithophores that are not represented by full genome sequences (Dyhrman *et al.*, 2006; Erdner and Anderson, 2006; Lidie *et al.*, 2005). Additional genome sequencing data will provide the foundation for the analysis of N fluxes in terms of whole genome responses on both the gene expression and proteomic level.

4. Nitrogen as a Nutrient

All organisms need to assimilate nitrogenous compounds for the biosynthesis of cellular material. Since nitrogen acquisition from the environment is largely mediated by active transport mechanisms (Falkowski, 1975) that obey Michaelis–Menton enzyme kinetics, variations in uptake kinetics among species (Eppley *et al.*, 1969) provide a fundamental mechanism for ecological competition. The uptake of inorganic nitrogen is also the basis of conceptual models of ''new'' and

''regenerated'' productivity (Dugdale and Goering, 1967), which couples nitrogen metabolism to the cycling and export of carbon (Eppley and Peterson, 1979). Although there are questions as to the relationship between productivity, nitrogen uptake, and export, measurements or estimates of nitrogen uptake are still often used to estimate export or ''new'' production (Armstrong, 1999; Laws *et al.*, 2000; McCarthy *et al.*, 1999; Yajnik and Sharada, 2003). Our understanding of nitrogen uptake and growth can be limited because these processes are often uncoupled in experiments measuring N incorporation rates. It is also difficult, if not impossible, to determine which organisms are responsible for observed N uptake. Furthermore, it is still a challenge to assess where and when N is a limiting nutrient for growth. The subject of N uptake and metabolism is extremely broad, and it is not our intent to review this subject comprehensively (we refer you to Chapter 7 by Mulholland and Lomas, this volume), but to provide examples of how molecular (and cellular) biological approaches can contribute to our understanding of nitrogen uptake in the sea.

5. Identifying Microorganisms Involved in Nitrogen Uptake

The biochemistry of nitrogen uptake involves membrane transport, assimilation (conversion of inorganic N into small organic metabolites), incorporation (synthesis of macromolecules) and catabolism (breakdown of macromolecules into small metabolites) (Frost *et al.*, 2005). Nitrogen uptake is typically studied by using isotopically labeled compounds (Lipschultz, 1995; and Chapter 31 this volume; Zehr *et al.*, 1989), but isotope uptake does not easily distinguish between transport, assimilation, and incorporation (Lomas, 2004). Rates of uptake are generally determined in bulk water, which makes it impossible to discern the contributions of individual microorganisms to N cycling. To overcome this problem, size fractionation methods are used to identify the major groups of microorganisms involved in N uptake (Wafar *et al.*, 2004). These experiments are often difficult to interpret because of the inefficiency of separating organisms with different functions, or even individual size classes, by filtration. For example, the potential contribution of heterotrophic bacteria to total community uptake is a major complication in interpreting inorganic nitrogen uptake data (Wheeler and Kirchman, 1986). Size fractionation experiments are also often used to separate larger phytoplankton from heterotrophic bacteria but these are confounded since the sizes of heterotrophic bacteria and smaller phototrophs, such as *Prochlorococcus,* overlap. Molecular methods can aid in the interpretation of size fractionation data since they can be used to identify species and populations in different size fractions, and more importantly, to assay the expression of functional (protein coding) genes to identify the major organisms involved in N assimilation in different size fractions.

It is clear that there are species level differences in the ability to use different nitrogen sources. Some of these differences can be explained by the simple presence or absence of key nitrogen assimilation genes. For example, some bacteria and

cyanobacteria cannot grow on nitrate or nitrite as their sole nitrogen source because they lack the necessary genes to assimilate these inorganic nitrogen sources. Some heterotrophic bacteria lack nitrate reductase genes necessary for the reduction of nitrate to ammonium (Allen *et al.*, 2002). Some marine *Synechococcus* strains cannot grow on nitrate as a sole nitrogen source (Moore *et al.*, 2002). A *Synechococcus* sp. (strain SH-94-5) isolated from a hot spring lacks both nitrate and nitrite reductase genes and cannot grow on nitrate or nitrite as a sole nitrogen source (Miller and Castenholz, 2001). Most strains of *Prochlorococcus* cannot grow on nitrate as a sole N source (Lopez-Lozano *et al.*, 2002; Moore *et al.*, 2002; Scanlan, 2003) and all sequenced *Prochlorococcus* genomes, to date, lack nitrate reductase (*narB*) genes (Dufresne *et al.*, 2003; Rocap *et al.*, 2003b). However, recent data from flow-cytometrically sorted *Prochlorococcus* populations from the Sargasso Sea show assimilation of significant fractions of nitrate; indicating that these organisms have yet to be cultivated (Casey *et al.*, 2007). *Prochlorococcus* also differ in their ability to assimilate nitrite. Some *Prochlorococcus* strains cannot assimilate nitrite (Moore *et al.*, 2002) and genome sequencing shows some *Prochlorococcus* to be lacking nitrate/nitrite transporters and nitrite reductase (*nirA*) genes (Dufresne *et al.*, 2003; Rocap *et al.*, 2003b). Therefore, the response of natural communities to different nitrogen sources partially reflects the genetics of the populations. Some species contribute to ''new'' production, and others do not, depending upon the presence of the genes for nitrogen assimilation. In many cases, this can only be determined by probing directly for the genes encoding the pathway of interest, such as nitrate reduction.

6. Nitrogen Uptake and Growth

Observed differences in nitrogen uptake between organisms can also be due to variations in genetic controls on each of the processes governing net uptake capacity. Experiments show that transport of inorganic nitrogen is more rapid than incorporation and assimilation (Fig. 30.3A; Zehr *et al.*, 1988; see also Chapter 7, Mulholland and Lomas, this volume) the latter processes controlled by the availability of cellular intermediates and the protein synthesis machinery (Fig. 30.3B). The difference between uptake/transport capacity and assimilation/incorporation results in ''enhanced nitrogen uptake'' that is related to growth rate and N status in phytoplankton (McCarthy and Goldman, 1979) and bacteria (Goldman and Dennett, 2000). Transport capability can exceed the ability of the intracellular machinery to assimilate N (Beardall *et al.*, 2001) if either the numbers of transporters increase with N limitation, or if the protein synthesizing machinery is regulated at a low growth rate. Over long time intervals, the uptake rate becomes equivalent to the assimilation rate, since the buildup of internal pools inhibits membrane transport, and the overall observed uptake rate becomes equal to the rates of assimilation and growth (Flynn, 1998; Glibert and Goldman, 1981; Goldman and Glibert, 1983). Nitrate uptake in excess of growth requirements may serve as an electron sink as well as a nitrogen source (Lomas and Glibert, 1999).
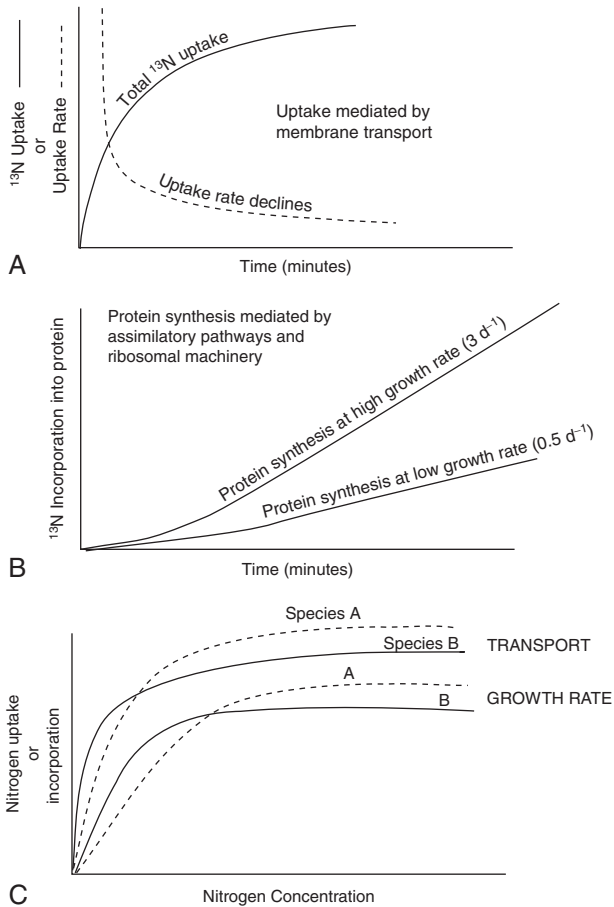
Figure 30.3 Difference between nitrogen uptake and growth kinetics from ^{13}N experiments, after Zehr *et al.* (1988). Short-term uptake kinetics (A) are driven by membrane transport processes, and usually exceed assimilation and incorporation into protein. Short-term uptake declines as feedback from internal pools and regulatory mechanisms are affected by influx of N. (B) Longer term N uptake is driven by protein synthesis rate, which is equivalent to the growth rate. (C) Model of competition between two species showing that competition can be affected by both transport and growth.

Some species have a high nitrate uptake capability that may provide a selective advantage in variable environments (Collos *et al.*, 1992; Fig. 30.3C).

Uptake capacity is dependent on the number and efficiency of membrane transport proteins. It is not really known how the numbers of transporters (e.g. ammonium or nitrate) per cell varies as a function of physiological state, or how the kinetics of transport may be a function of the protein structure of the transporters themselves. Some studies indicate that the number of transporters per cell increase with nitrogen limitation, but kinetic data suggests otherwise and that transporter numbers are more or less constant as a function of growth rate under N limitation

(Zehr *et al.*, 1988). This is an area where molecular biology and biochemistry will certainly provide new information for understanding the competition and coexistence of species. Relatively little is yet known about the functioning of ammonium, nitrate and nitrite transporters in marine microorganisms, but they have been genetically characterized in a variety of microorganisms, and tentatively identified in genomes of marine cyanobacteria and diatoms. Three ammonium transporters (*amt* genes) were described in the freshwater cyanobacterium *Synechocystis* PCC 6803, that varied in their uptake affinity (Montesinos *et al.*, 1998). Ammonium transporters were cloned from the diatom *Cylindrotheca fusiformis*, and were expressed most highly in N starved cells (Hildebrand, 2005). Although these proteins are related members of a gene family, one transporter is consistently expressed at much higher levels than others regardless of the N source (Hildebrand, 2005). Two nitrate transporter genes were also characterized in *C. fusiformis* and found to be regulated in response to nitrogen source (Hildebrand and Dahlin, 2000). Nitrate transporters have also been identified in several marine cyanobacteria and are nitrate/nitrate bi-specific permeases (*nrtP*) (Bird and Wyman, 2003; Wang *et al.*, 2000). The expression of *nrtP* was upregulated in the marine *Synechococcus* sp. WH 8103 in response to N starvation and nitrate addition and was more strongly repressed by ammonium in low-light conditions (Bird and Wyman, 2003). Many *Prochlorococcus* genes for nitrogen assimilation and transport are activated during N starvation (Tolonen *et al.*, 2006). Therefore, assaying the expression of nitrate transporters in diatoms and cyanobacteria can potentially identify conditions of N limitation or whether nitrate or ammonium are being used as nitrogen sources. Future experimental work holds much promise for determining how transporter abundance and activity are linked to the kinetics of cellular transport and possibly redox potential.

7. MOLECULAR CHARACTERIZATION OF NITROGEN ASSIMILATORY PATHWAYS

Molecular methods have been used extensively to study the activity of enzymes the major nodes of N assimilatory pathways in marine organisms. These enzymes include glutamine synthetase (ammonium assimilation), nitrate reductase (nitrate assimilation), urease (urea assimilation), and nitrogenase (nitrogen fixation), Table 30.1 (see also Berges and Mulholland, Chapter 32, this volume). The transcription of a plastid-localized glutamine synthetase (glnII) in the diatom *Skeletonema costatum* is induced to assimilate NH_4^+ derived from reduction of NO_3^- rather than from NH_4^+ directly transported into the cell (Takabayashi *et al.*, 2005). Therefore, quantification of glnII mRNA may be useful for indicating new production by phytoplankton. PCR primers have been developed for detecting assimilatory nitrate reductase genes in bacteria and cyanobacteria (Allen *et al.*, 2001; Jenkins *et al.*, 2006; Paerl *et al.*, in press) and for diatoms and other phytoplankton (Allen *et al.*, 2005) in the environment. Analysis of diatom nitrate reductase (NR) protein sequences have provided hints to the underlying mechanism of relatively low NR

Table 30.1 Catalytic enzymes essential for nitrogen transformations processes used in molecular studies

Process	Organism(s)	Function	Protein	Gene	Reference
Nitrogen assimilation	*Bacteria*	NO_3^- to NO_2^-	Assimilatory nitrate reductase	*nas*, *nar*	Allen *et al.*, 2001; Allen *et al.*, 2005; Jenkins *et al.*, 2006
		NH_3 to L-glutamine	Glutamine synthetase	*gln*	Gibson *et al.*, 2006
Nitrogen fixation	*Bacteria* and *Archaea*	N_2 to NH_3	Nitrogenase iron protein	*nifH*	Affourtit, 1997; Church *et al.*, 2005; Foster *et al.*, 2007; Jenkins *et al.*, 2004; Langlois *et al.*, 2008; Lovell *et al.*, 2000; Moisander *et al.*, 2007; Omoregie *et al.*, 2004; Piceno *et al.*, 1999; Short *et al.*, 2004; Steward *et al.*, 2004; Tiquia *et al.*, 2006a; Zani *et al.*, 2000; Zehr and McReynolds, 1989
			Nitrogenase alpha subunit	*nifD*	Holmes *et al.*, 2004; Roeselers *et al.*, 2007; Sroga *et al.*, 1996
DON processing	*Bacteria*	N-acetylglucosamine to oligosaccharides	Chitinase	*chiA*	Cottrell *et al.*, 1999
		Endohydrolysis of 1,4-β-D-glucosidic linkages in cellulose	Cellulase	*celM*	Cottrell *et al.*, 1999
		1,4-β-D-glucosidase	Cellulase (family 5)	GH5	Elifantz *et al.*, 2008

Denitrification	Bacteria and Archaea	NO_3^- to NO_2^-	Dissimilatory nitrate reductase (periplasmic) catalytic subunit	*napA*	Bru *et al.*, 2007; Cheneby *et al.*, 2003; Lopez-Gutierrez *et al.*, 2004; Philippot *et al.*, 2001; Flanagan *et al.*, 1999; Nogales *et al.*, 2002; Smith *et al.*, 2007
			Dissimilatory nitrate reductase (membrane) catalytic α subunit	*narG*	Bru *et al.*, 2007; Cheneby *et al.*, 2003; Lopez-Gutierrez *et al.*, 2004; Philippot *et al.*, 2001
		NO_2^- to NO	Cytochrome c and d containing nitrite reductase (*cd*1-NiR)	*nirK*	Braker *et al.*, 2000; Casciotti and Ward, 2001; Hallin and Lindgren, 1999; Liu *et al.*, 2003; Nogales *et al.*, 2002; Santoro *et al.*, 2006
		NO_2^- to NO	Copper-containing nitrite reductase (Cu–NiR)	*nirS*	Braker *et al.*, 2001; Braker *et al.*, 2000; Gruntzig *et al.*, 2001; Hallin and Lindgren, 1999; Jayakumar *et al.*, 2004; Liu *et al.*, 2003; Nogales *et al.*, 2002; Throback *et al.*, 2004; Tiquia *et al.*, 2006a; Tiquia *et al.*, 2006b
		NO to N_2O	Nitric oxide reductase cytochrome *b* subunit	*norB*	Braker and Tiedje, 2003; Casciotti and Ward, 2005
		N_2O to N_2	Nitrous oxide reductase	*nosZ*	Nogales *et al.*, 2002; Scala and Kerkhof, 1998, 1999

Table 30.1 Catalytic enzymes essential for nitrogen transformations processes used in molecular studies (*continued*)

Process	Organism(s)	Function	Protein	Gene	Reference
Anammox	Planctomycetes	N_2H_4 to N_2	hydrazine dehydrogenase (hydroxylamine/hydrazine oxidoreductase)	*hao*	Jetten *et al.*, 2003
Nitrification	Ammonia oxidizing bacteria	NH_4^+ to NH_2OH	Ammonia monooxygenase	*amoA*	Holmes *et al.*, 1995; Juretschko *et al.*, 1998; Mendum *et al.*, 1999; Nicolaisen and Ramsing, 2002; Nold *et al.*, 2000; Purkhold *et al.*, 2000; Rotthauwe *et al.*, 1997; Stephen *et al.*, 1999; Tiquia *et al.*, 2006a; Ward *et al.*, 2007; Ward and O'Mullan, 2002
		NH_2OH to NO_2^-	hydroxylamine oxidoreductase	*hao*	Bruns *et al.*, 1998; Urakawa *et al.*, 2006
	Ammonia oxidizing archaea	NH_4^+ to NH_2OH	Ammonia monooxygenase	*amoA*	Beman and Francis, 2006; Coolen *et al.*, 2007; Francis *et al.*, 2005; Hallam *et al.*, 2006; Mincer *et al.*, 2007; Wuchter *et al.*, 2006

Cited references were selected because they provide the foundation for targeting these functional genes in the environment. The use of these targets is expanding rapidly and a comprehensive list of every study referencing their use is much too long to include here. Therefore, researches interested in following a specific process should be sure to refer to current literature.

temperature optima in diatoms, as have studies of protein activity and protein abundance using immunoassays (Allen *et al.*, 2005; Gao *et al.*, 2000; Smith *et al.*, 1992). Berges and Harrison (1995) found that NR activity was the rate limiting step in nitrate utilization in a diatom, and thus probing for NR activity or gene expression can identify cells that are adapted to nitrate utilization, even on the single-cell level (Jochem *et al.*, 2000). Assays of gene expression also help to identify temporal patterns that relate to daily cycles of activity, and to identify constitutive and regulated responses. Daily cycles in nutrient uptake driven by light-dependent species-specific characteristics can influence competition for dissolved nutrients (Litchman *et al.*, 2004; Riegman *et al.*, 2000). The daily cycle of nitrate reductase activity in a diatom was suggested to be driven by transcriptional regulation (Vergara *et al.*, 1998). Shifts in gene expression in response to the presence of different N sources are implicated in the mechanism of preferential uptake and utilization of individual N sources.

There is complex feedback between use of different N sources (e.g. ammonium and nitrate) and internal N pools. The preferential use of reduced vs. oxidized forms of inorganic nitrogen sources has been recognized since early studies of nitrate and ammonium uptake in microalgae (Dortch and Conway, 1984) and thought to be based on energetic differences between transport and assimilation. More recently, it is recognized that both N sources can be used simultaneously (Collos, 1998; Collos *et al.*, 2003). Lomas (2004) measured the activity and expression of NR and urease and saw different responses in the uptake and assimilation of nitrate and urea when challenged with other N substrates, suggesting that NR and urease may have different internal feedback controls. The NR gene was cloned from the green alga *Dunalialla tertiolecta*, and found to be regulated in response to internal N pools but not to depletion of external sources (Song and Ward, 2004). As described above, the nitrate transporter in diatoms is also regulated in response to different nitrogen sources (Hildebrand and Dahlin, 2000). Whole genome microarray experiments with two *Procholorococcus* strains grown on different nitrogen sources show genes in common that are upregulated when cells are grown on nitrite, urea or cyanate as opposed to ammonium and genes unique to each nitrogen source (Tolonen *et al.*, 2006). Thus, there are clearly complex regulatory mechanisms in species level responses to the availability of nitrogen that are involved in determining overall nitrogen uptake and growth rates.

Regulation of gene transcription is central to the response of transporters and enzymes to nitrogen sources. In cyanobacteria, expression of genes in nitrogen assimilatory pathways is mediated by a signal transducer (P_{II}, encoded by *glnB*) and the regulatory protein NtcA (Tandeau de Marsac *et al.*, 2001). *ntcA* gene expression was shown to be correlated with nitrate use (Lindell *et al.*, 1998) and has been used as a target to assay for use of ammonium and nitrate and nitrogen stress in marine *Synechococcus* spp. (Lindell and Post, 2001). However, the *ntcA* regulatory mechanisms may vary between species and strains; Bird and Wyman (2003) found that the oceanic strain *Synechococcus* sp. WH8103 had a number of unusual characteristics, including the fact that *ntcA* did not appear to be regulated by ammonium. *ntcA* is regulated by ammonium in *Prochlorococcus* (Tolonen *et al.*, 2006), but there are no nitrate assimilation genes to regulate and the ammonium transporter appears not to be regulated by NtcA (Lindell *et al.*, 2002). NtcA appears to be central in balancing carbon and

nitrogen metabolism (Tandeau de Marsac *et al.*, 2001), but its role in *Prochlorococcus* may differ from that in *Synechococcus* (Lindell *et al.*, 2002). The P_{II} protein is found in bacteria and cyanobacteria and plays a variety of regulatory roles in N assimilation including control of N fixation. The P_{II} protein is reversibly phosphorylated in cyanobacteria in response to sensing N assimilation through the glutamine synthetase–glutamate synthase pathway (GS–GOGAT) and not simply N source availability (Forchhammer, 2003, 2004). Additionally, the P_{II} protein also senses fluxes in CO_2 levels and may link N and carbon (C) assimilatory pathways (Forchhammer, 2003, 2004). In *Prochlorococcus*, there are strain differences in *glnB* regulation under nitrogen stress, indicating that P_{II} function may be independent of N utilization (Tolonen *et al.*, 2006). Future studies of the activity of the P_{II} protein will be exciting because they may reveal how cyanobacteria cope with both N and C fluxes in dynamic marine environments.

7.1. Assimilation of DON

Most microorganisms can use some organic compounds as nitrogen sources. Our comprehension of the metabolism of organic nitrogen is limited by our understanding of the composition of DON in the oceans (see also Aluwihare and Meador, Chapter 3, this volume). DON, as well as dissolved organic carbon (DOC), are dynamic components of marine systems but are poorly characterized due to a variety of technical limitations in analyzing both their composition and utilization (Benner, 2002; Berman and Bronk, 2003; Bronk, 2002). Amino acids, as dissolved free (DFAA) or combined (DCAA) amino acid forms, and urea are DON constituents present in relatively low concentrations (Aluwihare and Meador, Chapter 3, this volume) but are important labile sources of nitrogen as they are turned over rapidly. Urea and amino acid uptake by bacteria (Cho *et al.*, 1996; Wheeler and Kirchman, 1986) and phytoplankton (Collier *et al.*, 1999; Wheeler *et al.*, 1974) has been known for many years. However, despite the fact that analysis of these compounds is more straightforward than analysis of the majority of DON constituents, relatively little is known about their uptake and metabolism. A mechanism for utilization of organic nitrogen by phytoplankton was shown by the discovery of amino acid oxidases on the cell surface of some marine phytoplankton (Palenik and Morel, 1990). These enzymes have not received much attention, but could be important in phytoplankton metabolism of organic nitrogen, and could be good targets for molecular or immunological approaches. Dissolved proteins are also metabolized by bacteria (Hollibaugh and Azam, 1983; Keil and Kirchman, 1992; Taylor, 1995), presumably by cell surface proteases (Hollibaugh and Azam, 1983). The role of proteases may be particularly important in the cycling of DON in the ocean, and much could be learned from targeting proteases to identify the types of microorganisms involved in protein turnover, and what controls their distribution and activity. Chitin, a dominant macromolecule in marine systems, also contains N and is decomposed by a number of microorganisms (Keyhani and Roseman, 1999; Svitil *et al.*, 1997). Chitin comprises 60–80% of reactive high molecular weight DON (Aluwihare *et al.*, 2005). Diverse chitinase genes have been reported from a variety of aquatic environments and were found in the Sargasso Sea environmental libraries (LeCleir *et al.*, 2004).

The composition of the bulk of dissolved organic matter (of which DON is a part) has always been controversial, but was largely believed to be a result of the recalcitrant properties derived from chemical or bacterial processing and reprocessing (Bronk *et al.*, 1994). Recent studies indicate that this material may largely be derived directly from photoautotrophic production; organic matter synthesized primarily by cyanobacteria (McCarthy *et al.*, 2004). This suggests that the biochemical pathways of organic matter synthesis and release need to be examined in detail, in order to determine the factors that control DOM synthesis and export in the oceans.

7.2. Nitrogen fixation

A variety of prokaryotic microorganisms can use atmospheric dinitrogen (N_2) as a source of nitrogen for growth. This capability, conferred by the enzyme nitrogenase encoded by the *nif* genes (Table 30.1), appears to be limited to prokaryotic microorganisms, but is widely dispersed in prokaryotic taxa. Nitrogen fixation is energetically expensive and sensitive to oxygen, and is highly regulated at transcriptional and post-translational levels (Zehr and Capone, 2002).

Nitrogen fixation in the marine environment occurs in benthic (sediments and microbial mats) and pelagic environments, and in salt marsh soils (see Carpenter and Capone, Chapter 4, this volume). Since very diverse microorganisms that span the Bacteria and Archaea domains of life can fix nitrogen, it is often difficult to ascertain which organisms are responsible for observed nitrogen fixation rates. Our discussion of the application of molecular approaches to the study of marine nitrogen fixation is brief because it has been reviewed extensively elsewhere (Carpenter and Capone, Chapter 4, this volume; Foster and O'Mullan Chapter 27, this volume; Scanlan and Post, Chapter 24, this volume; Paerl and Zehr, 2000; Zehr and Ward, 2002; Zehr *et al.*, 2003).

The *nifH* gene, encoding the nitrogenase iron protein is useful as a phylogenetic marker for identifying N_2 fixing microorganisms (Zehr *et al.*, 2003). The diversity of nitrogen fixing microorganisms has been studied by targeting *nifH* in a number of habitats including sediments (Burns *et al.*, 2002), salt marshes (Lovell *et al.*, 2000), marine microbial mats (Omoregie *et al.*, 2004b; Steppe and Paerl, 2002; Zehr *et al.*, 1995), hydrothermal vents (Mehta *et al.*, 2003), estuaries (Affourtit *et al.*, 2001; Jenkins *et al.*, 2004; Moisander *et al.*, 2007), and the open ocean (Falcon *et al.*, 2004; Hewson *et al.*, 2007; Mazard *et al.*, 2004; Zehr *et al.*, 1998, 2001). Current methods including DNA macro- and microarrays (Jenkins *et al.*, 2004; Moisander *et al.*, 2006, 2007) and quantitative PCR approaches (Church *et al.*, 2005a,b; Foster *et al.*, 2007; Short *et al.*, 2004) can provide information on the diversity of diazotroph communities and the abundance of individual phylotypes, respectively.

Expression of the *nifH* gene can indicate the activity of N_2 fixing microorganisms (Omoregie *et al.*, 2004a; Zani *et al.*, 2000), since nitrogenase is a highly regulated enzyme. Methods aimed at enumerating gene transcript abundance such as reverse-transcriptase quantitative PCR (RT-qPCR) can be used to identify *nifH* gene expression patterns; for example, diel cycles typical for cyanobacteria. These methods can also be used to assay for responses to environmental factors in experimental manipulations (Needoba *et al.*, 2007; Zehr *et al.*, 2007), or *in situ* (Church *et al.*, 2005b). Uptake

experiments can also be combined with gene expression assays. Experiments coupling *nifH* gene expression with $^{15}N_2$ uptake (Church *et al.*, 2005a; Montoya *et al.*, 2004; Zehr *et al.*, 2001), were important for showing the importance of previously unidentified unicellular cyanobacteria in the open ocean to upper water column nitrogen fixation. Prior to these experiments, it was traditionally believed that there were only a few dominant nitrogen fixing species in oligotrophic oceans, particularly the filamentous nonheterocyst forming cyanobacterium *Trichodesmium*, and the heterocystous cyanobacterial symbiont of diatoms, *Richelia* (Carpenter, 1983, pp. 65–103). Despite the identification of additional nitrogen fixing groups in the open ocean, in comparison, estuarine environments and coastal benthic environments contain much more numerous and diverse species of diazotrophs (Olson *et al.*, 1999; Omoregie *et al.*, 2004a,b; Jenkins *et al.*, 2004; Zehr *et al.*, 1995, 2003). A current challenge is to determine whether any of these groups in environments with dynamic N cycling are active and under what conditions they fix nitrogen. A recent study demonstrated nitrogen fixation in estuarine sediments from Narragansett Bay (Fulweiler *et al.*, 2007) and subsequently we detected *nifH* gene expression from the same N_2–fixing cores (Jenkins and Fulweiler, unpublished data). These experiments illustrate that use of molecular biological tools can be a powerful means for identifying new organisms active in N–cycling.

7.3. Identifying nitrogen limitation

Nitrogen availability constrains biomass (Liebig, 1840) and the rate of primary productivity (Blackman, 1905). This is true in marine systems (Falkowski and Raven, 1997) and has been noted early in the development of biological and chemical oceanography (Nixon and Pilson, 1983). It is still debated whether nitrogen, phosphorus or other nutrients are the proximate or ultimate (Tyrrell, 1999) limiting nutrients, and how nitrogen and phosphorus limitation constrains or is constrained by the elemental ratios of organisms in the sea (Falkowski, 2000; Geider and La Roche, 2002; Karl *et al.*, 2001; Klausmeier *et al.*, 2004; Redfield, 1958) (see Hutchins and Fu, Chapter 38, this volume). Information at the cellular or molecular level can be used as a proxy for nutrient stress by assessing physiological responses to nutrient limitation. Unbalanced growth results in shifts in gene expression and protein abundance that can be indicators of limitation in the Blackman sense (i.e. by one nutrient or the other). Molecules impacted by nutrient limitation include transporter molecules (see above), proteases (Berges and Falkowski, 1998), and other proteins (Geider *et al.*, 1993; LaRoche *et al.*, 1993; Leonardos and Geider, 2004; Plumley and Schmidt, 1989). A nitrogen regulated protein on *Emiliania huxleyi* cell surfaces was identified (Palenik and Koke, 1995) and could be used to interrogate individual cells for nitrogen stress. Monitoring expression of the global nitrogen regulator, *ntcA*, described above, is useful for identifying N limitation in cyanobacteria and their dependence upon different N sources in both the laboratory and in field populations (Lindell and Post, 2001; Lindell *et al.*, 2005). A variety of other genes and proteins in cellular metabolism, from transporters to response regulators and metabolic enzymes (e.g. glutamyl-tRNA synthetase in cyanobacteria) (Luque *et al.*, 2002) may be good indicators of physiological status that can be used to interrogate natural populations.

> ## 8. MOLECULAR BIOLOGICAL TOOLS FOR PROBING THE USE OF NITROGENOUS COMPOUNDS FOR CELLULAR ENERGY

8.1. Denitrification

Denitrification is the use of oxidized nitrogen compounds as terminal electron acceptors in respiration, resulting in the formation of dinitrogen gas. For a comprehensive discussion of marine dentirification, please see Devol, Chapter 6, this volume. Dentrification is important for fixed N budgets in the oceans since it is a primary loss mechanism for N. The balance between denitrification and N_2 fixation controls N limitation over long time scales (Capone, 2000; Codispoti *et al.*, 2001; Falkowski, 1997). Denitrification results in the production of N_2O and NO, contributing to greenhouse gas emissions which affects climate (Capone, 2000; Lashof and Ahuja, 1990). Denitrification is largely an anaerobic process, but can occur aerobically as well under some conditions (Robertson *et al.*, 1995). Denitrification occurs in organic–rich sediments at continental margins, and oxygen minimum zones (OMZ) in intermediate waters, such as the Arabian Sea, the eastern tropical North and South Pacific Oceans and the Baltic Sea (Gruber and Sarmiento, 1997) (see Devol, Chapter 6, this volume). The capacity for denitrification is widespread amongst prokaryotic lineages and cannot be deduced from 16S gene comparison. Therefore, studies targeting functional genes involved in denitrification pathways are invaluable for determining the distribution and function of denitrifying microorganisms in the environment.

During the denitrification process, nitrate is sequentially converted to nitrogen gas by the action of four metalloenzymes: the periplasmic and membrane bound nitrate reductases (encoded by the *narG* and *napA* genes) reduce NO_3^- to NO_2^-, the cytochrome cd_1 and Cu containing nitrite reductases (encoded by the *nirS* and the *nirK* genes, respectively) reduce NO_2 to NO, nitric oxide reductase (encoded by the *norB*) reduces NO to N_2O, and nitrous oxide reductase (*nosZ*) reduces N_2O to N_2. All the genes encoding the enzymes involved in denitrification contain conserved regions (Bothe *et al.*, 2000) which facilitates the development of molecular probes that target diverse species, Table 30.1.

Studies using functional genes as markers to study denitrifier diversity in the ocean demonstrate a high diversity of denitrifiers that are divergent from previously cultivated strains. The spatial and temporal heterogeneity of denitrifiers in sediments was demonstrated based on phylogenetic analysis of denitrification genes off the New Jersey shore (Scala and Kerkhof, 2000) and from Washington coast and Puget Sound sediments (Braker *et al.*, 2000). There appear to be similarities between communities in different layers of sediment cores even though steep redox gradients were present in the core (Braker *et al.*, 2001). The authors hypothesized that invertebrate bioturbation may be responsible for the relative homogeneity of denitrifiers along vertical gradients. In contrast, denitrifier diversity along vertical profiles of water columns show differences in community structure between depths as a function of oxygen concentration (Liu *et al.*, 2003). Principle component analysis showed that nitrate and oxygen levels may control denitrifier community structure off the Pacific coast of Mexico (Liu *et al.*, 2003). The denitrifier community

composition is influenced by O_2, NO_3^- and NO_2^- gradients and depth within the water column in the Chilean OMZ (Castro-Gonzalez *et al.*, 2005). Similarly, Jayakumar *et al.* (2004) observed the highest denitrifier diversity in Arabian Sea samples with high nitrate concentrations and the lowest diversity in a surface water sample with undetectable nitrate, implying a correlation between ecosystem chemistry and genetic diversity.

Although diversity studies reflect microbial community composition, they do not identify members of the community that may be actively denitrifying. Studies of cultivated denitrifiers show that expression of denitrification genes is induced in the presence of nitrogen oxides at low oxygen tensions. In estuaries with anthropogenic loading of nutrients, denitrifiers are often active in the sediments that have high levels of nitrate. Nogales *et al.* (2002) assayed the expression of *narG*, *napA*, *nirS*, *nirK*, and *nosZ* genes in two sediment samples taken from the River Colne estuary with high denitrification rates and found low expression of *nirS* genes and barely detectable expression of *nosZ* genes, even though all five genes were present in the samples. In a more recent study in the same estuary, Smith *et al.* (2007) found higher copy numbers of expressed denitrification genes at the estuary head where the rates of denitrification are highest. They also found higher copy numbers of the nitrate reductase genes (*narG* and *napA*) than for three *nirS* subgroups at each site sampled (Smith *et al.*, 2007). Although it is unclear at this time whether expression levels between different denitrification pathway genes are due to differences in transcript abundances at the time of sampling, or differences in detection limits between different genes, it seems that gene expression assays are progressing for studying marine denitrifiers.

It is also possible to use antibody based approaches to assay the accumulation of enzymes involved in denitrification pathways in environmental samples. Ward and Cockroft (1993) developed antisera to nitrite reductase from a marine *Pseudomonas stutzeri* isolate, and assayed sediment, water column and microbial mat samples. The antibody identified nitrite reductase in mat samples and was more specific than a DNA probe designed against the same species (Ward and Cockroft, 1993). More recently, other strains of denitrifiers have been isolated from both sediment and water column samples. Sequence information regarding the denitrifying genes should prove invaluable for future refinement of both sequence based approaches and the development of additional antibodies to denitrifiers important in the marine environment.

8.2. Anaerobic ammonia oxidation (anammox)

Although denitrification was thought to be the major sink of fixed N in the marine nitrogen budget, another process of converting fixed nitrogen to N_2 gas by anaerobic ammonium oxidation (anammox) has recently been shown to be important in marine environments (recently reviewed in Francis *et al.* (2007); Jetten *et al.*, 2003; Kuypers *et al.*, 2003) (see Devol, Chapter 6, this volume). Anammox bacteria are unusual chemolithoautotrophs first discovered in sewage treatment plants (Jetten *et al.*, 2003). They oxidize ammonia with nitrate as a terminal electron acceptor under anoxic conditions producing N_2 gas. Anammox bacteria (in the genera *Kuenenia, Brocadia, Scalindua,* and *Anammoxglobus*) form

a monophyletic cluster in the 16S rRNA tree that branches deeply in the order Planctomycetales (Kuypers *et al.*, 2003). Therefore, this monophyletic group can be targeted with 16S rRNA probes by PCR and FISH. Bacteria in this group also localize anammox reactions to a subcellular compartment surrounded by membranes with a unique ladderane lipid signature (van Niftrik *et al.*, 2004). Detection of these lipids in the environment can also be used to indicate the presence of anammox bacteria.

Anammox activity has been described in marine sediments (Amano *et al.*, 2007; Engstrom *et al.*, 2005; Hietanen, 2007; Penton *et al.*, 2006; Rich *et al.*, 2008; Risgaard-Petersen *et al.*, 2004; Rysgaard *et al.*, 2004; Schmid *et al.*, 2007; Thamdrup and Dalsgaard, 2002; Trimmer *et al.*, 2005) and in anoxic water columns (Dalsgaard *et al.*, 2003; Hamersley *et al.*, 2007; Jaeschke *et al.*, 2007; Kuypers *et al.*, 2003, 2005; Lam *et al.*, 2007; Thamdrup *et al.*, 2006) and is thought to account for 30–50% of total N_2 produced in oceans (Devol, 2003). Kuypers *et al.* (2003) identified Planctomycetes in the Black Sea on the basis of 16S rRNA sequences that corresponded to peaks of ladderane lipid profiles and nitrate concentrations. Using fluorescence *in situ* hybridization (FISH) they determined an anammox cell density of $\sim$1900 $\pm$ 800 cell ml^{-1}, enough to account for their observed ammonium oxidation rates (Kuypers *et al.*, 2003). An optimized set of PCR primers to the 16S gene from the *Candidatus* Scalindua group of annamox bacteria was developed recently and was used to show widespread distribution of these groups in marine and freshwater sediments (Penton *et al.*, 2006). Genomic approaches have proven fruitful for illuminating the physiology of anammox bacteria which have yet to be isolated in pure culture. Strous *et al.* (2006) used environmental genomics methods to assemble the genome of the uncultured anammox bacterium *Kuenenia stuttgartiensis* from a complex bioreactor community. The authors identified candidate genes responsible for ladderane biosynthesis and biological hydrazine metabolism which provide a blueprint for future physiological studies and could be good targets for anammox function in the environment.

8.3. Ammonia oxidation and nitrification

The oxidation of ammonia to nitrate is catalyzed by chemoautotrophs and is particularly important in the formation of ''new'' nitrate in the deep ocean (Ward, 2000). The major significance of nitrification is converting reduced N into an oxidized form, nitrate, that can lead to the removal of nitrogen from the system through denitrification (Ward, 2000 and Chapter 5, this volume). Ammonia oxidation is typically an aerobic process that occurs in aerobic or microaerophilic habitats or at interfaces between anaerobic and aerobic environments (Ward, 2000). The oxidation of ammonium to nitrate provides relatively little energy per mole of substrate oxidized. Therefore, ammonia-oxidizers and nitrifiers are slow growing microorganisms that depend on relatively large concentrations or fluxes of substrate. Cultivation of these microorganisms can be difficult and studying them *in situ* is limited by low abundances (<0.1% of total prokaryotes in marine water column) and low rates of activity typical of most environments. Thus, molecular approaches prove particularly useful for studying nitrification because they provide a sensitive means by which to assay organisms that are not abundant and have slow growth rates.

Molecular and immunological approaches have been used to characterize the distribution or diversity of nitrifiers. For a long time, there were only two known monophyletic lineages of aerobic ammonia-oxidizers found in the γ- and β-Proteobacteria (reviewed in Kowalchuk and Stephen, 2001). Recently, an exciting discovery was made; ammonia-oxidizer was isolated that belongs to the marine Crenarchaeota (Konneke *et al.*, 2005). Crenarchaeota are found in high numbers in cold oxic oceans (DeLong, 1992; Fuhrman *et al.*, 1992). Therefore, a numerous group of marine microbes have the potential to oxidize ammonia and may be major primary producers in environments deficient in organic nutrients and sunlight. For a more extensive discussion of marine nitrification we refer you to Ward, Chapter 5, this volume.

Non-marine ecosystems are dominated by β-Proteobacterial ammonia-oxidizers in the *Nitrospira* and *Nitrosomas* groups and, therefore, can be studied using phylogenetic (e.g. rRNA) approaches designed to target this subdivision. However, ammonia-oxidizers in the marine environment are found in both β- and γ- Proteobacteria subdivisions and, therefore, it is more difficult to use 16S rRNA directed studies to comprehensively study these organisms in marine systems. Therefore, many studies of ammonia-oxidizers in the marine environment focus on the β subclass of Proteobacteria and ignore the γ subclass. These studies have found that different populations occupy different niches in the marine environment. For example, distinct *Nitrosomas* groups correlated with gradients of salinity, ammonia, and oxygen in an estuary (de Bie *et al.*, 2001), *Nitrosomas* groups were shown to be associated with particulate matter in the Mediterranean Sea whereas *Nitrospira* groups were found in planktonic samples (Phillips *et al.*, 1999). *Nitrospira*-like 16S rRNA phylotypes were found to be widely distributed, even in polar oceans (Hollibaugh *et al.*, 2002). Nitrite oxidizers are found in several taxonomic groups, but they have not been studied much in any environment, including the marine environment.

The gene encoding the α-subunit of AMO (*amoA*) has been sequenced from many cultivated ammonia-oxidizers in the Proteobacteria and in general, *amoA* phylogenetic trees correspond well with those of 16S rRNA. The *amoA* gene also has greater sequence variation in comparison to 16S rRNA (Rotthauwe *et al.*, 1997) so it provides better phylogenetic resolution for the ammonia-oxidizers. The *amoA* gene is useful for studying ammonia-oxidizer diversity in the marine environment because it is possible to detect all Proteobacterial lineages simultaneously (Table 30.1). Nold *et al.* (2000) used *amoA* gene phylogeny to show that ammonia-oxidizers in Washington coast and Puget Sound sediments were dominated by β-Proteobacteria. Mortimer *et al.* (2004) measured nitrate concentrations and nitrification in vertical profiles of sediment samples from an organic-rich marine fjord and compared those profiles to microbial populations in the sediment cores by PCR and RT-PCR of 16S rRNA sequences. A shift in ammonia-oxidizing bacterial community composition was seen at the same depth in a sediment core where a major nitrate peak occurs at an anoxic depth, suggesting a correlation between bacterial community composition and nitrate generation (Mortimer *et al.*, 2004). Physiological studies on *Nitrosomonas europea* and *Nitrosomonas eutropha* suggest that anaerobic oxidation of ammonia may also be carried out by β-Proteobacterial

ammonia-oxidizing bacteria (Schmidt and Bock, 1997, 1998). Ammonia oxidation may be coupled to the reduction of manganese in continental shelf sediments (Mortimer *et al.*, 2004).

The archaeal *amoA* gene has also been targeted in the marine environment and distinct ammonia-oxidizing archaeal communities were found at the base of the euphotic zone, suboxic water columns, and in estuarine and coastal sediments (Francis *et al.*, 2005). A time series study in the North Sea showed the archaeal *amoA* correlates with a decline in ammonium concentrations and was 1–2 orders of magnitude higher than *amoA* from bacterial nitrifiers (Wuchter *et al.*, 2006). A similar patter was seen in Monterey Bay and the North Pacific Subtropical Gyre (Mincer *et al.*, 2007). Archaeal *amoA* is more abundant that bacterial *amoA* in estuarine sediments and shows a phylogentic patterning that changes with space across the estuary (Beman and Francis, 2006). Therefore, this recently discovered group of crenarchaeal nitrifiers may have a significant impact on the cycling of N and C in the ocean.

9. Summary and Perspective

The increasing application of molecular biological techniques to studies in marine nitrogen cycling demonstrates the feasibility of assigning functional roles in biogeochemical transformations to individual organisms. Molecular biological methods have been instrumental for identifying the presence of organisms for major nitrogen transformation pathways that may have long remained undetected otherwise. Gene based detection methods often uncover a vast diversity of uncultivated organisms which may in part explain missing N budget terms. For example, part of the oceanic fixed N deficit may be accounted for by the activity of new groups of N fixing organisms which are unaccounted for in current budgets (Codispoti, 2007). However it is also important to keep in mind that although functional gene diversity may be related to ecosystem biocomplexity and stability, genetic diversity is not necessarily related to the rate or extent of geochemical transformations encoded by those genes (Zehr and Ward, 2002). Measurements of the expression of specific genes make it possible to determine the distribution of individual genotypes that are physiologically active. It is now feasible to determine the correlations between the distributions of individual genotypes, expression of genes and physico-chemical factors in the marine environment. It may be years from now before this explosion of molecular and genetic data can inform predictive ecosystem models directly, but knowledge of the genetic potential and responses of organisms in the environment can provide a blueprint for refining considerations impacting ecosystem function. For example, Doney *et al.* (2004) suggest, ''Such an approach would be based on determining the environmental factors that regulate specific functions analogous to genetic regulation; that is models would start with a simulated ocean genotype, one much richer than presently resolved, and then predict, under a range of conditions the expression of a simulated phenotype, which could then be compared with genomic data''.

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Isotope Tracer Methods for Studies of the Marine Nitrogen Cycle

Fredric Lipschultz

Contents

1. Introduction

The use of the stable nitrogen isotope, ^{15}N, as a tracer is central to the study of the nitrogen biogeochemistry of marine ecosystems and the physiology of marine organisms. Studies of ecosystem processes such as denitrification or nitrification would be extremely limited without direct rate measurements; although attempts are often made to infer rates from net changes in concentrations of various nitrogen species, through the use of inhibitors, and other indirect assessments. Similarly, the application of ^{15}N tracer methods has enhanced our understanding of organismal metabolism by allowing us to make

direct observations of transport, uptake, and assimilation of N compounds by organisms (see Mulholland and Lomas, this volume), and to identify and quantify transformations such as nitrification or nitrate reduction to ammonia (see Ward, this volume).

Advances in the use of ^{15}N in pelagic marine systems were initially compiled by Harrison (1983) over 20 years ago. At that time, the number of studies using ^{15}N was still quite small due to the difficulty of making the isotopic measurement itself, and the limited ability to isolate the various dissolved components of the nitrogen cycle. Most studies therefore focused on movement of ^{15}N from the dissolved to the particulate pool since particles are easily isolated by filtration. A decade later, Glibert and Capone (1993) described methods for studying nitrogen cycling in aquatic and sediment systems. Included in their discussion were issues such as isotope dilution; a problem that had become apparent as techniques were developed to isolate dissolved source pools such as nitrate or ammonium. Glibert *et al.* (1982), for instance, demonstrated that consumption and production of ammonium could be sufficiently large to dramatically change the ammonium isotopic ratio over the course of an incubation. These changes permitted the calculation of remineralization rates, which if not corrected for can introduce significant errors in calculations of uptake rates. Standard methods for uptake studies using ^{15}N were canonized for the JGOFS program (Knap *et al.*, 1996) and by Dugdale and Wilkerson (1986).

This review covers improvements in tracer methods as applied to pelagic marine over the past decade and attempts to point out promising new directions for the future. Applicable methods from the large literature on benthic nitrogen cycling studies are highlighted, but a complete review is beyond the scope of this effort. The focus is on the methods rather than on ecological or physiological applications of the methods, as the applications are covered in other chapters of this volume. However, salient applications of the methods will be mentioned to provide context as to why one might want to isolate and analyze the isotopic composition of a particular analyte. The review begins with major changes over the past years, discusses the basic experimental design of tracer experiments, then covers the analytical separation of the different constituents of the nitrogen cycle, and concludes with the ^{15}N analysis itself and interpretation of the resulting isotope data using various models.

Two major developments in analytical chemistry have greatly enhanced our ability to conduct nitrogen isotopic analyses in the ocean; improvements in the actual instrumentation for ^{15}N analysis, and advances in chemical detection that provide an ability to more accurately measure low concentrations of ammonium and nitrate (see also McCarthy and Bronk, this volume). Coupling of mass spectrometers to CHN (carbon, hydrogen, nitrogen) analyzers (e.g., Owens, 1988), combined with advances in computers that permitted extensive automation, has opened up the field to a far wider range of practitioners as well as permitted a vast expansion in the speed of analysis that can be completed. These advances have resulted in CF-IRMS (continuous flow isotope ratio mass spectrometry) essentially supplanting the alternative technology of emission spectroscopy for ^{15}N analysis.

The ability to measure low nanomolar concentrations of dissolved nutrients opened up the possibility of conducting true tracer experiments in the vast bulk of the oceans. Experiments using tracer level additions (typically considered to be 10% of the ambient source concentration) had been limited to high nutrient environments in coastal and estuarine habitats. In oligotrophic regimes, investigators commonly added a fixed tracer

addition (e.g., 30 to 100 nM) to all samples with concentrations near or below the detection limit, resulting in major perturbations to the resident microbial community. Precise detection of low nanomolar concentrations of ammonium progressed from concentrating methods, first by extraction (Brzezinski, 1987) and then by diffusion (Jones, 1991), to direct analysis by fluorescence (Holmes *et al.*, 1999). Most recently, long-pass capillary cells have permitted nanomolar analysis of ammonium using colorimetry (Li *et al.*, 2005). Parallel improvements occurred for nitrate/nitrite by increasing the sensitivity of colorimetric analysis (Adornato *et al.*, 2005; Oudot and Montel, 1988; Yao *et al.*, 1998; Zhang, 2000) and application of chemiluminescent detection (Garside, 1982; Braman and Hendrix, 1989). Improvements have continued with automation and the ability to analyze multiple chemical species at low concentrations (e.g., Hales *et al.*, 2004; Masserini and Fanning, 2000). Coupled with new techniques to isolate and extract dissolved nutrients for isotopic analysis, these improvements permitted measurements of uptake rates in the oligotrophic Pacific (McCarthy *et al.*, 1996) and Atlantic Ocean (Harrison *et al.*, 1996; Lipschultz, 2001; Rees *et al.*, 1999).

Another major improvement since the 1983 review is not analytical, but conceptual; there is an increasing awareness of the complexity of the nitrogen cycle. Our improved ability to study various fluxes means that investigators should assess not only movement of N compounds into the particulate pool, but also transformations among the dissolved pools such as ammonium regeneration and nitrification on a regular basis. Nitrogen fixation, routinely dismissed as insignificant prior to the mid-1990s, must now be considered in any study of new production (see Carpenter and Capone, this volume). The interplay between denitrification, anammox, dissimilatory nitrate reduction to ammonium (DNRA) and nitrification in suboxic systems is far more complex than imagined a few years ago, and new approaches are rapidly being developed (see Section 3.4; see also Devol, this volume and Naqvi, this volume). However, there is still no canonical set of rate measurements that must be made in every ecosystem, nor set of standard methods to be employed, and there remains sometimes sharp disagreement over the interpretation of tracer results (e.g., DON production).

2. EXPERIMENTAL DESIGN

The basic terminology used in this review (Fig. 31.1A) includes the precursor and product, where the precursor can either be labeled or unlabeled. When labeled, the precursor will also be referred to as the ''source'' since it supplies tracer to the progressively ^{15}N enriched product pool. The process of isotope dilution refers to the addition of an unlabeled precursor to the source pool resulting in a decrease in the source isotopic ratio. The precursor could be known, such as for NO_3^- isotope dilution where NO_2^- is the only option, or unknown as with NO_2^- isotope dilution where NO_3^- or NH_4^+ could be the precursor. Isotopic equilibrium is achieved when the product pool has the same isotopic composition as the precursor pool and it is important to note that isotope dilution fosters this condition. At equilibrium, a rate can no longer be discerned by comparing the isotopic ratios of the pools. As suggested by the unidirectional arrows in Fig. 31.1, an assumption of the tracer method is that isotope recycling does not occur. However, we know this
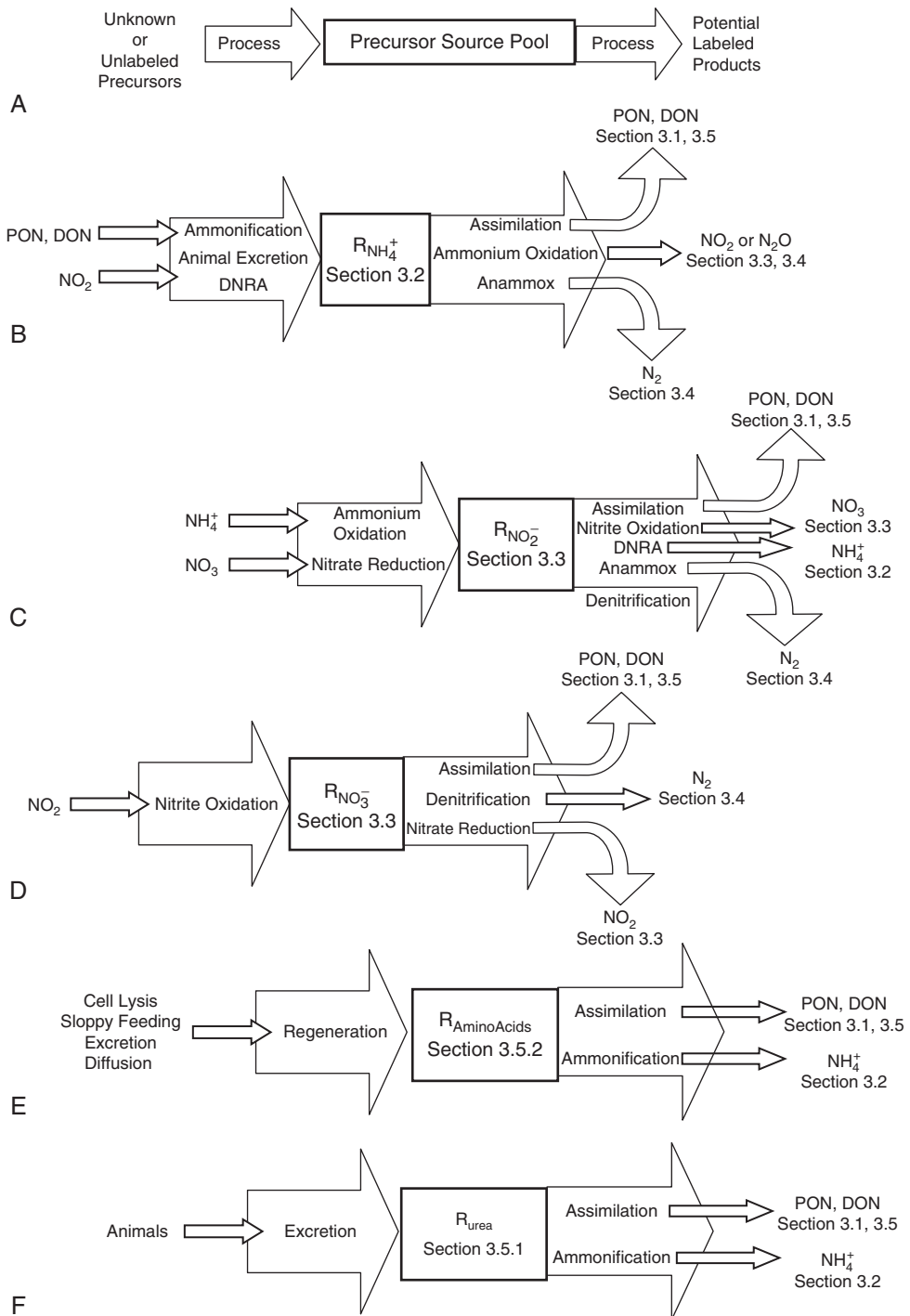
Figure 31.1 Illustration of the different analytical pools, their potential inputs of unlabeled precursors, and losses of labeled analyte to potential product pools. The various metabolic processes affecting the central pool are listed, as well as the chapter section reference for information on the appropriate analytical separation techniques. (A) Cartoon of sources and sinks of a central pool, (B) Ammonium, (C) Nitrite, (D) Nitrate, (E) Amino acids, and (F) Urea.

in fact can occur for instance, when ammonium is assimilated but the organism is then eaten and ^{15}N is excreted from the animal back into the ammonium pool.

2.1. Initial planning

The initial planning of tracer studies using ^{15}N to measure nitrogen cycle rates requires consideration of several practical issues. Two aspects are inextricably linked to determine length of the incubation; turnover rate of the relevant pools and the detection limits for the analytical methods. The turnover rate (pool size^{-1} × uptake rate) needs to be estimated for both source and product pools to ensure that sufficient tracer can be detected in the product pool while dilution of the source pool is either insignificant or small enough so that isotopic equilibrium, isotopic recycling, or severe isotopic dilution does not become an issue (see Section 5.2.1) (Glibert *et al.*, 1982; Harrison and Harris, 1986). A balance therefore needs to be found between increasing the incubation time to enhance detection of tracer in the product pool and minimizing the incubation time to prevent potential issues affecting the source pool. Given the detection limit of mass spectrometers and a best-guess turnover rate for the product pool, one can estimate the relative trade-offs between incubation time and changes in isotopic labeling of the source and product pools at a given enrichment level.

Turnover rate × incubation time × source enrichment = change in enrichment, e.g., $0.1\ d^{-1} × 0.2\ d × 10\% = 0.2\%$.

Generally the shortest incubation possible would resolve this dichotomy for biogeochemical processes such as nitrification or denitrification. However, complications arise for uptake studies since short incubations have the potential to primarily detect transport (movement across the cell membrane) whereas longer incubations favor assimilation into organic compounds such as amino acids or proteins. This problem can be especially serious if the source isotopic enrichment increases the nutrient concentration sufficiently to significantly perturb the uptake kinetics of the resident microbial community. A sudden, large concentration increase can lead to "surge" transport during which transport and assimilation are uncoupled, that is a cell's internal pools swell rapidly and assimilation lags (Goldman and Glibert, 1982; Kanda *et al.*, 1988).

Another, more subtle, point to consider whenever the receiving pool is heterogeneous (e.g., PON or DON) is underestimating the turnover time due to achieving isotopic equilibrium between source and receiving pools. For instance, since the particulate pool typically includes detrital nitrogen, the isotopic difference between the two pools will never approach zero even when the live component is at equilibrium with the source pool. Hence, although no further flux between the compartments can be detected, the rate calculation will always yield a finite, albeit continuously declining apparent rate. This type of issue arises due to confusion of equating a pool, the operational entity actually measured, with a compartment, the well-mixed and kinetically homogeneous mathematical construct used in models (see Section 5).

Final points to consider include the degree of replication and treatment of the initial, or time zero, concentrations and enrichment of both the product and source pools. The ability to replicate analyses has been greatly enhanced by the advent of automated CF-IRMS so that there is no longer a reason not to measure duplicate samples as a minimum. However, given the high analytical precision of modern mass spectrometers, the replication should be from the field by incubating replicate bottles

from a single sample depth (a measure of natural variability) rather than from duplicate analyses from the same bottle (e.g., a measure of analytical error). Assumptions made concerning the initial time points can also greatly affect the calculated rates when product enrichments are small. Researchers will often assume that particles have constant natural abundance values, or only assess the natural abundance periodically during the study to account for small, natural (‰) level changes. Others add the tracer to the incubation bottle, deploy the incubation bottles and then immediately filter an initial time point for comparison with the final values. One then needs to decide how to interpret the initial time point given there is often a substantial time between tracer addition and filtration (often 30 min). During this interval, the population may experience abnormal conditions (e.g., high light exposure or surge uptake due to nutrient perturbation) prior to filtration. In oligotrophic samples from the base of the euphotic zone, this can be a significant fraction of the total uptake. Similarly, the initial source pool enrichment can simply be calculated from the initial concentration and the ^{15}N addition, or actually measured. Given the potential matrix effects on the final value, it is preferable to directly measure both the initial and final source enrichments with the same analytical procedure to reduce potential errors.

2.2. Incubation protocols

In addition to the aforementioned issues of the length of the incubation, there are a host of additional choices required during the planning of any incubation experiment, regardless of the involvement of ^{15}N. The primary production literature is replete with cautionary tales of bottle effects such as trace metal (Fitzwater *et al.*, 1982) or sampling (Williams and Robertson, 1989) contamination. Although these issues have not been directly tested for nitrogen cycling, there is no reason not to follow all the same precautions learned the hard way from other metabolic studies. One particular area of concern is potential trace metal contamination of the ^{15}N stock solution, an issue of major concern for ^{14}C work requiring passing the tracer stock solution through a Chelex column or diffusing gaseous CO_2 from the commercial source into a new container. As far as I am aware, such precautions are not routine in ^{15}N work (or at least not often reported). At a minimum, the stock solutions should be analyzed for trace metals to assess the potential for contaminating the incubation water. Another contamination concern is the actual material of the incubation vessel. Again as for primary production studies, incubations with ^{15}N should use polycarbonate or polystyrene bottles that can be adequately cleaned of trace metals and are transparent. Studies where UV is an important factor, such as nitrification, need to consider the wavelength transmission curves for the incubation vessel.

The decision whether to incubate on deck or to use an *in situ* deployment, such as a spar buoy, rests partially with the effects of light and temperature, but also with the issue of incubation length. Deck incubations generally use neutral density screens to adjust the light levels, and pump surface water through the incubator to control temperature. A potential concern with pumping surface water is whether there is a strong temperature gradient between the surface and the depth of one's deepest samples, as the organisms could become temperature-shocked due to the sudden temperature change. As with primary production studies, it is also possible to use

wavelength filters to adjust the light quality to better mimic the water column. Deck incubations have a significant advantage in that they permit time course analyses of uptake or sequential short-term incubations to study the effects of the diurnal light cycle or entrained rhythms on uptake. *In situ* deployments are often chosen to best capture the ambient light field experienced by the organisms. In addition, since primary production is often determined using an *in situ* spar buoy (e.g., JGOFS standard protocol (Knap *et al.*, 1996)), there are also advantages to parallel deployment of incubations for nitrogen cycling. The ability to conduct both ^{13}C and ^{15}N measurements simultaneously also has favored use of *in situ* incubations. However, such deployments typically last from dawn to dusk because exposure of incubation bottles from deeper, low light depths to surface light levels during deployment or retrieval in the middle of the day could bias the uptake rate. Since light period incubations can be as long as 18 h, issues raised earlier of pool turnover times potentially can be of concern.

2.3. Pathways

All pathways of the nitrogen cycle are now amenable to probing with ^{15}N, a capability reached over the past decade that continues to improve with the development of new methods such as for anaerobic ammonium oxidation (anammox) (see Devol, this volume). Although chapters are included in this volume covering individual pathways, it is worthwhile to review our current view of the entire nitrogen cycle from the viewpoint of tracking ^{15}N precursor additions to the different potential product pools (Fig. 31.1). The analytical techniques to separate the various pools are described in Section 3, the isotopic measurements of the pools in Section 4, and models for data analysis of the tracer movement between compartments in Section 5.

   Although it is rarely necessary, or desirable, to isolate all intermediate pools in the nitrogen cycle, it is important to consider their potential effect on rate calculations (see also Section 5). For instance, a study of denitrification rates might add $^{15}NO_3^-$ and extract the dinitrogen pool, but since nitrite and N_2O are intermediates, the tracer could become "trapped" in the intermediate forms until they reach a sufficient degree of labeling that the tracer becomes detectable in the subsequent, more reduced form. Clearly the true rate of dinitrogen formation would only be achieved once the intermediate pools are at isotopic equilibrium with the purported source pool, in this case nitrate. The salient point to consider is the turnover time of the intermediates and hence whether their isotopic ratio needs to be directly determined or whether they come into equilibrium rapidly enough to be considered homogeneous with the precursor pool. The issue of pools versus compartments is also central to the discussion concerning the rate of DON production (e.g., Slawyk and Raimbault, 1995; Flynn and Berry, 1999), which itself echoes the same, earlier debate over use of ^{14}C for studies of DOC production. The same issue affects the interpretation of attempts to measure the uptake of DO^{15}N (Bronk and Glibert, 1993) since the degree of labeling of each DON compound in the operational pool undoubtedly varies and one cannot discern which compound or compounds are actually being utilized. Additionally, the DON might simply be remineralized to ammonium and the tracer taken up from that pool, further aggravating the interpretation.

Another consideration while planning a tracer experiment is achieving mass balance, or accounting for the entire amount of added tracer. Although a common criterion for radioisotope experiments, it was not generally considered for ^{15}N work until an apparent loss of tracer during incubations was reported (Bronk and Glibert, 1994; Eppley and Renger, 1992; Laws, 1984; Slawyk et al., 1990a; Thybochristensen and Blackburn, 1993; Ward et al., 1989). Since the lost tracer was suspected to be in the DON pool, new methods were developed to study the nitrogen flux through the DON pool (see Section 3.5), although depending on the methodology, losses could also occur to other pools. For instance, achieving balance in $^{15}NO_3^-$ uptake experiments may require isolation of the nitrite pool in addition to PON due to phytoplankton excretion of nitrite, if the nitrate extraction protocol does not include nitrite. Such excretion can occur due to changes in light level or to poor coordination of nitrate and nitrite reduction within the cells, processes that appear to account for maintenance of the primary nitrite maximum in the ocean (Lomas and Lipschultz, 2006). Net nitrate uptake into PON would thus only equal gross uptake if all excreted nitrite was assimilated during the incubation. Filtration artifacts have also been suggested to cause loss of label, but direct analyses have not found it to be a significant problem (although see Wilkerson and Grunseich, 1990). Although mass balance should always be a goal as discovery of unaccounted losses stimulates new research, not all studies that have tested mass balance have found problems (Lipschultz et al., 1986; Slawyk et al., 1998).

3. ANALYTICAL SEPARATIONS

3.1. Particles

The simplest and most common method to remove particles from incubation water is by filtration (but see Slawyk et al., 1998 for an alternative approach). Glass–fiber filters, typically with 0.7 μm nominal cutoff (GF/F), are often the choice (Kanda et al., 1998) although other options have been explored such as Teflon, polycarbonate or silver filters. As has been shown for studies of primary production, care should be taken to minimize the pressure differential across the membrane to minimize cell rupture. For instance, Wilkerson and Grunseich (1990) reported 20–70% reduction in ^{15}N uptake with 13 cm Hg pressure differential compared to gravity filtration. Altabet (1990) tested aluminum oxide filters for natural abundance measurements since they are available in precisely defined pore size that would facilitate size fractionation studies. Raimbault et al. (2000) compared the two filter types for $^{15}NO_3^-$ uptake and found that while the GF/F filter failed to capture up to 60% of the PON, uptake rates were similar on both filters, implying that the small particles escaping filtration were not utilizing nitrate. Interestingly, although Altabet (1990) noted that the polycarbonate ring around the filter had to be removed via combustion before use, Raimbault et al. (2000) did not pre-combust the aluminum oxide filters but did not state how they dealt with it. Libby and Wheeler (1997) compared 0.2 μm teflon filters against GF/F filters for PON analysis and also found 60% higher PON on the teflon. Prior to analysis, one would either need to remove

the particles from the filter or to digest the material on the filter by persulfate oxidation (Raimbault and Slawyk, 1991). One could also use Ag filters that have known pore sizes and can be directly combusted, but they are obviously expensive. GF/F filters have been found to retain only ~50% of bacteria (Taguchi and Laws, 1988) which could partially account for the discrepancy between the 0.2 and 0.7 μm filter sizes (for more on PON analysis, see Chapter 28 by Bronk and McCarthy, this volume).

In addition to incomplete retention of particles, glass–fiber filters adsorb dissolved organics on the high surface area of the filter (Maske and Garcia-Mendoza, 1994). For ^{15}N studies, although mass retention of DON is not a major issue, retention of the highly labeled incubation water on the filter has the potential to affect the isotopic ratio of the retained particulates so that wetting the filter prior to filtration and rinsing afterwards with filtered seawater is a common practice. Filtered seawater is used to prevent osmotic shock of the living particles.

Regardless of the type of filter, the particle load needs to be preserved prior to mass spectroscopic analysis. Common options include drying or freezing the filter, with storage of the filter in Whirlpak bags, microcentrifuge tubes, alumninum or tin foil, or plastic Petri dishes. Lorrain et al. (2003) assessed the effects of removing inorganic carbonates and of preservation on suspended particulate samples for natural abundance ^{13}C and ^{15}N analysis. They observed that fuming with HCl vapors followed by drying resulted in no change, whereas dilute acid wash or freezing were capable of altering the signal. Since any changes were at natural abundance levels, either freezing or drying should be effective for tracer experiments. If fuming with HCl is used, note that filters should not be wrapped in tin or aluminum since the fumes are corrosive.

Determination of the size class of active consumers of nitrogen is a common objective and the typical approach is sequential filtration through different pore size filters. A filter with precise filtration characteristics, such as a 5 μm Nuclepore filter, is often used to remove large particles after incubation with ^{15}N, followed by removal of smaller particles onto a GF/F filter (Probyn, 1985; Probyn et al., 1990; Glibert et al., 1992; Hasegawa et al., 2000; Ward and Bronk, 2001). Uptake by the larger particles can be assessed either by removal of the large particles from the Nuclepore filter and capture onto a glass–fiber filter for analysis, or a parallel sample can be filtered onto a GF/F to determine uptake by the entire size spectrum from which is subtracted the uptake of the <5 μm particles. Alternatively, the wet oxidation method of Raimbault and Slawyk (1991) can be employed directly with the Nuclepore filter. An alternative to size fractionation is density fractionation, recently employed by Hamilton et al. (2005) who used gradient centrifugation to separate benthic algae from detritus.

Flow cytometry, where various properties of individual particles can be interrogated as a basis for cell sorting, can also be used for size fractionation. Lipschultz (1995) for instance, found that phytoplankton >10 μm preferentially used nitrate over ammonium compared to cells <10 μm. Flow cytometry also has the distinct advantage over all other particle analysis methods by permitting fractionations based on pigment content that allows isolation of phytoplankton from bacteria, or separation by pigment type to permit isolation of cyanobacteria from algal cells. Current flow cytometry (FCM) and CF-IRMS technology now permits dual ^{13}C and ^{15}N

analysis on cells as small as *Prochlorococcus* spp. (Casey *et al.*, 2007). Flow cytometry has also been applied for studies using other tracers such as ^{35}S (Zubkov and Tarran, 2005; Zubkov *et al.*, 2001) and ^{13}C (Li, 1994; Pel *et al.*, 2003). It is surprising that flow cytometry has not been more widely applied, but hopefully the continuing advances in instrumentation and availability of molecular probes will attract more researchers to this versatile tool.

Size fractioned particles can also include individual macroscopic particles ranging from large phytoplankton to seagrasses to zooplankton that have sufficient mass for analysis. Villareal and Lipschultz (1995) analyzed the internal nitrogen pool of individual cells of large, solitary phytoplankton such as the giant diatom *Ethmodiscus rex*, and Villareal *et al.* (1993) isolated visible aggregations of *Rhizosolenia* spp. for isotopic analysis. Nitrogen fixation by individual colonies of the cyanobacteria, *Trichodesmium*, has also been amenable to stable isotopic analysis (Orcutt *et al.*, 2001) using the low mass protocol of Owens and Rees (1989). Larger organisms such as seagrasses or zooplankton (Hasegawa *et al.*, 2001) can also readily be isolated, although care is required to prevent exceeding the mass limit of the CF-IRMS instrument or to adequately homogenize the sample to ensure analysis of a representative fraction.

3.2. Ammonium

As the primary inorganic nitrogenous nutrient in most marine ecosystems, tracing the fate of ammonium is critically important. Initial approaches to isolating ammonium for isotopic analysis by steam distillation were derived from the soil literature. Difficulties distilling samples with the typically low ammonium concentrations present in marine waters has led to development of a range of alternative techniques, with few researchers still utilizing distillation. In all methods involving ammonium, great care is required to minimize ammonium contamination from the atmosphere (e.g., cleaning compounds such as Windex), from sample/reaction containers and from reagents.

3.2.1. Organic extractions

Since the typical analysis of ammonium concentration relies on formation of a colored compound, indophenol, investigators have extended the analysis to extract and concentrate the dye for isotopic analysis, either by solvent extraction (Dudek *et al.*, 1986), or by solid phase extraction (Selmer and Sorensson, 1986; Wheeler *et al.*, 1989). In any such method, inadvertent extraction of dissolved organic nitrogen is a crucial concern that can strongly affect the blank and that can vary by depth or location. To overcome this problem, Preston *et al.* (1996b) further developed the extraction by derivatizing the indophenol dye after solid phase extraction and then analyzing the derivatized dye by gas chromatography–mass spectrometry (GC–MS). Even if compounds other than indophenol are derivatized, the GC separation, as well as monitoring the specific molecular ions for the derivatized indophenol, greatly reduces the potential interference from unknown compounds. Additionally, the GC–MS requires far less mass than typical CF-IRMS analysis, so that analysis of 50 nM ammonium in 50 ml samples becomes possible rather than extracting several liters or adding carrier ammonium to reach sample masses of

~1–4 μmol. The precision is also more than sufficient for studies of isotopic dilution (~0.05–0.22 at.% excess at 7% atom percent abundance). The main drawback is the multiple steps required for the analysis: dye formation, extraction, derivatization and isotopic analysis. Clark *et al.* (2006) recently extended the Preston *et al.* (1996b) approach by addition of a deuterated internal standard that minimizes the accumulated errors arising during the extensive sample processing, presenting results for ammonium regeneration rates from the North Atlantic at ammonium concentrations as low as 10 nmol kg^{-1}.

A recent improvement builds on the fluorometric analysis of ammonium concentration (Holmes *et al.*, 1999) with OPA (ortho–phthalaldehyde). Johnston *et al.* (2003) extracted the OPA-NH$_4^+$ derivative (1-sulfonato-iso–indole) by solid phase extraction and found that derivatized amino acids were not retained along with the indole on the column so that the method was specific. Since they developed the method for natural abundance work, the requisite high precision obliged analysis by CF-IRMS and its attendant high mass requirement (7.14 μmol N for a standard deviation of 0.5‰) that is difficult to achieve in oligotrophic waters. Since tracer experiments do not require such high precision, a lower mass could likely be used with the inherently lower precision GC–MS.

Another derivatization approach was developed by Gardner *et al.* (1991, 1993, 1995) based on high performance liquid chromatography. Ammonium is directly separated from the sample using cation exchange and then detected fluorometrically by post–column derivatization with OPA. Although not completely separated from ^{14}NH$_4^+$, increasing proportions of ^{15}NH$_4^+$ increased the retention time of the single peak, hence permitting construction of a calibration curve. The method has the advantage of using small sample size (<5 ml but high concentration) and not requiring extensive chemistry prior to the HPLC analysis, but does require careful matching of standards to sample salinity. It also requires large tracer additions to achieve a reasonable precision (standard error of 2 at.%), which limits its utility to habitats such as high nutrient coastal regimes where turnover times are fast and the perturbation is not thought to greatly affect the experimental interpretation.

3.2.2. Gaseous extraction

The second class of methods for isolating ammonium from samples relies on conversion of the ionic to gaseous form at high pH, with differences arising from the techniques used to remove the ammonia. As noted earlier, initial protocols were adopted from the extensive soil science literature on steam distillation (e.g., Glibert, 1982). However, this approach suffered from sample carryover effects and a high blank, both of which were partially responsible for the extensive literature as various approaches were taken to resolve the problems. Rather than actively forcing the gas from solution by heating, a range of methods have been developed that rely on passive diffusion into an acidic solution (Blackburn, 1979; Holmes *et al.*, 1998; Kristiansen and Paasche, 1989; Laima, 1993; Risgaard–Petersen *et al.*, 1995). Although these techniques generally require long periods of time to transfer the gas from the solution to the acidic trap, they do not require active intervention. For instance, Holmes *et al.* (1998) sandwiched a glass–fiber filter saturated with acid between two Teflon filters and floated it on the high pH sample while oscillating the

solution to breakdown the diffusion boundary layer and gently heating (40°C). Ammonia slowly diffused through the Teflon filters to the acidic filter over 14 days, whereupon the filter was recovered, dried and directly analyzed by CF-IRMS. A variant of this approach was employed by Lipschultz and Cook (2002) who extracted $^{15}NH_4^+$ from the cytoplasm of a sea anemone by pumping the samples through Teflon tubing surrounded by a static solution of acid that was then assayed for isotopic composition. As noted below, the diffusion methods also are often employed for sequential removal of ammonium, nitrate and DON after conversion of the additional components to ammonium (e.g., Slawyk and Raimbault, 1995).

3.3. Nitrite and nitrate

Isotopic analysis of these oxidized species requires conversion to forms amenable for entry into the mass spectrometer. The methods can be loosely grouped by the level of reduction, with the simplest being conversion of nitrate to nitrite, followed by conversion to dinitrogen, or complete reduction to ammonium. A range of methods exists for the separation of nitrite via organic extraction (Section 3.3.1). The analysis of ammonium has been discussed above (Section 3.1), so this section focuses only on the actual conversion of oxidized nitrogen to ammonium (e.g., Devarda's alloy, mossy Cd (Jones, 1978), or Zn dust (Gardner *et al.*, 1995)). Conversion to N_2 or N_2O will be mentioned, but direct analysis of these gases for denitrification studies will be covered in Section 3.4.

A common practice is to reduce NO_3^- to NO_2^- via cadmium reduction prior to isotopic analysis so that both species are combined for analysis. Although NO_2^- concentrations in many marine systems are very small compared to NO_3^-, there are instances where one is interested in both separately or in just NO_3^-. One approach is to measure the concentrations of both species and then, by assuming no tracer is present in NO_2^-, one can back calculate the actual $^{15}NO_3^-$. Alternatively, removal of NO_2^- prior to reduction of NO_3^- can be achieved by several approaches. Olson (1981) used sulfamic acid, however since the destructive action of the strong acid is reactivated during the subsequent diazotization reaction with NO_3^- after its reduction to NO_2^-, the yield of the final azo dye can be low and variable (Lipschultz, 1984). Yakushiji and Kanda (1998) took advantage of the reaction of sulfanilic acid with NO_2^- (see below). Rather than forming the final azo dye, the diazonium ion can be destroyed by heating which effectively destroys the initial NO_2^- present in the sample. Two new techniques remove nitrite by reaction with sodium azide in acetic acid buffer to produce N_2O (McIlvin and Altabet, 2005) or via reaction with ascorbic acid to produce NO which is then removed bubbling (Granger *et al.*, 2006).

3.3.1. Organic extractions

The earliest approaches to measuring the concentration of nitrate and nitrite relied on the conversion to a highly colored azo dye. Initial approaches to analysis of the isotopic content of nitrate and nitrite relied on similar conversion followed by extraction of the dye into an organic solvent in a separatory funnel. The organic phase was then removed, evaporated and the extracted dye analyzed for isotopic content (Olson, 1981; Schell, 1978; Wada and Hattori, 1972). The extraction was

improved by Kator *et al.* (1992), who employed the same basic solid phase technique as had been applied to analysis of ammonium (Section 3.2.1). Yakushiji and Kanda (1998) suggested use of sulfanilic acid as the initial coupling reagent rather than aniline that had been used by prior workers, as they found the aniline derivative was difficult to effectively extract. As with ammonium, the same issues of large, variable blanks are present with these methods since other organic compounds are extracted that can co-elute with the dye. Yakushiji and Kanda (1998) found that the blank can be greatly reduced by passing the effluent from the NO_3^- reduction step through the solid phase column prior to formation of any dye, hence removing adventitious organic nitrogen contamination prior to derivatization. This approach also worked sufficiently well for Johnston *et al.* (2003) that they were able to make natural abundance measurements. One still has to consider contamination from the aniline reagent used for the derivatization, but this should be far more constant than unknown DON compounds.

As for ammonium, Preston *et al.* (1998) resolved the blank issue for nitrate by derivatization of the eluted azo dye to a compound that was amenable to GC–MS analysis. An additional advantage was the ability to analyze very small amounts of nitrate or nitrite, which meant that low concentrations could be analyzed without addition of carrier nitrogen. Recently, Clark *et al.* (2007) modified the GC–MS approach (Preston *et al.*, 1998) by addition of an HPLC preconditioning step to maintain sensitivity of the mass spectrometer and use of a deuterated internal standard to increase accuracy. An extension of this type of approach eliminates the entire dye formation and extraction step by directly reacting nitrate with penta-fluorobenzyl bromide (PFB) to form an ester that can be analyzed by GC–MS (Miyajima *et al.*, 2005). Although developed for DON analysis after oxidation to nitrate (see Section 3.5.3), it could be adapted to $^{15}NO_3^-$ analysis itself. Interestingly, as well as reacting with NO_3^-, PFB will also directly react with NO_2^-, so that each analyte can be measured independently and simultaneously as the products have different masses. However, the method does suffer from poor derivatization of NO_3^- in the presence of Cl^-, which is clearly an issue for seawater samples! Miyajima *et al.* (2005) note that strict care is required to calibrate the procedure with standards at the same salinity as the samples.

3.3.2. Conversion to gaseous forms

An alternative approach for analysis of NO_3^- or NO_2^- is conversion to a gaseous form such as N_2O, N_2, or NH_3. Conversion to ammonia commonly employs Devarda's alloy combined with moderate heating. Sigman *et al.* (1997) observed that the alloy can add a significant blank that varies with the batch, and note that combustion of the alloy failed to reduce the blank while also reducing the reduction efficiency. They also suggest that Devarda's alloy may cause breakdown of some components of the DON pool in seawater, resulting in contamination of the $^{15}NO_3^-$ isotopic signal. Tanaka and Saino (2002) replaced Devarda's alloy with an aluminum reagent that reduced the blank and permitted analysis of nitrate at low concentrations. Once ammonia is formed, it can be analyzed by any of the methods described in Section 3.2.

Several investigators have used biological reduction of NO_3^- or NO_2^- to gaseous forms amenable for direct analysis via mass spectrometry. Risgaard-Petersen *et al.* (1993) and Jensen *et al.* (1996) used cultures of denitrifying bacteria to respire NO_3^- and NO_2^- to N_2 which was then stored for analysis by mass spectrometry. Given the large atmospheric burden of N_2 that represents a constant threat of massive contamination, Sigman *et al.* (2001), building on earlier efforts (Hojberg *et al.*, 1994), chose to work with N_2O by employing a mutant strain of a denitrifying bacteria that only produced N_2O. This approach had several advantages over chemical reduction to N_2 such as a 100-fold reduction in the sample size requirement, a reduction in the blank size, and a reduced time requirement of the analysis. A chemical method using sodium azide in acetic acid buffer to produce N_2O from NO_2^- has been successfully employed by McIlvin and Atlabet (2005) for natural abundance measurements in aquatic systems, and could thus been useful for tracer work.

3.4. Gaseous nitrogen—N_2O and N_2

In addition to the previously discussed methods where production of N_2 or N_2O from other forms of nitrogen is an analytical step, there are related methods for analysis of the gases where the gas itself is the form of interest. For instance, studies of denitrification have been transformed by the application of the isotope pairing technique (IPT) (Nielsen, 1992) that requires analysis of all three isotopic variants of N_2 ($^{28}N_2$, $^{29}N_2$, $^{30}N_2$). An excellent review of the IPT approach, including compilation of studies, and a comparison and discussion of various approaches to using IPT such as batch mode versus flow-through systems, was provided by Steingruber *et al.* (2001). The technique accounts for the two potential pathways for the appearance of the ^{15}N: either denitrification or coupled denitrification–nitrification where the source of some of the N is from nitrification (see Chapter 6 by Devol, this volume). The critical difference between direct analysis of the gas, and gas produced by various reduction techniques (Section 3.3), is that no information is retained in the isotopic forms of N_2 after reduction and one can simply employ standard mass spectroscopic analysis where the ion current in all three collectors is considered together. Although emission spectroscopy is no longer often used, it is capable of analysis of only the total isotopic ratio rather than all of the isotopic combinations. The comprehensive review of all extant denitrification methods for both terrestrial and aquatic systems by Groffman *et al.* (2006) should be consulted before embarking on an assessment of denitrification.

Recent work has demonstrated that denitrification is not the only pathway producing N_2. The anaerobic ammonium oxidation (anammox) pathway has received considerable attention and appears to account for the lack of ammonium in some denitrifying regimes (e.g., Dalsgaard *et al.*, 2003; Kuypers *et al.*, 2003). In addition to $^{15}NO_3^-$ to trace denitrification, parallel incubations with $^{15}NH_4^+$ are used to track the anammox pathway, $NH_4^+ + NO_2^- = N_2 + 2H_2O$ (Dalsgaard and Thamdrup, 2002; Risgaard-Petersen *et al.*, 2003, 2004; Rysgaard and Glud, 2004; Thamdrup and Dalsgaard, 2002;). Risgaard-Petersen *et al.* (2003) provide details on the calculations for the IPT (N.B. erratum by Risgaard-Petersen *et al.*, 2004) when both denitrification and anammox occur simultaneously because anammox alters the expected distribution of the N_2 isotopomers produced solely from denitrification.

An additional complication is the observation of DNRA in culture and the field by anammox bacteria (Kartal *et al.*, 2007). They reported production of ^{15}N:^{15}N from *Kuenenia stuttgartiensis* provided $^{15}NO_3^-$–with production of $^{15}NH_4^+$ that then fed the anammox pathway and thus masqueraded as denitrification.

Extraction of N_2 from water samples, generally stored in a gas–tight container sealed with a septum (e.g., Exetainer), involves partitioning of the gas into a suitable headspace such as helium. The gas is allowed to equilibrate and the headspace is sampled for introduction of the gas phase into the mass spectrometer (Capone and Montoya, 2001). Hamilton and Ostrom (2007) provide a detailed exploration of the use of exetainers and demonstrated high fidelity with the classic, large-volume extraction protocol. Rather than extraction into a headspace, several investigators have employed membrane inlet mass spectrometers (MIMS) to directly sample N_2 for denitrification and nitrogen fixation studies (An *et al.*, 2001; Jensen *et al.*, 1996). MIMS can also be used directly to measure denitrification by monitoring changes in the N_2/Ar ratio over time since the Ar composition reflects changes in temperature and other physical factors while change in the N_2 concentration reflects the balance between nitrogen fixation and denitrification (Kana *et al.*, 1998). An advantage of combining MIMS with the isotopes is the ability to separate the two contributions to the net flux. MIMS also obviates the need for the generally slow, separate extraction step. The membrane inlet configuration varies but always features a gas–permeable membrane that separates the mass spectrometer from the solution (Hartnett and Seitzinger, 2003; Kana *et al.*, 1994). A probe inlet (Hartnett and Seitzinger, 2003) permits analysis of depth zonation of rate processes in sediments but has only been employed for N_2/Ar measurements. Either a quadrupole or magnetic sector mass spectrometer can be used for the isotope pairing analysis, but the quadrupole can also simultaneously determine the N_2/Ar ratio.

Special mention goes to Sameshima-Saito *et al.* (2004) who developed a gas chromatographic method for $^{15}N_2$ analysis. Interestingly, they found that using a N_2 carrier gas of natural abundance with a thermal conductivity detector (TCD) permits detection of the enriched $^{29}N_2$ and $^{30}N_2$ species due to slight differences in their respective thermal conductivity from the $^{28}N_2$ carrier. However, although the method only requires the commonly available TCD-GC, does not suffer from atmospheric N_2 contamination problems, and is fairly precise, it is incapable of separating the varying contributions of $^{29}N_2$ and $^{30}N_2$ as both peak elute simultaneously. Thus, as with the MIMS approach, it is only suitable to screen for total N_2 production.

Analysis of $^{15}N_2O$ during nitrification and denitrification rate studies has been limited. Punshon and Moore (2004) further developed the method of Barnes and Owens (1998) to extract $^{15}N_2O$ produced from either $^{15}NH_4^+$ or $^{15}NO_3^-$ by natural samples. Using purge-trap gas chromatography with a quadrupole mass spectrometer, they were able to determine production rates for N_2O from nitrification. No production was observed with $^{15}NO_3^-$, which was not surprising since denitrification is not expected in the oxic water samples. The IPT for $^{15}N_2$ production from two different precursor pools has been extended to N_2O analysis by Bergsma *et al.* (2001) for production of N_2O from soils, and Master *et al.* (2005) explored the theory of isotopic pairing in IPT to include N_2O production in sediments. Nicholls *et al.* (2007) have recently applied a suite of ^{15}N additions to identify the pathways and rates of N_2O production in the suboxic regime of the

Arabian Sea. After extracting the gases by equilibration, they utilized cryofocusing of N_2O with separation from CO_2 on a capillary column before isotopic analysis by CF-IRMS of the N_2O. They found the vast bulk of N_2O was produced from NO_2^- reduction, with little contribution from nitrification at any depth within the suboxic waters. Their results also suggested an alternate source of nitrogen for dentrification, such as DON or DNRA via internal coupling to N_2 production.

Nitrogen fixation studies do not require analysis of the precursor N_2 isotopic composition, but rather the particulate receiving pool of prokaryotes and possibly DON or ammonium if there is significant leakage of the tracer to those pools during the course of the incubation (e.g., Glibert and Bronk, 1994). Tracer methods for nitrogen fixation have not been popular due to the ease of applying the acetylene reduction method (for a discussion, see Chapter 4 by Carpenter and Capone, this volume). However, as clearly analyzed by Montoya *et al.* (1996), use of $^{15}N_2$ has some significant advantages that have led to increased use in recent years. Among the advantages are increased sensitivity, ability to partition the total fixation rate into different pools (i.e., DON) or different organisms, and increased certainty since the acetylene reduction method always requires a potentially variable conversion factor. An example of these advantages is the recent batch culture study of Mulholland *et al.* (2004) that quantified the appearance of $^{15}N_2$ as NH_4^+ and DON, finding about 93% of the tracer was retained within *Trichodesmium* in 2 h incubations over the first 11 days of the experiment with little production of DON. They also found that a 4:1 conversion factor for the acetylene based rate best approximated the gross $^{15}N_2$ fixation rate. Capone and Montoya (2001) provide methodological details on both denitrification and nitrification using ^{15}N and acetylene approaches.

3.5. Dissolved organic nitrogen

Development of methods for analysis of the bulk DON pool have accelerated due to increased interest in the cycling of dissolved organic carbon (DOC). Analysis of this pool generally requires considerable processing to remove dissolved inorganic nitrogen (DIN) that would affect the DON isotopic ratio and to remove salts that are deleterious to the mass spectrometer. Additionally, in contrast to most other tracer applications, the interpretation of the experimental results is still open to discussion due to uncertainty in both the identity of the precursor pool and the huge heterogeneity of the DON pool. For instance, Bronk and Glibert (1993) isolated phytoplankton derived $DO^{15}N$ and employed it as a tracer for DON uptake, forcing the assumption that the pool was uniformly labeled and wasn't simply remineralized to ammonium prior to uptake by the particulate pool. Improvements in the ability to isolate specific fractions for analysis (Nunn and Timperman, 2007) will certainly help improve our understanding of the role of DON in the marine nitrogen cycle (see Section 6).

3.5.1. Urea
The basic approach for analysis of ^{15}N–urea is to use the enzyme urease to convert urea to ammonium. The few available methods differ primarily in handling the removal of pre-existing ammonium and the extraction of newly produced ammonium (see Section 3.2 for options). Hansell and Goering (1989) used ^{15}N–urea as a

precursor, but did not isolate the urea for isotopic analysis, using the breakdown rate for ^{14}C-urea to estimate the turnover time for urea and so correct for isotope dilution. Slawyk et al. (1990b) tested both simple diffusion and a combined distillation followed by diffusion approach to remove the ab initio ammonium and then the ammonium produced by the urease enzyme. The ammonia diffusion was clearly superior and they reported high precision despite only removing ~90% of the initial ammonium. They found extremely rapid turnover of urea with an 80% recovery of their tracer, proposing the missing tracer might be found as ^{15}NH$_4$$^+$, which suggests the need to monitor that pool rather than simply discard the pre-existing NH$_4$$^+$. Indeed, Lipschultz (2001) found rapid appearance of ^{15}N in ammonium during incubations with ^{15}N-urea in the Sargasso Sea. Rysgaard and Risgaard-Petersen (1997) used cation exchange to remove ammonium from seawater samples prior to enzymatic hydrolysis, followed by their micro-diffusion method (Risgaard-Petersen et al., 1995) to remove the ammonium and convert it to N$_2$ for mass spectrometry. Impressively, the cation exchange removed all of the 20 μM ^{15}NH$_4$$^+$ from a solution so that no residual tracer could be detected by mass spectrometry. Although only 10 nmol of urea was required, the 2 ml sample used in their protocol would require addition of carrier or rescaling of the volumes to handle the typically 10–100× lower concentrations found in natural waters. Lomas et al. (2002) modified Slawyk et al.'s (1990a,b) protocol to be able to use their normal distillation procedure and were able to remove 95% of the NH$_4$$^+$ with little urea loss and subsequently recover 95% of the NH$_4$$^+$ produced from the urease step.

Another approach (Patterson et al., 1993), that has not been attempted in aquatic systems, uses the same basic derivatization scheme for both amino acids and for urea that was employed by Preston et al. (1996a) for amino acids in seawater. Using N-methyl-N-(tert-butyldimethysilyl)trifluoroacetamide (MTBSTFA), they quantified the isotopic composition of doubly and singly labeled ^{15}N-urea in blood plasma, which suggests the method should be transferable to marine systems.

3.5.2. Amino acids

Two similar methods for isotopic analysis of amino acids in marine systems permit calculation of the turnover time of many of the individual amino acids (Preston et al., 1996a). In the earlier work, free amino acids were first extracted onto a Chelex 100 column, then eluted and derivatized for GC–MS analysis. The same derivatizing agent and GC column is used as Preston et al. (1998) used for their nitrate procedure (Section 3.3.1), making experience with one protocol readily applicable to the other. Most of the common amino acids can be simultaneously determined in the resulting ion chromatogram, but some such as Tyr, Glu and His suffer from low derivatization yield and are not detected. Sommerville and Preston (2001) extended the earlier work by an initial derivatization with IsoBOC (isobutylcarbonyl) to permit extraction with solid phase chromatography (rather than Chelex 100) before the same derivatization and GC–MS analysis as used by Preston et al. (1996a). By selective ultrafiltration and hydrolysis to free amino acids, they were also able to analyze the isotopic composition of amino acids in dissolved combined amino acids, dissolved peptides and dissolved proteins. These methods also permit measurement

of the production of amino acids from $^{15}NH_4^+$ or $^{15}NO_3^-$ rather than taking a black-box approach of extracting the entire DON pool.

One can also extract and analyze the amino acids within an organism, either as dissolved free or combined amino acids, as well as from proteins after hydrolysis with strong acid. Dohler and Hagmeier (1997) used 80% ethanol to extract amino acids from field populations of phytoplankton for ^{15}N analysis. Kanda (1988) used hot water, and Lipschultz and Cook (2002) used trichloroacetic acid (TCA) to extract the low molecular weight (LMW) pool, which includes the amino acids, for ^{15}N analysis. McClelland *et al.* (2002) measured the natural abundance of amino acids in proteins of zooplankton after hydrolysis by forming *N*-pivaloyl-*i*-propyl (NPP)–amino acid esters. Rather than analyzing the derivatized compounds by GC–MS, they separated the compounds by GC, then combusted the compounds exiting the GC for analysis by CF-IRMS as N_2 gas (GC-c-IRMS). Piniak *et al.* (2003) used the same derivatization to measure the production of free amino acids within an anthozoan after exposure to $^{15}NH_4^+$. Recently, Veuger *et al.* (2005) developed an analytical protocol similar to McClelland *et al.* (2002) using GC-c-IRMS to trace assimilation of ^{15}N into either D- or L-alanine. This provides a measure of bacterial versus algal uptake since only the bacteria synthesize D-Ala. Veuger *et al.* (2007) have presented an update to address potential artifacts such as racemization and varying bacterial D/L ratios. In addition, either method can be used for analysis of the full suite of amino acids. Extraction of internal pools is mentioned again in Section 3.6, and compartmental analysis of such data is discussed in Section 5.2.2.

3.5.3. Bulk DON

Separation of the overall DON pool from samples presents a wealth of sequential analytical choices that has fostered considerable disagreement over the reliability of the final results (Bronk and Ward, 2000). In general, the protocols require initial removal of PON, followed by removal of DIN, and finally conversion of the remaining DON using wet oxidation procedures based on standard methods for measuring DON concentrations. The resulting NO_3^- can then either be converted to a form amenable for mass spectrometric analysis (Section 3.3), or be reduced to ammonium before analysis (Section 3.2). Taking an historical approach in the next section, I summarize the two core methods in common use and the disagreements between their advocates. However, in addition to extant issues over the quality of the data, there are disagreements over the proper interpretation of the tracer during uptake of DI^{15}N that will be discussed in Section 5.2.2.

Bronk and Glibert (1991) initially employed an ion retardation column to remove DIN after filtration of particles, leaving only the DO^{15}N for mass spectrometric analysis. With this simple procedure, the DON was ready for isotopic analysis after evaporating the water. Unfortunately, the manufacturer no longer makes an adequate resin (Bronk, personal communication) and so this procedure is defunct. In addition, they analyzed the internal, LMW pool of the particulates using TCA to precipitate the higher molecular weight compounds. This step was motivated by a desire to directly analyze the presumptive precursor for the external DON rather than to assume the precursor was the DI^{15}N, since the tracer would be diluted within the cells when the newly synthesized, labeled organic compounds mixed with pre-existing ones or with ones created via catabolic processes. To overcome

the precursor problem, Bronk *et al.* (1994) combined the separated particulate and dissolved organic pools to calculate the ''gross'' uptake of $DI^{15}N$.

Slawyk and Raimbault (1995) developed a different approach, based on the methods of Axler and Reuter (1987), that employed standard methods for DON concentration measurements. Again beginning with filtration of particles, they tested both distillation and diffusion to remove the DIN before oxidizing the DON with persulfate to NO_3^-, which was then reduced to NH_4^+ using Devarda's alloy. The resulting NH_4^+ was finally captured for analysis by either distillation or diffusion. They recommended diffusion to remove NH_4^+ due to better recovery. Subsequently, and for the same reasons as Bronk *et al.* (1994), they retained the particulates with the DON so that the oxidation step included both pools, permitting calculation of gross uptake of $DI^{15}N$ (Slawyk *et al.*, 1998). By comparing gross uptake (they used the term WOPMC for ''without particulate material collection'') to the standard uptake measurement into just the particles, they were able to estimate DON uptake.

Without access to the ion resin, Bronk and Ward (1999) switched to vacuum distillation to remove NH_4^+, followed by peroxide oxidation under UV radiation to convert DON to NO_3^-. The nitrate was then reduced to nitrite and isolated by solvent extraction using tricholoroethanol (Olson, 1981) (Section 3.3). As noted above, Miyajima *et al.* (2005) have recently also analyzed DON by conversion to nitrate but employed GC–MS analysis of the PFB derivative. Their approach has the advantage of requiring small sample sizes, which also permits simply drying the sample to remove NH_4^+ during the DIN removal step. Clearly, any of the methods for $^{15}NO_3^-$ analysis (Section 3.3) would be suitable for this purpose too.

The positives and negatives of the different approaches to removal of DIN (vacuum distillation vs diffusion) and to convert DON to an extractable form are still a matter of disagreement (Bronk and Ward, 2000). Primary issues of concern include premature conversion of DON to DIN during the initial removal of DIN, and poor oxidation efficiency of DON to nitrate. Under the warm basic conditions used to remove DIN, DON can be lost as a result of basic hydrolysis of amino groups and subsequent volatilization of NH_3 (Bronk and Ward, 2000; Sigman *et al.*, 1997). With respect to poor oxidation efficiency for DON, a large community-wide comparison of DON oxidation methods indicates that proper use of the persulfate oxidation, UV oxidation, and high temperature combustion methods all yield similar results (Bronk *et al.*, 2000). Care should be taken, however, to monitor the performance of UV lamps over time when using the UV oxidation method and to take extreme care in keeping blanks low when using the persulfate oxidation method (e.g., Knapp *et al.*, 2005). The best approach to removing DIN prior to this step though remains unresolved at this point and should be evaluated by each investigator for their particular circumstances.

3.6. Internal pools

The isotopic composition of the internal constituents of cells has occasionally been determined to understand metabolic pathways. Separation of ^{15}N-labeled cellular constituents based on solubility in TCA was used by Wheeler *et al.* (1982) and Glibert and McCarthy (1984). Bronk and Glibert (1991) also used TCA extraction, followed by ultrafiltration to extract the soluble LMW component of field samples.

Kanda *et al.* (1988) similarly used solubility, but employed hot ethanol rather than TCA. Both approaches separate LMW compounds into the soluble fraction, and macromolecular compounds into the insoluble fraction. However, care should be taken comparing these different approaches, not just due to differential solubilities, but also the operational definition of "soluble" since filtration with micron pore size filters (e.g., GF/F) will produce a sharply different molecular weight cutoff compared to ultrafiltration or centrifugation. Although not using ^{15}N, Dortch and coworkers (Clayton *et al.*, 1988; Dortch *et al.*, 1984; Thoresen *et al.*, 1982) examined a range of techniques for such separations, including hot water rather than ethanol. Although it is not clear exactly where the molecular weight cut-off actually lies with these techniques, the soluble fraction is assumed to be primarily dissolved free amino acids and some portion of the dissolved combined amino acid pool. Kanda *et al.* (1988) used a simple compartmental model (see Section 5) of time course data to calculate the assimilation of $^{15}NH_4^+$ into the LMW pool and the movement of ^{15}N into, and out of, the macromolecular fraction. They found that exponentially growing diatom cells maintained a small internal LMW pool that rapidly (1–2 h) came into isotopic equilibrium with the external source and subsequent assimilation reflected macromolecular synthesis rates.

Appearance of ^{15}N into specific components of the heterogeneous and ill-defined LMW DON pool, such as individual amino acids within cells, either free or as components of proteins, has occasionally been determined (e.g., Dohler and Hagmeier, 1997). Piniak *et al.* (2003) measured the appearance of $^{15}NH_4^+$ into individual free amino acids in the sea anemone, *Aiptasia pallida*, but were not able to quantify rates since the isotopic composition of the precursor $^{15}NH_4^+$ inside the cells was not determined. The appearance of $^{15}NH_4^+$ inside cells was determined by Lipschultz and Cook (2002) in the anemone *Condylactis* using a teflon membrane to separate NH_4^+ from the remainder of the LMW compartment, trapping the NH_3 into an acidic solution on the other side of the membrane. Mulholland and Bernhardt (2005) extracted intracellular NH_4^+ from *Trichodesmium* IMS101 using boiling, Milli-Q water and then isolated NH_4^+ in the extract using solid phase extraction after creating indophenol dye (Section 3.2.1). Application of Preston *et al.*'s (1996a) method would permit analysis of the turnover of internal amino acids in a manner similar to that employed by Hama *et al.* (1987) using ^{13}C. Both McClelland and Montoya's (2002) or Veuger *et al.*'s (2005) approaches for ^{15}N-amino acid analysis of proteins also opens access to study of amino acid metabolism of marine organisms ranging from bacteria to zooplankton.

4. ^{15}N Measurement

Detailed reviews of the theory and practical aspects of the instrumentation for ^{15}N measurements are available in Preston (1993) for emission and Mulvaney (1993) for mass spectrometry.

4.1. Emission spectrometers

Emission spectrometers use the difference in optical properties of N_2 that arise from the increased mass of the different isotopomers to calculate the isotope ratio. The equipment is less expensive than mass spectrometers due to the lower complexity of

the equipment. The simplicity and low cost of the instrument, along with greater reliability, led to its initial popularity. Although the technique lacks precision compared to mass spectrometry, it is generally sufficient for tracer experiments (Preston, 1993). Greatly improved precision was achieved using diode array imaging technology (Lipschultz, 1993), but inherent limitations of the optical technique have stymied further improvements. Although it is possible to couple emission spectrometry to combustion systems and to automate the analysis (Therion *et al.*, 1986), rapid developments in mass spectrometry have resulted in this approach losing favor so that emission spectrometry currently is rarely employed.

4.2. Mass spectrometers

As noted above, major improvements in the design of mass spectrometers (e.g., Preston and Owens, 1983) have led to far wider use of the technology. Additional advances include the ability to take the instrument to sea (Owens, 1988), allowing feedback on experiments during the expedition. In contrast to most users of mass spectrometers, ocean scientists are often limited by the amount of mass available for analysis. A major problem with analysis of small masses is correcting for the blank and the differential amplification of the isotope beam currents in the mass spectrometer. Although a solution is simply to add sufficient ''carrier'' nitrogen of known isotopic content to each sample, care must be taken to ensure accurate, precise, and consistent additions to be able to retrieve the true sample values. Owens and Rees (1989) were able to analyze samples as small as 10 nmol N with sufficient precision for tracer studies by shifting the timing of the oxygen pulse used to combust the samples later in the analysis cycle. Since the oxygen pulse was a major contributor to the blank, the shift removed its contribution from the sample peak while still allowing complete combustion. Fry *et al.* (1992) presented a correction method for the blank using a mass series of known isotopic composition. Later, Fry *et al.* (1996) used cyrofocusing for small samples to improve the signal to noise ratio of the sample peak and enable analysis at natural abundance levels. Pearson *et al.* (2004) make note of their implementation of a ''nano-combustion'' interface to their CF–IRMS that permitted use of $\sim$1 nmol C rather than the typical 1 µmol C for stable isotope precision measurements. Such an interface would obviously be tremendously useful in a range of applications. In addition to improvements in the basic equipment, there are a host of alternatives to magnetic sector mass spectrometers that have not been explored by ocean scientists such as time-of-flight mass spectrometry that could permit analysis of spatial patterns or of individual cells (see Section 6).

Mass spectrometers are also now capable of simultaneously measuring the ^{13}C isotopic ratio and investigators have increasingly conduced dual ^{13}C and ^{15}N experiments. Slawyk and coworkers (Slawyk, 1979; Slawyk *et al.*, 1977) conducted the earliest studies that predated the advent of CF–IRMS. Subsequent applications include Collos and Slawyk (1984), and more recently Dauchez *et al.* (1995), Kudela and Cochlan (2000), Mousseau *et al.* (2000), Marba et al. (2002), Fan and Glibert (2005). As discussed below, one uses the same equation for ^{13}C uptake as for ^{15}N uptake studies (Legendre and Gosselin, 1996).

By far the most common type of marine sample analyzed by mass spectrometry is particulate, which means the analyst must cope with the filter used for separation (see Section 3.1). The most commonly used filter, the glass fiber GF/F, presents several challenges. First is reducing the blank, which is accomplished by pre-combustion of the filter (generally 2–4 h at 450°C) prior to use and storage so that the filters do not become recontaminated via adsorption of atmospheric nitrogen compounds. A second challenge is preparing the filter for introduction into the combustion system, as too large a filter package will result in jamming of the automated sample introduction apparatus. Artisanal solutions abound for folding and compressing the filter to fit into the tin foil cup used to contain the sample. Beside the obvious solution of using the smallest possible filter (typically 25 mm) that still permits adequate filtration time, compression can be achieved with lever-arm pill machines or simply smashing a rod down on the filter with a hammer (e.g., www.stableisotopefacility.ucdavis.edu/sampleprep.htm). Kanda *et al.* (1998) present a diagram for a custom-made tablet press capable of compressing 47 mm filters for use with automated sample trays on CHN analyzers coupled to mass spectrometers.

The actual combustion of the glass filter presents additional problems. The filters melt and potentially clog the combustion tube, and attempting to analyze too many samples in one run can fill the tube so that the final samples are no longer adequately combusted. The filters can also chemically interact with the quartz combustion tube and greatly shorten its life, resulting in leaks and high operating costs. The simplest approach is to replace the tube and its chemicals after each run, absorbing the cost as part of the analysis. However, many analysts use various types of sacrificial ''liners'' that are placed inside the combustion tube. These include quartz glass tubing that is discarded after each run or ceramic tubes (Kanda *et al.*, 1998) that last ~20 samples before discarding. Steel liners (SAE-J405–30310) can be obtained that will withstand multiple runs (>100) of ~50 filters with removal of the glass slag from each run accomplished using a hammer (pers. obs.).

Finally, glass fiber filters are commonly used as a solid support for other forms of nitrogen such as organics (Sections 3.2.1 and 3.3.1) or ammonium (Section 3.2.2). As there is no need for a small pore size to capture particles, a GF/C can be used as the reduced amount of glass is less a problem in the combustion system. It is possible to place the organic nitrogen compound in its solvent directly into a tin cup for drying, but some manufacturer's cups permit the solvent to leak through minute holes with resultant loss of sample.

5. Data Analysis

5.1. Basic equations

Once the appropriate isotopic measurements have been completed, there remains the need to choose the correct model to calculate the rate. Although typically denoted ''the rate equation'', in reality it is a model that bears with it assumptions that should be understood and appreciated. Sheppard (1962) set forth the basis for

interpretation of any tracer, stable or radioactive, and detailed the mathematics for compartmental analysis. His essential equation for transfer of a tracer from a source compartment to a sink compartment

$$\frac{dR_{sink}}{dt} = V(R_{source} - R_{sink}) \tag{31.1}$$

holds regardless of whether one considers DIN uptake into particles or ammonium oxidation to nitrite. R_{sink} is the isotopic ratio of the product or sink compartment, R_{source} is the isotopic ratio of the ^{15}N source compartment, and V is the transfer coefficient (AKA turnover rate with units T^{-1}). There are several important considerations that are often overlooked, but become apparent when the typical equation that is actually employed by most investigators Eq. (31.2) is compared to the above.

$$\frac{R(t)_{sink} - R(t-1)_{sink}}{\left(R_{source} - R(t-1)_{sink}\right)\Delta t} = V \tag{31.2}$$

The derivative is now approximated by a difference equation based on the single final measurement often conducted on the sink pool, $R(t)_{sink}$, and $R(t-1)$ is $R(t=0)$ which is assumed to be the natural abundance of N_2. The derivative makes clear that as the two compartments approach isotopic equilibrium, the rate of change of R_{sink} also asymptotically declines so that V remains constant.

However, when measuring nitrogen uptake by particles in the marine system, the presence of detrital nitrogen on the filter along with the living pool causes two problems. First, in the denominator, $R(t)_{sink}$ can never reach equilibrium with the ^{15}N source even though the active component (phytoplankton + bacteria) within the operational pool (PON) may actually be at equilibrium. Considering potential growth rates of the active pool on the order of 1 day, a long incubation may in fact permit substantial progress towards equilibrium, especially when R_{source} is declining due to dilution (e.g., Glibert et al., 1982). As a concrete example, if the living PON is 10% of the total and reaches 99% of equilibrium with an $R_{source} = 10\%$, the denominator will be 9% rather than 0.1%, resulting in a major underestimate of the true rate. Another issue arises from approximating the derivative by the difference since the trajectory of $R(t)_{sink}$ is curved whereas it is linear with the assumptions of Eq. (31.2). Finally, if $R(0)_{source}$ is used, then again the result can be an underestimate if isotope dilution is significant (Section 5.2.1). Many of these problems can be minimized by restricting the incubation period (see Section 2.1) or by ensuring that the operational pool is really a compartment (i.e., kinetically homogeneous and well-mixed).

Dugdale and Wilkerson (1986) discussed the equations and experimental considerations for determining uptake rates into particles (ρ) in marine applications, clearly pointing out that the turnover rate, V (Eq. (31.2)), should not be used since $R(t)_{sink}$ included the detrital component. They recommended using a 2–6 h incubation and the following equation:

$$\rho = \frac{R(t)_{\text{sink}} - R(0)_{\text{sink}}}{\left(R_{\text{source}} - R(0)_{\text{sink}}\right)\Delta t} \times \text{PON}(t) = V \times \text{PON}(t) \qquad (31.3)$$

where the numerator is the at.% ^{15}N excess in the sink pool (particulates) and $R(0)_{\text{sink}}$ is the initial at.% of the sink pool. They noted that multiplying V by the total PON gave the uptake rate, ρ, into the active pool. The turnover rate would only have well-defined meaning if the detritus was not present as during a bloom, or the living component was isolated (e.g., Lipschultz, 1995). Dugdale and Wilkerson (1986) also recommended that PON(t), the value for the particulates at the end of the incubation, be used rather than an alternative approach using PON(0). Legendre and Gosselin (1996) revisited the computation of rates to compare equations for ^{13}C and ^{15}N uptake, showing that by making some approximations (i.e., that the at.% ^{15}N initially was zero rather than 0.366 and hence that the at.% ^{14}N was 100%, and that R_{source} was about 9% greater than a nominal 10% addition), that Eq. (31.3) was identical to the standard equation for ^{14}CO$_2$ or ^{13}CO$_2$ uptake

$$\rho = \frac{R(t)_{\text{sink}} - R(0)_{\text{sink}}}{(R_{\text{DIC}})\Delta t} \times [\text{DIC}] \qquad (31.4)$$

where DIC is the concentration of dissolved inorganic carbon in seawater. One then need only substitute [DIN] for [DIC] and R_{DIN} for R_{DIC}. These assumptions make sense for radiotracers since the tracer additions are typically miniscule compared to the standard 10% stable isotope addition. Cobelli et al. (1987, 1992) earlier discussed the same issue at length. Several considerations of the effects of uptake of a single labeled DIN source in the presence of uptake of multiple other nitrogen sources have led to the conclusion that Eq. (31.3) is accurate (Collos, 1987; Legendre and Gosselin, 1996).

5.2. Specific models

5.2.1. Isotope dilution
Models for calculating isotope dilution are based on the assumption of a single, well–mixed compartment losing uniformly labeled material while simultaneously gaining unlabeled material. Although assessment of just the isotopic content over time permits calculation of the turnover time of the compartment, when combined with concentration measurements one can also calculate transport rates into, and out of, the compartment. Application to marine systems originated with Blackburn (1979) and Caperon et al. (1979), and was then extended by Glibert et al. (1982, 1985) and Laws (1984). The extent of dilution was tested in a range of environments over the ensuing years (Glibert et al., 1992; Harrison and Harris, 1986; Kanda et al., 1987; Lipschultz and Cook, 2002; Lipschultz et al., 1986) with the extent ranging from considerable to limited, suggesting that each investigator should assess this likelihood independently. Although attention has primarily focused on ammonium,

there have also been reports of isotope dilution for nitrate (Lipschultz, 2001), urea (Slawyk *et al.*, 1990b), and amino acids (Preston *et al.*, 1996a).

As with the equations for transfer between two compartments Eqs. (31.1–31.4), the same equation for isotope dilution (Glibert *et al.*, 1982) applies to any single compartment that can be measured (see Section 2).

$$P(t) = P(0) + (d - u) \times t \qquad (31.5)$$

and

$$\ln[R(t) - R(0)] = \ln[R(0) - \text{NA}] - [d/(d - u)] \\ \times [\ln(P(t) - P(0))] \qquad (31.6)$$

where d is the dilution or input of unlabeled material to the compartment, u is the loss from the compartment with the same isotopic composition (R) as the compartment, and NA is the natural abundance value prior to the addition of the tracer. The concentration of the material (P) at any time, t, is the sum of the net change over time, $(d-u) \times t$, from the initial amount $P(0)$. Glibert *et al.* (1982, 1985) discuss the various permutations when the concentration doesn't change or the concentrations are undetectable. They pointed out that R_{source} in Eqs. (31.1–31.4) needs to reflect any isotope dilution and that u is not equivalent to particulate uptake (ρ in Eq. 31.3) unless there are no other losses from the dissolved pool such as nitrification in the case of ammonium isotope dilution. Practically, isotope dilution can be calculated using the equations outlined in Mulholland and Bernhardt (2005).

It is worth noting that the term isotope dilution has also been applied to applications where the concentration of a compound is determined isotopically rather than by direct chemical determination. The most familiar application is the determination of the magnitude of a blank (pre-existing ^{14}N in an analysis) by adding a known amount of ^{15}N to a sample and determining the decrease from the expected isotopic value of the tracer addition. This concept can also be used in field samples, such as for determining the concentrations of dissolved and combined amino acids.

5.2.2. Compartmental analysis

Compartmental analysis is an extension of the simple, single compartment models (Sections 5.1 and 5.2.1) to include multiple compartments. It is far more commonly applied in medical studies or in other ecosystems than the marine environment. Even for ^{15}N, the soil, plant, and limnological communities have applied the technique with great success (e.g., Tank *et al.*, 2000). It also has a long history of use in conjunction with radioisotopes such as carbon (Smith and Geider, 1985; Smith *et al.*, 1979) or phosphorus (Perez-Martinez *et al.*, 1995; Smith *et al.*, 1985). However, to implement such an approach requires analysis of the mass and isotopic composition of nearly all compartments, as well as over multiple times, which represents a major analytical undertaking for stable isotopes. However, a more

complete analysis of the nitrogen cycle not only ensures that there is mass balance of the tracer (or at least identifies clearly identifies a problem if some tracer is missing), but also ensures that rates of individual processes are placed in their biogeochemical context. One also gains more confidence in the calculated rates due to the additional constraints of multiple assessments of the same processes. For instance, measuring the $^{15}NH_4^+$ loss via isotope dilution and ^{15}N appearance in particles as well as NO_2^- means that the sum of uptake and ammonium oxidation are constrained to equal (in a statistical least–squares sense) the net NH_4^+ loss term. The most complete example of compartmental analysis of the aquatic nitrogen cycle provides an example of the power of the technique (Lipschultz et al., 1986).

   A simple example is that of Kanda et al. (1988) who coupled the internal LMW and macromolecular compartments together to calculate uptake of external $^{15}NH_4^+$ into the LMW, as well as rates of macromolecular catabolism and anabolism. Bronk (1999) isolated the intracellular DON pool and calculated an intracellular transformation rate, which quantifies the flow of nitrogen from the intracellular NH_4^+ pool to the intracellular organic N pool. LaRoche and Harrison (1987) explored a range of three compartment models to explain the observed exchange of ^{15}N between particulate and ammonium pools. The third compartment, DON, was not directly measured but was tested as a sink for the missing ^{15}N that initially disappeared and then reappeared from the mass balance calculation (see Section 2.3). The best model was one lacking in biological veracity as the tracer could move from NH_4^+ directly to DON without passing through a living cell (PON), although this is the same conceptual model underlying some estimates of phytoplankton DON loss. This model would work operationally if the DON precursor pool within the cells rapidly came to isotopic equilibrium with the external DIN pool during the incubation. Here, defining ''rapid'' is the key issue and likely a contributor to some of the differences between estimates of DON release since a long (relative to the precursor reaching equilibrium) incubation would result in a very different result than a shorter incubation. Without direct measurements of all precursor compartments (including actually knowing the appropriate precursor's identity), as was attempted by Bronk and Glibert (1991), it is best to take a step back and combine both DON and PON, as is done in the calculation of gross nitrogen uptake rates (Bronk et al., 1994; Slawyk et al., 1998). Flynn and Berry (1999) should also be consulted. It should, however, be possible to better understand the mechanisms of DON loss by measuring ammonium and amino acid isotope dilution of both internal and external compartments now that improved methods are available (e.g., Mulholland and Bernhardt, 2005; Mulholland et al., 2004).

5.3. Comparisons between ^{15}N rates and other methods

Confidence in rates of nitrogen cycling can be gained not only by redundant ^{15}N measurements such as described above (Section 5.2.2), but also by comparison to other analytical approaches. Comparison of rates measured by tracer with those measured by net changes in concentration is the most common approach, but requires the assumption that net concentration change is really due to only a single

input or output. For instance, comparing $^{15}NO_3^-$ uptake into particles to $\Delta[NO_3^-]$ requires assuming no nitrification.

5.3.1. Nitrification

There have been very few comparisons of different methods for determination of nitrification rates. Enoksson (1986) compared three isotopic methods, ^{15}N, $^{14}CO_2$ incorporation with N-serve to inhibit nitrification, and NO_3^- isotope dilution. She found ammonium oxidation measured with ^{15}N was 2–7 times greater than determined by the other methods, but pointed out that both different incubation times (from 1 to 12 days) and different substrate additions were used which would greatly alter the measured rates. Jones (1992) found a good relationship between a ^{14}CO method and ^{15}N for measuring ammonium oxidation. Although most researchers have switched to direct assessment using ^{15}N, there is still usage of the $^{14}CO_2$ method (e.g., Brion et al., 2000).

5.3.2. Denitrification

By far the majority of denitrification studies have been in sediments, with limited successful direct measurements in anoxic water columns (see Chapter 6 by Devol, this volume). There have been several comparisons between the most commonly used denitrification assay, the acetylene block technique, and ^{15}N based measurements (reviewed in Cornwell et al., 1999). The acetylene block has generally been found to underestimate denitrification since it also blocks nitrification within the sediments that acts as a major source of oxidized nitrogen in a process termed coupled nitrification–denitrification (Seitzinger et al., 1993). Incomplete blockage of reduction of N_2O to N_2 is also a potential issue. ^{15}N based methods initially suffered from inability to resolve the sedimentary from water column contribution due to lack of labeling in the sedimentary $^{14}NO_3^-$ precursor. The IPT method (Section 3.4, Nielsen, 1992) resolved this issue. Welsh et al. (2001) compared the IPT with acetylene block in sediments from a seagrass bed where assumptions of the IPT approach might not be met. Rates were similar in the dark, but rates in the light were twice as high with acetylene blockage. Assuming the IPT was correct, they suggested the excess N_2O arose from dissimilatory nitrate reduction to ammonium (DNRA) activity. Bernot et al. (2003) compared the ^{15}N-MIMS IPT (Section 3.4) with acetylene block rates in estuarine sediments, finding that addition of chloramphenicol was need during the acetylene block incubations to limit changes in the microbial community's response to the ideal conditions created during the assay. They recommended the use of short-term acetylene block assays as a cost-effective substitute for ^{15}N based analysis.

The early approach to denitrification rates of direct measurement of net N_2 fluxes resulted in rates that were often too high due to long incubation times (Seitzinger et al., 1993). However, Hamersley and Howes (2005) have recently optimized the protocol for direct measurements after carefully considering a range of variables. Rather than a batch or closed incubation, Risgaard-Petersen et al. (1993) used a continuous flow system and found excellent agreement with the IPT approach. A variation of the direct measurement technique employs the N_2/Ar

ratio measured using MIMS (Hartnett and Seitzinger, 2003; Kana *et al.*, 1998) that doesn't require long incubations. However, these direct measurements of net N_2 flux cannot resolve any losses of N_2 due to nitrogen fixation. As noted above (Section 3.4), An *et al.* (2001) have developed a ^{15}N method to resolve both coupled nitrification–denitrification as well as N_2 fixation. Eyre *et al.* (2002) found that N_2/Ar could be affected by interaction of O_2 with N_2 in the mass spectrometer, resulting in artificially higher rates. Removing oxygen prior to MIMS analysis resulted in comparable rates with the IPT method. Along with suggested improvements, they present a detailed analysis of the assumptions of both techniques. Kana and Weiss (2004) noted that their particular instrument did not suffer the same oxygen effect and so their rates and technique were valid. Eyre *et al.* (2004) retorted that indeed their instrument had the effect and each investigator should test the magnitude on their equipment. They noted that they consistently found slightly lower rates with N_2:Ar and suggested that nitrogen fixation was the cause.

5.3.3. Uptake

After a spate of findings that ^{15}N uptake did not match the net loss of nutrient during incubations (e.g., Eppley and Koeve, 1990; Eppley and Renger, 1992; Slawyk *et al.*, 1990a; Ward *et al.*, 1989), more recent comparisons have found far better agreement. Boyd *et al.* (1995) reported a 1:1 correspondence between net nitrate loss and $^{15}NO_3^-$ uptake into particles over a range of NO_3^- concentrations, without the need to invoke loss to DON or filter losses. Raimbault *et al.* (2000) concluded that large discrepancies between net losses and uptake were likely erroneous since their results were in agreement once a small DON loss (∼10%) was included. They also found little loss of $^{15}NO_3^-$ to submicron particles passing the standard GF/F filter (see Section 3.1). There was also no detectable isotope dilution of nitrate during their 12 or 24 h incubations, although it is important to note that at the lowest concentrations (as low as 3 nM), ^{15}N additions were 42 nM so that isotope dilution would likely only have been detectable if true tracer additions were utilized. For instance, Lipschultz (2001) observed significant isotope dilution of a 1 nM $^{15}NO_3^-$ addition with constant 10 nM concentrations during incubations in the Sargasso Sea. In such a case, there was no net concentration change so that ^{15}N would be the only way to measure a rate.

Besides phytoplankton, the only comparison for other autotrophic systems is that of Naldi and Wheeler (2002) and O'Brien and Wheeler (1987) who both found good agreement between uptake by macroalgae and ^{15}N-based uptake rates, with little loss of DON or ammonium.

6. Future Directions

The past two decades since Harrison's review in 1983 has seen good progress in techniques to isolate and analyze the isotopic composition of the dissolved inorganic pools. Progress has been far slower for the dissolved organic, and particularly the

particulate, nitrogen fractions, with both DON and PON essentially still dealt with as a black box. For PON, flow cytometry holds great promise as a tool to study different size fractions or pigment classes (Lipschultz, 1995; Casey *et al.*, 2007 submitted for publication), but has not been widely adopted for metabolic studies. Use of ^{13}C rather than ^{14}C removes the concern of contaminating the instrument and permits dual isotope (^{13}C and ^{15}N) experiments. Vital stains open the window for a range of metabolic studies of living cells, and molecular techniques now permit exploration of a wide range of taxonomic or even metabolic groupings. For instance, Pel *et al.* (2003) used pyrolysis GC–MS to analyze for ^{13}C labeled fatty acids in very small amounts of filamentous cyanobacteria isolated by fluorescence-activated cell sorting. Such a technique might be applicable to marine species such as *Trichodesmium* and analysis of ^{15}N. Chen *et al.* (2000) developed an *in situ* PCR method for prokaryotes that permitted flow cytometric analysis of the expression of a specific gene inside bacterial cells. This type of approach could also be coupled to isotopic analysis to truly relate enzyme activity to rates.

A range of molecular techniques that have been coupled to stable isotopes are poised to make important contributions such as permitting attribution of rates to the responsible organisms. Radajewski and coworkers (Hutchens *et al.*, 2004; Radajewski *et al.*, 2000) developed stable isotope probing (SIP) which uses ^{13}C labeled growth substrates to selectively recover "heavy" ^{13}C-labeled DNA by density gradient ultracentrifugation. Radajewski *et al.* (2003) provides an excellent review of the advantages and disadvantages of the different SIP approaches. The labeled DNA can then be amplified, cloned, and sequenced to identify which organisms are actively growing on the substrate. Unfortunately, this approach requires long incubations and very high substrate labeling to achieve sufficient separation of the labeled DNA. MacGregor *et al.* (2002) recovered rRNA using biotinylated oligonucleotide probes to bind the RNA to streptadivin-coated magnetic beads on a column. Although the application involved determining the natural abundance ratio of the RNA, they note potential application in tracer studies. More recently, Pearson *et al.* (2004) replaced the biotinylation step with oligo-dT paramagnetic beads to separate rRNA for isotopic analysis. To overcome the significant amount of material previously required, they developed a "nano–combustion" interface to the CF-IRMS so that ~3 nmol C was required for 0.3‰ precision. For nitrogen, Cadish *et al.* (2005) recently explored protocols for ^{15}N-DNA SIP, finding an 40 at.% labeling of the DNA was required which would limit the application of this approach. A major limitation of this approach is the co-mingling of heavy, isotopically labeled DNA with unlabeled DNA with high G+C content causing smearing of the signal. This was overcome by Buckley *et al.* (2007) who employed sequential CsCI density gradient centrifugation, with the second gradient including bis–benzimide.

Another interesting suite of approaches that undoubtedly will be further developed employs SIMS—secondary isotope mass spectrometry (also know as multiple-isotope imaging mass spectrometry (MIMS)). Orphan *et al.* (2001) applied FISH–SIMS or fluorescent *in situ* hybridization SIMS to detect isotopically light carbon in archaeal cells, identifying the Archaea by FISH and using SIMS to quantify the isotopic composition of individual cells by ion microprobe. Finzi *et al.* (2006) applied nanoSIMS to visualize uptake of $^{15}N_2$ and $^{13}CO_2$ by individual cells of a

single filament of *Trichodesmium*. Lechene *et al*. (2007) demonstrated the power of this new technology by imaging isotopically labeled, individual nitrogen-fixing bacteria within shipworms, and the transfer of ^{15}N to adjacent tissues. Rather than FISH to visualize the cells, TEM sections allowed precise registration of the two images of intact shipworms. Note that these approaches identify particular organisms involved in a particular process rather than the rate of an entire population of organisms, for which flow cytometry is far better suited.

Although beyond the scope of this review, it is worth mentioning two other areas where ^{15}N tracers could be applied to studies of marine organisms. There is burgeoning interest in a subset of proteomics that employs stable isotopes such as ^{15}N. Of particular interest is the potential to conduct $DI^{15}N$ incubations and then separate the proteome to detect appearance of the tracer into specific proteins using a wide range of techniques reviewed recently by Tao and Aebersold (2003). Application of electrospray ionization – fourier transform ion cyclotron resonance – mass spectrometry (ESI-FTICR–MS) to analysis of DON (Nunn and Timperman, 2007) could also be used to identify $DO^{15}N$ produced by phytoplankton after exposure to $DI^{15}N$. Nuclear magnetic resonance (NMR) can also be employed with ^{15}N to study metabolism of a wide range of organisms (Mesnard and Ratcliffe, 2005). Applications have included cytoplasmic storage of NH_4^+ or NO_3^- in cells as well as assimilation pathways.

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ENZYMES AND NITROGEN CYCLING

John A. Berges *and* Margaret R. Mulholland

Contents

1. PRINCIPLES AND PRACTICE

1.1. Importance of enzyme-mediated reactions

Enzyme–mediated reactions are responsible for most of the transformations of nitrogen (N) that occur in the marine environment, thus measurements of enzymes have figured prominently in oceanographic work for some time, especially in terms of N assimilation (see Falkowski, 1983).

Many different measurements of enzymes are undertaken to acquire different types of information. For example, the presence of genes for an enzyme has been used to infer whether organisms are capable of performing particular functions, the expression of those genes or the appearance of the enzyme protein is used to indicate if and under what conditions the gene is functioning, while assay of the activity of the enzyme has been used to infer rates of particular processes (see Section 2). In fact, virtually all measurements classified as ''molecular'' and most rate measurements of ''uptake'' are, in fact, measurements of enzymes. Because strictly molecular methods (i.e., the capacity to perform a reaction; see Chapter 30 by Zehr and Jenkins, this volume) and N uptake (i.e., the net result of enzymatically mediated processes; see Chapter 6 by Mulholland and Lomas, this volume) are discussed elsewhere in this book; in this chapter, we will focus primarily on measurements of enzyme activities.

Conceptually, measurements of enzyme activity are easier to interpret than measurements of the presence or absence of particular enzymes, genes, or transcripts or rates of transcription and translation, and are more likely to be related to physiological rates. Measurements of enzyme activity have often been proposed as quantitative indices/estimators of biological rate reactions. Moreover, enzyme activity can often be measured quickly and using conventional ocean-going equipment, so that these measurements are more easily incorporated into oceanographic studies. That said, assays of enzyme activity are subject to different biases than molecular methods, and the interpretation of enzyme activity relies on specific knowledge of reaction pathways and their regulation (see Section 1.3.5). Interpretation of enzyme activity is often complicated when pathways are branched, cyclic, or reversible; when different enzymes produce similar products or compete for similar substrates; or when different pools of the same enzyme are regulated differently.

Paradoxically, as we have gained more information about enzymes from the molecular perspective, the interpretation of enzyme activity measurements and the classification of enzymes have actually become more complicated. First, there are different classification schemes for gene or protein sequences (derived from molecular biology) and biochemical function (derived from classic enzymology). For example, enzyme genes and proteins are classified in term of homologous structures and sequences. In contrast, in biochemical classification schemes, enzyme nomenclature is based in the reaction that is mediated (the International Union of Biochemistry and Molecular Biology (IUMB) uses Enzyme Commission (EC) numbers for this purpose; Table 32.1). Thus, two evolutionary divergent enzymes that catalyze the same reaction would fall into the same EC category, while their functional proteins might be quite distant based on genetic analysis. In addition, enzymes that catalyze processes such as membrane transport, where there is no actual chemical change, cannot easily be accommodated in the biochemical classification scheme. For example, because they mediate no chemical transformation ''permeases'' or ''translocases'' are generally not included in enzymological classifications, yet are frequently adopted in the molecular biology literature.[1] In this discussion, we will generally classify enzymes according to activity (i.e., EC numbers) but we will also consider pathways that mediate no chemical transformation.

In 1983, Falkowski observed, ''there is little information on the N enzymology of marine organisms'' and also noted that there was ''scanty'' information about transporters (Falkowski, 1983). At that time, most of the information available for enzymes was based on activity assays with relatively few attempts to purify enzymes and characterize their structural and functional diversity, and very few attempts to use molecular or other techniques to probe the full range of enzymes mediating

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1 Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). Enzyme Nomenclature: Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzyme-Catalysed Reactions. Published in Enzyme Nomenclature 1992 [Academic Press, San Diego, California, ISBN 0-12-227164-5 (hardback), 0-12-227165-3 (paperback)] with Supplement 1 (1993), Supplement 2 (1994), Supplement 3 (1995), Supplement 4 (1997), and Supplement 5 (in *Eur. J. Biochem.* 1994, **223**, 1–5; *Eur. J. Biochem.* 1995, **232**, 1–6; *Eur. J. Biochem.* 1996, **237**, 1–5; *Eur. J. Biochem.* 1997, **250**, 1–6, and *Eur. J. Biochem.* 1999, **264**, 610–650, respectively) [Copyright IUBMB] and online at http://www.chem.qmul.ac.uk/iubmb/enzyme/index.html).

Table 32.1 Enzyme activities relevant to marine nitrogen cycling

Enzyme name		EC numbers
(a) Uptake, reduction and assimilation		
Membrane transporters		3.6.3
	Polar-amino-acid-transporting ATPase	3.6.3.21
	Nonpolar-amino-acid-transporting ATPase	3.6.3.22
	Oligopeptide-transporting ATPase	3.6.3.23
	Nitrate-transporting ATPase	3.6.3.26
	Polyamine-transporting ATPase	3.6.3.31
	Quaternary-amine-transporting ATPase	3.6.3.32
	Taurine-transporting ATPase	3.6.3.36
	Guanine-transporting ATPase	3.6.3.37
	Peptide-transporting ATPase	3.6.3.43
Nitrate reductase (assimilatory)		1.7.1.1(NADH-specific)
		1.7.1.2(NADH(P)H-bispecific)
		1.7.1.3(NAD(P)H-specific)
		1.7.7.2(Ferredoxin-specific)
Nitrite reductase (assimilatory)		1.7.7.1(Ferredoxin),
		1.7.1.4(NAD(P)H-bispecific)
Glutamate dehydrogenase		1.4.1.2 (NADH)
		1.4.1.3(NAD(P)H)
		1.4.1.4(NADPH)
Glutamate synthase		1.4.1.13(NADPH)
		1.4.1.14(NADH)
		1.4.7.1(Ferredoxin)
Glutamine synthetase		6.3.1.2
Nitrogenase		1.18.6.1 (Ferredoxin)
		1.19.6.1(Flavodoxin)
Urease		3.5.1.5
Urea carboxylase		6.3.4.6
Allophanate hydrolase		3.5.1.54

(*Continued*)

Table 32.1 Enzyme activities relevant to marine nitrogen cycling (*continued*)

Enzyme name		EC numbers
(b) N compounds as electron sources or sinks		
Nitrate reductase (dissimilatory)		1.7.99.4
		1.9.6.1 (Cytochrome)
Nitrite reductase (dissimilatory)		1.7.2.1 (NO-forming)
Nitric oxide reductase		1.7.99.7
Nitrous oxide reductase		1.7.99.6
Nitrite reductase		1.7.2.2 (Ammonia-forming)
Hydroxylamine oxidase		1.7.3.4
Nitrite oxioreductase		1.7.99.4
Hydroxylamine reductase		1.7.99.1
Hydroxylamine oxioreductase		1.7.99.8
(c) Synthetic pathways for secondary compounds		
(d) Turnover and maintenance of internal N		
Proteases	Aminopeptidases	3.4.11.x
	Dipeptidases	3.4.13.x
	Du/tripeptidyl-peptidases	3.4.14.x
	Peptidyl-dipeptidases	3.4.15.x
	Serine carboxypeptidases	3.4.16.x
	Metallocarboxypeptidases	3.4.17.x
	Cysteine carboxypeptidases	3.4.18.x
	Omega peptidases	3.4.19.x
	Serine endopeptidases	3.4.21.x
	Cysteine endopeptidases	3.4.22.x
	Aspartic endopeptidases	3.4.23.x
	Metalloendopeptidases	3.4.24.x
	Threonine endopeptidases	3.4.25.x
	Other endopeptidases	3.4.99.x

D-/L-Amino acid oxidases	D-Aspartate oxidase	1.4.3.1
	L-Amino-acid oxidase	1.4.3.2
	D-Amino-acid oxidase	1.4.3.3
	Amine oxidase (flavin-containing)	1.4.3.4
	Amine oxidase (copper-containing)	1.4.3.6
	D-Glutamate oxidase	1.4.3.7
	Ethanolamine oxidase	1.4.3.8
	Putrescine oxidase	1.4.3.10
	L-Glutamate oxidase	1.4.3.11
	L-Lysine oxidase	1.4.3.14
	D-Glutamate(D-aspartate) oxidase	1.4.3.15
	L-Aspartate oxidase	1.4.3.16
	Glycine oxidase	1.4.3.19
Transaminases		2.6.1.x
(e) Other enzymes relevant to N cycling		
Rubisco		4.1.1.39
Isocitrate dehydrogenase		1.1.1.41 (NAD)
		1.1.1.42 (NADP)
Succinate dehydrogenase		1.3.99.1
Cytochrome c oxidase		1.9.3.1
Citrate synthase		2.3.3.1, 2.3.3.3, 2.3.3.8
Carbon–nitrogen lyases		4.3.x.x
Forming carbon–nitrogen bonds		6.3.x.x
Acting on carbon-nitrogen bonds, other than peptide bonds		3.5.x.x
Chitinase		3.2.1.14

reactions affecting the marine N cycle. Much of the information regarding N assimilation was based on analogies with processes in higher plants and freshwater green algae. Remarkably, much of this information has proven to be correct.

Since 1983, the technical aspects of enzyme measurements have changed radically. For perspective, the first monoclonal antibodies were produced less than a decade before (Köhler and Milstein, 1975), practical DNA sequencing methods were in their infancy (Maxam and Gilbert, 1977; Sanger et al., 1977), mainstays such as the polymerase chain reaction had yet to be put forward (Saiki et al., 1988), and automated DNA sequencers were still the stuff of science fiction (Smith et al., 1986). A remarkable suite of molecular measurements are now considered routine (see Chapter 30 by Jenkins and Zehr, this volume). While the principles of enzyme activity measurements remain largely the same, technical innovations such as improved spectrophotometers (including diode array detectors), compact scanning fluorometers, more sophisticated optics for fluorescence microscopes, and multiwell plate readers (e.g., Butler et al., 1988), as well as the development of highly fluorescent enzyme substrates (e.g., Molecular Probes, Bachem) have now revolutionized the practice. We contend that in order to understand how genetic capacity is expressed in the environment, it is critical to couple molecular measurements with rate estimates of activity in the environment.

In this chapter, our goal is to review the practical aspects of enzyme activity measurements and their interpretation and to illustrate how such measurements have advanced our knowledge of marine N cycling. While our main focus will be the assimilatory pathways found in marine photoautotrophs, we will also consider degradation pathways and secondary metabolism, and will point out other groups of marine organisms where enzyme activity has proven useful in examining aspects of the N cycle.

1.2. Overview of enzymes involved in marine nitrogen cycling

There are an enormous number of enzymes that are relevant to the marine N cycle and we still know little about many of them. Indeed, since all enzymes are proteins, all enzymes are intrinsically part of the N cycle. We can broadly divide enzymes into: (1) those involved in inorganic N acquisition (including uptake, reduction, and assimilation, and acquisition of organic N), (2) those involved in using N compounds as electron sources or sinks (i.e., dissimilatory enzymes), (3) those involved in incorporating N into macromolecules or synthesizing secondary compounds that contain N, (4) those responsible for turnover and maintenance of internal N, and (5) those involved in pathways strongly coupled to N metabolism. Table 32.1 provides a summary of relevant enzymes and enzyme classes, including prokaryotic and eukaryotic autotrophs and heterotrophs.

1.2.1. Nitrogen acquisition
Mulholland and Lomas (this volume, Chapter 7) have reviewed the general uptake and assimilation pathways for photoautotrophs (summarized in Fig. 7.2). Transporter proteins are not enzymes in the strictest sense because they do not catalyze particular chemical transformations, but facilitate entry of specific compounds into

cells. A distinct Transporter Classification (TC) system exists that is analogous to the EC system (http://www.tcdb.org), although there remain areas of overlap (e.g., ATPases are in TC 2. 2.2.1, but also fall under EC 3.6.3.x; Table 32.1). Although it is possible to make measurements of nutrient transport and infer ''activities'' of transporters using techniques that measure membrane potential such a patch-clamping (Boyd and Gradmann, 1999a,b; Taylor and Brownlee, 2003), it is not possible to unambiguously measure such activities using traditional enzyme assays. Immunochemical and gene sequencing methods have been most useful for isolating transporters (Hildebrand and Dahlin, 2000; Lara *et al.*, 1993; Tischner *et al.*, 1989; Chapter 30 by Jenkins and Zehr's review, this volume).

Reduction of nitrogen compounds to NH_4^+ may be viewed as a committing step on the way to assimilation. Enzymes mediating these reactions are attractive targets for study because they generally represent linear pathways relating enzyme activity to metabolic flux of particular compounds: nitrogenase (N_2), nitrate reductase (NO_3^-), and nitrite reductase (NO_2^-) (Table 32.1). Assimilation of nitrogen generally refers to the synthesis of small organic molecules, such as amino acids, from inorganic N and some cellular C compound, and enzymes responsible for this process include glutamine synthetase (GS), glutamate synthase, and glutamate dehydrogenase (Table 32.1).

There are also a number of enzymes that degrade organic N compounds, inside or outside the cell, thereby making N available for assimilation or uptake, respectively. Examples we will consider include urease, amino acid oxidases, and extracellular peptidases (Mulholland *et al.*, 2002; Mulholland and Lee, in revision, Chapter 7 by Mulholland and Lomas, Fig. 2, this volume; Table 32.1). In addition, for heterotrophic organisms, digestive enzymes, especially proteases, are important for internal N cycling, recouping cellular N, and excretion.

1.2.2. Nitrogen compounds as electron sources or sinks

In addition to N assimilation, nitrogen compounds in nature can also be used as electron sources or sinks, especially by prokaryotes (but not exclusively, see Zvyagil'skaya *et al.*, 1996). The enzymes that mediate these dissimilatory reactions have been studied extensively in the context of inputs and losses of fixed N in the marine N budget. Many of these measurements have been based on enzyme activities (e.g., dissimilatory nitrate reductase). Since dissimilatory reactions have been reviewed elsewhere in this volume (Chapter 5 by Ward and Chapter 6 by Devol, this volume), these will not be discussed further and we refer the reader to these chapters.

1.2.3. Incorporation of nitrogen into macromolecules and secondary compounds

Incorporation of nitrogen is typically viewed as flux of inorganic N into macromolecules such as proteins and nucleic acids. Pathways of protein and nucleic acid synthesis are complex, and highly regulated so that single enzyme activities are unlikely to provide useful information regarding their production and turnover. In addition to what we think of as ''conventional'' metabolites, marine organisms contain a bewildering array of N-containing secondary compounds, many of which are responsible for

toxic effects, cell–cell communication, and other processes we still know little about. In particular, enzymes of EC classes 4.3 (C–N lyases), 6.3 (forming C–N bonds), and 3.5 (acting on C–N bonds, other than peptide bonds) are likely to be important in cells, as are transaminases and carboxylases (Table 32.1). Few of these enzymes have been measured in marine organisms, especially with respect to N cycling. However, there is increasing recognition of the importance of these enzymes, for understanding toxic secondary metabolites such as domoic acid (Pan *et al.*, 1998; Shimizu, 1996), and there have been recent advances in appreciating the importance of such enzymes including the role of *N*-sulfotransferase in saxitoxin production (Sako *et al.*, 2001).

1.2.4. Turnover and maintenance of internal nitrogen

Intracellular nitrogen turnover is poorly understood in marine species despite the fact that N turnover is often used to estimate productivity. In addition, N turnover is often estimated based on C turnover and Redfield stoichiometry but these may not be in balance in natural systems (Mulholland *et al.*, 2006). Since the major pool of cellular N in organisms is in protein, understanding protein turnover is likely to shed light on cellular N turnover in general. Based on our understanding of eubacteria, plants, and animals (e.g., Bond and Butler, 1987; Gottesman and Maurizi, 1992; Vierstra, 1993), it seems likely that the major catabolic pathways for proteins are conserved amongst all of these groups (Fig. 32.1).

Protein turnover is essential for acclimation to changes in the external environment (e.g., irradiance or N supply), release of stored nitrogen during N-limited periods, removal of damaged or mis-synthesized N-containing compounds, reorganization of cell morphology to generate resting stages, production of specialized structures such as
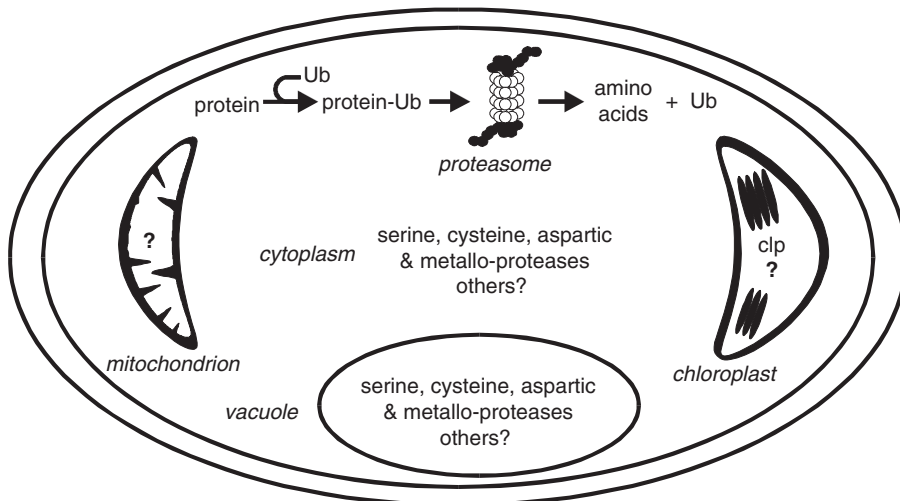
Figure 32.1 Pathways of protein turnover and maintenance in a hypothetical unicellular eukaryotic photoautotroph (after Collos and Berges, 2002). Ub represents ubiquitin and polyubiquitin. Question marks indicate current uncertainties about proteases present in different cell compartments.

heterocysts, cell cycle functions, cellular defense mechanisms, and the creation and removal of intra- and intercellular signaling compounds. Within cells, degradation of protein is accomplished by the activity of hydrolytic enzymes and is regulated by cellular compartmentalization, selectively activating or inactivating particular groups of proteases, or other energy dependent pathways (see Vierstra, 1993). In some cases, the degradation products are then removed from cells or cellular compartments through specific pathways, e.g., urea cycle enzymes, or glutamate dehydrogenase in the case of heterotrophs (e.g., Mayzaud, 1987).

Proteases are traditionally divided into four classes, based on the key amino acids found in the active site of the enzyme: serine proteases, cysteine proteases, aspartic proteases, and metalloproteases (Bond and Butler, 1987), although more recently a number of enzymes have been characterized that defy such a classification systems because they exhibit mixed characteristics and have multiple activities (Liu *et al.*, 2003). Protease activities are also classified by the nature of the cleavage they catalyze: endoproteases (i.e., cleaves within a peptide chain; also called proteinases) versus exoproteases (cleave at the ends of peptide chains) such as aminopeptidases or carboxypeptidases. Proteases are sensitive to pH and as such, their location in cellular compartments may vary (Berges and Falkowski, 1998). In eukaryotic cells large (600–900 kDa), multisubunit proteases with multiple activities (proteasomes) are present. Many details of proteasomes remain unclear (Liu *et al.*, 2003), but this protease complex is involved in the ubiquitin–dependent proteolytic pathway. Ubiquitin (Ub) is a highly-conserved 76-amino-acid peptide that is covalently coupled to proteins targeted for degradation (Kerscher *et al.*, 2006). Proteins labeled with multiple Ub molecules (polyubiquitinated proteins) are rapidly degraded by proteasomes. It has also become clear that there are several other polypeptides found in cells that share similarities with Ub and function in protein modification, e.g., SUMO, ISG15, Nedd8, and Atg8 (Kerscher *et al.*, 2006). For example, SUMO is a 100-amino-acid peptide with 20% homology to Ub. While some of these polypeptide-tagging systems may have proteolytic functions (e.g., SUMO, Colby *et al.*, 2006), others may function to regulate transcription, signal transduction, and the cell cycle (Kerscher *et al.*, 2006).

There is evidence that the majority of protein turnover within higher plant cells may occur via the Ub/proteasome pathway (Rock *et al.*, 1994; Vierstra, 2003); the few data available from marine organisms suggest that the pathway is very well conserved (e.g., Moriyasu and Malek, 2004; Tonon *et al.*, 2003; Berges, unpublished annotation from *Thalassiosira pseudonana* genome sequencing project). Within pro-karoytes and organelles of eukaryotic cells that have evolutionary roots in the prokaryotic domain, Ub and proteasomes are not found, but ATP-dependent proteases such as the Clp, Lon, and Ftsh proteases are thought to have similar functions (Clarke *et al.*, 2005; Sakamoto, 2006).

Beyond proteases, there is virtually nothing known about other enzymes mediating N turnover, e.g., the nucleases that degrade RNA and DNA (cf., Falkowski and La Roche, 1991), or about the degradation of complex molecules like toxins and phytochelatins. Some details are available for chlorophyllases from marine diatoms and they may be compartmentally regulated, because degradation proceeds very rapidly when cells are homogenized (Owens and Falkowski, 1982).

1.2.5. Other enzymes connected with nitrogen cycling

A number of other enzymes deserve consideration because they are involved in pathways that are closely linked to nitrogen metabolism, such as Rubisco and other carboxylases that are critical to the coupling of C and N metabolism (Huppe and Turpin, 1994), or electron transport system (ETS) enzymes such as isocitrate dehydrogenase that are involved in respiratory metabolism (Roy and Packard, 2001). Others are relevant to N losses (e.g., esterases that are used as indices of cell lysis; Agusti *et al.*, 1998 or proteases associated with active cell death processes, e.g., Berman–Frank *et al.*, 2004) (Table 32.1).

1.3. Measuring enzyme activity

What we generally want to know is the rate at which enzymes are catalyzing reactions in nature. True *in situ* assays of enzymes are not yet practical outside of the laboratory, although technological developments may make these sorts of observations possible in the future. In practice, enzyme-specific assays are developed based on the chemical reaction catalyzed (Table 32.2). Because all EC enzymes consume substrates to generate products, the chemical reaction and its energetics are examined to determine logical, and chemically measurable, endpoints for evaluating enzyme activity. Commonly endpoints are selected based on: disappearance of a substrate, production of an end-product, consumption of cellular energy, production of byproducts, or oxidation of a reducing equivalent (Table 32.2).

Unfortunately, enzyme activity measurements are considerably more complex than simply quantifying enzyme proteins or the production of metabolites. Achieving efficient extraction of proteins of interest can be challenging and the cellular environment or environments in which the enzyme operates may be difficult to simulate *in vitro*. In this section we consider general approaches to activity assays (e.g., strategies and types of assays), practical considerations (e.g., precautions and controls), kinetic measurements, and interpretation (e.g., deciding whether an enzyme activity can serve as an index of a metabolic rate).

1.3.1. Approaches to enzyme activity measurements

Assaying enzyme activity is often a trade off between the desire to measure activity in living cells under natural environmental conditions (a strict *in vivo* assay) and extracting and purifying the enzyme and making measurements under precisely controlled conditions (a strict *in vitro* assay). While maintaining natural, and thus relevant, conditions is often important in understanding rate reactions *in situ*, it is often difficult to measure the reaction in a way that is satisfactory. Therefore, in practice, all assays fall somewhere in between the two extremes: even the gentlest *in vivo* assay involves some perturbation to the natural environment, and even the most rigorous *in vitro* assay involves assumptions (see Kornberg, 1990).

In general, the best candidates for *in vivo* assays are extracellular or cell surface associated enzymes because there are a variety of fluorescent or fluorogenic substrates that can be added to intact cells in natural seawater whose disappearance or products can be measured (e.g., leucine aminopeptidases (LAPs), cell surface amino

Table 32.2 Overview of N assimilatory enzymes, their chemical reactions, and assay endpoints

N Assimilation	Reaction catalyzed	Energy	Reductant	Cofactors	Inhibitors	Assays
Nitrate reductase	$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O$	ATP	NAD(P)H; NADH; NADPH; ferredoxin		NH_4^+, CN^-, hydroxylamine, vanadate CN^-	NO_2^- production, NADH oxidation
Nitrite reductase	$NO_2^- + 6e^- + 8H^+ \rightarrow NH_4^+ + 2H_2O$		Ferredoxin; NAD(P)H			Disappearance of NO_2^-, NH_4^+ production
Nitrogenase	$N_2 + 8H_2 \rightarrow 2NH_4^+$	ATP	Ferredoxin or flavodoxins	Mg^{2+}		Acetylene reduction, $^{15}N_2$ uptake
Urease	$CO[NH_2]_2 + 2H_2O \rightarrow 2NH_4^+ + CO_2$		Ferredoxin or flavodoxins		High urea	NH_4^+ production
Urea carboxylase/ allophanate hydrolase	$CO[NH_2]_2 + 3H_2O + HCO_3^- \rightarrow 2CO_2 + 2NH_4^+$	ATP		Biotin	Avidin	$^{14}CO_2$ production
Glutamate dehydrogenase	$NH_4^+ + \alpha\text{-ketoglutarate} \rightarrow$ glutamate		NAD(P)H; NADH; NADPH		Methionine sulfoxamine azaserine, albizziine	NAD(P)H oxidation or NAD(P)$^+$ production
Glutamine synthetase	$Glu + NH_4^+ \leftrightarrow Gln$	ATP		Mg^{2+}; Mn^{2+}		Hydroxylamine, P_i formation, ATP hydrolysis and NADH oxidation, glutamine production

(Continued)

Table 32.2 Overview of N assimilatory enzymes, their chemical reactions, and assay endpoints (*continued*)

N Assimilation	Reaction catalyzed	Energy	Reductant	Cofactors	Inhibitors	Assays
Glutamate synthase	Gln + α-kg → 2Glu					^{14}C glu production; NADH oxidation
Extracellular proteases	Various		NADH; NADPH; ferredoxin			Fluorogenic and fluorescent substrates; substrate disappearance; product formation
Amino acid oxidases	Amino acid + H_2O + O_2 → NH_4^+ + keto or other acid + H_2O_2					H_2O_2 production; fluorescent substrates; substrate disappearance; product formation

acid oxidases, and peptide hydrolysis; Hoppe, 1983; Mulholland *et al.*, 1998, 2002, 2003; Pantoja and Lee, 1994, 1999; Pantoja *et al.*, 1997). Because enzymes catalyzing similar or even identical reactions can be located both intracellularly and on the cell surface, for assays of cell surface enzymes, it is best to disturb the sample as little as possible to keep cells from bursting and mixing intracellular and extracellular enzyme pools. If truly extracellular enzymes are the assay target, then it is also important to do filtered controls to distinguish enzymes released to the environment (exoenzymes) from those associated with cells (ectoenzymes).

Intracellular enzymes are usually measured in chemically defined homogenates *in vitro* (e.g., nitrate reductase, Berges *et al.*, 1995). Recently, novel noninvasive assays have been developed using magnetic resonance techniques (e.g., Callies *et al.*, 1992; Mesnard and Ratcliffe, 2005), and some of these may be applicable to natural plankton assemblages (see Feuillade *et al.*, 1995). There are also a number of hybrid assays, where intracellular enzymes are measured by permeablizing cell membranes without extraction (sometimes referred to as ''*in situ*'' assays, see Corzo and Niell, 1991). In some respects, hybrid assays represent the worst of both worlds: permeablization (typically with alcohols) alters the local environment of the enzyme so *in vivo* conditions are not realized, yet the substrate concentrations are not controlled as in *in vitro* assays (see Berges, 1997). The availability of membrane-permeable substrates and the ability to monitor changes in them on a single-cell basis using flow cytometry has also led to the development of new enzymological methods, and though these have yet to be applied to enzymes of the N cycle, there is enormous potential for a growing number of reactions for which fluorescent substrates are available (Collier, 2000; Jellett *et al.*, 1996; Jochem, 1999).

The vast majority of *in vitro* enzyme assays measure activity in a reaction vessel to which reductant, substrates, and cofactors are supplied in excess (so-called V_{max} assays, see Section 1.3.3). Under these conditions, one assumes that enzyme activity is not limited by any chemical factor so the enzyme-mediated reaction proceeds at its optimal rate. Consequently, V_{max} assays are sometimes referred to as measuring ''potential'' activity, even though this term can be deceptive (see Section 1.3.5). *In vivo* assays, on the other hand are thought to better estimate ''actual'' physiological rates *in situ* because reductant, substrates, and cofactors are not augmented. However, it is conceivable that energy supply or substrates may limit activity, at least over time in a closed reaction vessel, and so care must be taken to select appropriate time scales over which to measure activity. As a further complication, there may be more than one enzyme catalyzing the same reaction in an organism or extract, or multiple organisms with different forms of the same enzyme in a given samples. In this case, kinetic parameters at subsaturated substrate concentrations may well be impossible to interpret.

1.3.2. Assays

Several considerations are common for both *in vivo* and *in vitro* assays. For example, many enzymes can be assayed using artificial rather than physiological substrates. This may simplify detection and quantification of substrate or product pools, but rates measured are not necessarily equivalent to those achieved using natural substrates. Enzyme assays are usually either stop-time (i.e., a substrate is added, the

reaction is allowed to proceed, and is then stopped at a precise time; comparing initial and final time points, depletion of substrate or increase in product can be calculated), or continuous (i.e., a variable such as absorbance or fluorescence is continuously monitored over time, and changes in these properties can be related to changes in substrate or product) (see Rossomando, 1990). Absorbance measurements have traditionally been used, based on specific peaks (e.g., NADH absorbs strongly at 340 nm), but fluorescence measurements are more precise (since two wavelengths are specified, e.g., fluorescein isothiocyanate (FITC)-labeled substrates have absorption peaks near 480 nm and fluorescence peaks close to 530 nm), and generally offer detection limits over an order of magnitude lower. Care must be taken in selecting fluorescent compounds because many substrates developed for medical use have red fluorescence (e.g., Texas Red), and are almost useless in organisms that have chlorophylls; green fluorescing substrates (e.g., FITC) are generally more adaptable.

1.3.2.1. *Storage and extraction* Because enzymes are highly labile, care must be taken during sample collection, storage, and handling to preserve enzyme activity. Appropriate holding times for preserved samples, holding temperature, and selection of preservatives or preservation methods all need to be considered on an enzyme-specific basis. For example, algal nitrate reductases are extremely labile and require immediate storage at liquid N_2 temperatures (e.g., $-196^{\circ}C$) to maintain activity (see Berges and Harrison, 1995a, Young *et al.*, 2005), while many algal proteases retain activity at room temperatures for many hours (e.g., Berges and Falkowski, 1998). Enzymes must be protected during sample handling and homogenization to avoid pH fluctuations, oxidation of cellular material, and other compounds such as phenolics or exogenous heavy metals (Gegenheimer, 1990; Volkin and Klibanov, 1989). In addition, selection of extraction buffers is important because, in addition to controlling the pH, activities of different enzymes are enhanced or depressed by different buffers (Berges and Harrison, 1995a; Stoll and Blanchard, 1990). Finally, intracellular proteases can degrade enzymes, either specifically or nonspecifically. A number of protease inhibitors can be used to prevent this (unless of course proteolytic enzymes are the target of assays) (Gegenheimer, 1990; Volkin and Klibanov, 1989); however, in phytoplankton and macroalgae, there is evidence that commonly-used protease inhibitors are not effective (see Berges and Falkowski, 1996; Perez-Llorens *et al.*, 2003).

1.3.2.2. *Assay conditions* To measure realistic enzyme activities, pH, temperature, and ionic strength should be kept as close to physiological conditions as possible, but in many cases we may not know the conditions that are relevant to a particular enzyme within a cell or cellular compartment. One strategy is to assume that the conditions that produce maximum activity are those for which the enzyme is adapted. However, while changing pH and temperature may produce higher rates of activity in a test tube, the resulting measurements often become further distanced from reality. For example, raising the assay temperature generally increases reaction rates up to a point. However, high temperatures can also denature enzymes and result in lower enzyme activity (e.g., Berges *et al.*, 2001). Rather than ''maximizing''

activity, a better process might be "optimization," wherein temperature, pH, and ionic strength are kept close to what are believed to be physiological conditions, and substrate and cofactor concentrations are adjusted so as to be nonlimiting for a given assay duration (see also Newsholme and Crabtree, 1986).

In the case of *in vivo* assays, there are tradeoffs in selecting the concentrations of substrates (including fluorescent or fluorogenic compounds being used as tracers) to add to incubations. Concentrations of substrates are ideally adjusted to levels found in the environment. However, sometimes these concentrations are not known or ambient concentrations are so low that substrates are rapidly depleted in bottle incubations, precluding an accurate rate measurement. It is therefore paramount that enzyme assays are conducted on appropriate time scales (and these may vary temporally, spatially and depending on the enzyme and organism) so that substrates are not significantly depleted in incubation bottles. Conversely, if substrate additions are too high relative to ambient environmental concentrations, enzyme activity can be stimulated over natural conditions (e.g., see Chapter 7 by Mulholland and Lomas, this volume). A common strategy is to add substrates at saturating levels. This allows for stable measurements, and can be an indication of maximal rates of activity under the environmental conditions. These so-called "potential" rates should be used cautiously because rate processes in organisms often depend on nutrient and environmental "prehistory," and because other elements and factors also in short supply in nature may become limiting over time in incubation bottles.

For intracellular enzymes, the picture is complicated because we do not have a clear idea of physiological substrate concentrations and turnover times inside cells; indeed, compartmentalization, metabolic channeling, and tight coupling of reactions make it difficult to estimate intracellular concentrations even under ideal conditions (Albe *et al.*, 1990; Srere, 1987). For these reasons, intracellular enzyme assays are typically run at saturating substrate concentrations (i.e., V_{max} assays); the reaction rate should then depend only on the quantity of active enzyme (see Section 1.3.3).

1.3.2.3. Quality-control measures

There are a number of basic quality-control steps that need to be taken to ensure that rate measurements are not biased by assay artifacts. First, it must be demonstrated that the rate reaction is linear over time for the period of the assay. If this is not the case, then either the enzyme is not stable, the substrates are being depleted over the course of the assay, or there is some sort of inhibition of the reaction over time, perhaps by a reaction product. Second, the activity should vary directly with the amount of enzyme (or homogenate) added. If this is not true, it suggests a problem with the assay such as degradation of enzyme or exhaustion of substrates. Third, for a V_{max} assay, all reaction substrates must be saturating. Typically, this is evaluated by running parallel reactions varying the concentration of each substrate independently; however, even this is not simple because reactions can also be inhibited by high substrate concentrations.

1.3.2.4. Scaling activity

Once activity measurements have been made, they must be scaled to something ecologically or physiologically relevant. *In vitro* enzyme activity is typically scaled to cell protein because it is straightforward to make protein

determinations in the same homogenate that is used for assays, but this may not always be appropriate (see Berges and Harrison, 1995b). In many studies of N cycling, it is relevant to simply express enzyme activity as the amount of nitrogenous product generated or substrate consumed per unit time per unit biomass. Alternative scaling variables include cell number, cellular N and C, or chlorophyll *a* biomass; the choice of scaling factors depends largely on the process being measured and the context in which the measurement was made. Enzyme activity is typically expressed as units (U) (or, less commonly, International Units, IU), where one unit is the amount of enzyme needed to catalyze the conversion of one micromole substrate to product in one minute. Since minutes and micromoles are not SI units, some investigators prefer to use moles per second, although this results in small values. The katal (kat), a derived SI unit representing the catalytic activity that will raise the rate of reaction by one mole per second in a specified assay system is technically correct, but is not often used (see Dybkaer, 2000).

1.3.3. Enzyme kinetic measurements

Beyond the instantaneous rate measurements described above, determining how enzyme activity varies with substrate concentration can provide useful information about enzyme capacity and the rates that are likely to be observed under different environmental conditions. These kinetic measurements probably work best in *in vivo* assays of exo- and ectoenzymes where substrate concentrations can be measured directly from the external environment and there are no metabolic intermediary pools or reactions to complicate the picture. Kinetic measurements also have limitations as there can be bottle effects, diffusion boundary layers around organisms can be important, and it is often difficult to make measurements at the "low" substrate end-members in all but the most oligotrophic environments. Further, kinetic parameters are physiological variables themselves, dependent on the preconditioning of cells and so can vary widely even in the same organism, across environments (see Chapter 7 by Mulholland and Lomas, this volume).

For *in vitro* assays, it is extremely difficult to determine the relevant intracellular concentrations of substrates due to compartmentalization of particular reactions, competing reactions that produce and consume common substrates, and metabolic channeling (Albe *et al.*, 1990; Srere, 1987). Consequently, *in vitro* kinetic measurements may not be very useful for assessing *in situ* or *in vivo* reaction rates. However, provided assays conditions are carefully controlled, kinetic parameters may be very useful for characterizing enzyme isoforms and enzymes from different metabolic pools, different organisms, or from organisms collected from different environments.

In most cases where enzyme kinetics have been measured in marine settings, simple Michaelis–Menten models have been used to characterize enzyme activity. This is sensible, because such rectangular hyperbolic models suit many biological processes (e.g., Berges *et al.*, 1994). Aquatic biogeochemical models use a "classical" kinetics formulation to describe N uptake (e.g., Fasham *et al.*, 1990; Moore *et al.*, 2002). Similarly, physiological models use enzyme kinetic principles to formulate mathematical equations describing enzymatically mediated N assimilation (Packard *et al.*, 2004). However, it is still unclear whether such kinetic descriptions are ecologically meaningful for the reasons described earlier. In addition, many enzymes

display non-Michaelis–Menten kinetics due to regulatory pathways, and multiple enzymes or enzyme isoforms accomplishing the same reaction but with different substrate affinities. For example, allosteric enzymes have multiple subunits, regulatory sites that bind modulators other than the substrate, and/or multiple binding sites for substrate. Michaelis–Menten kinetics assume that there is no interaction among binding sites when in fact, binding of a modulator can cause activation/inhibition of enzyme activity, while binding of a substrate on another subunit may increase or decrease binding at another subunit (cooperativity) (Cornish-Bowden, 2004; Ottaway, 1988). Such mechanisms make it difficult to interpret enzyme activity, especially in the context of the commonly used kinetic models (see also Section 1.3.5).

1.3.4. Enzyme inhibitors

Enzyme inhibitors represent powerful tools for isolating specific enzyme activities in crude homogenates (e.g., proteases; Berges and Falkowski, 1996), verifying assays, and determining whether alternative metabolic pathways exist. They can also provide a means to differentiate the roles of different taxa that perform identical reactions. For example, cyanobacterial assimilatory nitrate reductases are quite distinct from eukaryotic forms and so the relative contributions of the two groups to total nitrate assimilation might be distinguished using a carefully selected inhibitor. Very few specific inhibitors are known (e.g., vanadate for nitrate reductase, methionine sulfoximine [MSX] for GS, and azaserine for glutamate synthase), and so their use has been limited.

To be used effectively, the mechanisms through which inhibitors exert their effects need to be understood. For example, competitive inhibitors compete with physiological substrates for binding sites. Their effect depends on the relative concentrations of substrates and the inhibitor and the degree of inhibition depends on the number of active sites occupied by the inhibitor versus the metabolic substrate. In contrast, noncompetitive inhibitors bind to parts of the enzyme other than the substrate binding site, so the degree of inhibition depends only on the inhibitor and not the substrate concentration. This type of inhibition is typically irreversible and reduces the amount of total enzyme available to catalyze a particular reaction. Uncompetitive inhibition occurs when the inhibitor binds to the enzyme–substrate complex and prevents the reaction from being catalyzed.

There are also inhibitors that affect enzyme synthesis. Inhibitors of transcription (e.g., dibromothymoquinone [DBMIB]) and inhibitors of translation (e.g., cycloheximide [CHX]) are available but these are not specific to particular enzymes. In addition, because protein synthesis takes place within the chloroplast and mitochondrion in eukaryotes, prokaryotic protein synthesis inhibitors (e.g., chloramphenicol [CAP]) may be necessary to distinguish prokaryotic versus eukaryotic activity (e.g., Segovia and Berges, 2005).

1.3.5. Interpreting enzyme measurements

There are many factors that can affect enzymes *in vivo* that are not simulated in *in vitro* assays. Consequently, *in vitro* enzyme activities may or may not reflect actual *in situ* rates. In nature enzymes may not be substrate-saturated as they are in *in vitro* assays;

N is often limiting in the marine environment. Further, many enzymes are sensitive to cellular substrate concentrations rather than extracellular concentrations and it is difficult to measure the relevant intracellular metabolite pools. *In vitro* assays may affect the conformation of enzymes and the degree to which they are modified. For example, allosteric effects (see Section 1.3.3) may be modified under *in vitro* conditions. Many enzymes undergo posttranslational regulation wherein enzyme activity is affected by binding of activator/inactivator proteins and covalent modification of the enzyme (e.g., adenylylation, phosphorylation or carbamylation) (Ottaway, 1988). When there is posttranslational modification of enzymes, enzyme activity measured in assays may be unrelated to *in vivo* activity (see Section 2.2.1) and there are few ways to determine the extent of enzyme modification in nature.

To understand why it is often difficult to interpret *in vitro* enzymes assays, it is useful to consider the principles of metabolic control theory, derived from engineering control theory (Raven, 1981). Consider a series of enzyme catalyzed reactions, where *a, b, c*, and *d* represent substrates and or products of reactions catalyzed by enzymes represented by A, B, and C:

$$a \xrightarrow{A} b \xrightarrow{B} c \xrightarrow{C} d$$
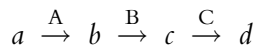
Control of the whole series of reactions generally rests at one point (the rate-limiting step), which is often the first step (Newsholme and Crabtree, 1986), catalyzed in this case by enzyme A. Further, it has been argued that it is advantageous that control of a metabolic pathway not shift between steps as conditions change, otherwise regulation would be unreasonably complex, i.e., in this example, the step catalyzed by A should continue to be rate-limiting. For this to be true, the concentration of *b* and substrates downstream must always be below saturation for the enzymes catalyzing their transformations, such that they do not accumulate and enzymes B and C do not become rate limiting. It follows that enzyme A is always functioning at close to its capacity, an thus V_{max} assays for A would give information about the rate of flux through the whole pathway, but that V_{max} assays for enzymes B and C would not. Another way cells could maintain a single rate-limiting step in a metabolic pathway is for downstream enzymes (e.g., B and C) to have K_m values that are close to the steady state concentrations of *b* and *c*, respectively, when the pathway is functioning at its maximum rate (see Heinrich and Hoffmann, 1991). In this way, even small changes in *b* or *c* will result in relatively large changes in the rates of enzymes B and C (respectively) and thus prevent these enzymes from becoming rate limiting. Although this example is theoretical, there are considerable empirical data supporting such relationships (see Albe *et al.*, 1990; Pettersson, 1991). Moreover, if conditions change so that fluxes through a pathway increase or decrease, parallel adjustments in concentration occur in all the enzymes along the particular metabolic pathway (see discussion in Fell, 2005), opening the possibility that even nonrate-limiting enzymes (e.g., B or C) could provide information about prevailing metabolic fluxes (see Newsholme and Crabtree, 1986).

In addition to determining where metabolic pathways are regulated it is also essential to understand how particular enzymes are regulated in order to interpret

enzyme activity assays. In the case where an enzyme or enzyme pathway is controlled by an enzyme whose concentration or activity is regulated at the level of synthesis and degradation, an *in vitro* enzyme activity is likely to be a good reflection of *in situ* rate. It is much more difficult to interpret enzyme activity for enzymes that are regulated allosterically or posttranslationally because simple V_{max} measurements may not reflect enzyme availability or potential.

Analysis of metabolic networks may help us properly interpret enzyme activities from enzyme abundance, and ultimately extrapolate to the whole organism using models. Application of such computational modeling to enzyme systems is still limited but promising. For example, Su *et al.* (2006) were able to develop computational models of N assimilation in *Synechococcus* sp. WH 8102 that closely corresponded to results from microarray analyses of gene expression.

Ultimately, if enzyme activity measurements are to be useful we need to be able to validate them in cultures under controlled laboratory conditions, and then in controlled field settings where there is basic understanding of the important processes, resident organisms, and their metabolic pathways. In natural samples, it is often difficult to specifically target organisms of interest using bulk collection methods. As a result, it is not always clear to whom enzyme activities can be attributed (e.g., cooccurring organisms or endosymbionts). Unfortunately, the field-oriented focus of aquatic sciences, lack of appropriate cultured organisms, and the time-pressures of most project funding cycles mean that such validations are seldom done.

2. Case Studies of Specific Enzymes

2.1. Inorganic nitrogen acquisition

Inorganic N is taken up by microbes and marine plants in a variety of forms and uptake is facilitated through a variety of transporters (see Chapter 7 by Mulholland and Lomas, this volume). Inorganic N compounds must first be reduced to ammonium prior to their assimilation into organic compounds and these reduction steps are enzymatically mediated and are described here.

2.1.1. Nitrogenase
2.1.1.1. Overview Nitrogenase, the enzyme complex catalyzing N_2 fixation, is found in phylogenetically diverse prokaryotes (archaea and bacteria, including cyanobacteria), ranging from aerobic heterotrophs, aerobic and anaerobic phototrophs to strict anaerobes (see Chapter 4 by Carpenter and Capone, this volume). The enzyme is extremely sensitive to O_2 and N_2 fixation is energetically expensive, "costing" a cell up to 16 molecules of ATP per molecule N_2 reduced (Postgate, 1998). Nitrogenase enzymes are numerous but biochemically similar and so the whole family of enzymes is referred to as "nitrogenase" even though the name does not conform to systematic enzyme nomenclature (Postgate, 1998). The enzyme complex nitrogenase consists of two proteins, a molybdenum iron protein (Protein 1) that binds N_2 (dinitrogenase reductase) and a smaller iron protein (Protein 2) that acts

as an electron donor to Protein 1 (nitrogenase reductase), although alternative nitrogenases that contain vanadium or only iron in the larger subunit have been identified. As well as catalyzing the reduction of N_2 to NH_3, nitrogenase can reduce other small triply bonded molecules, including acetylene, azide, and cyanide, and nitrogenase reduces hydrogen ions to gaseous hydrogen, even when N_2 is present. Many assays of nitrogenase activity take advantage of these competing reactions. Although N_2 consumption or H_2 production theoretically can be used to estimate N_2 fixation rates, N_2 concentrations in aquatic systems are high (and generally saturated in seawater) and simultaneous production and consumption of H_2 both intracellularly and extracellularly through a variety of processes confounds interpretation of this measurement.

2.1.1.2. Assay techniques   Because robust *in vitro* assays have not been developed, nitrogenase activity has been estimated based the use of isotopic tracers and substrate analogs. Nitrogen fixation rates have been estimated based on $^{15}N_2$ uptake using tracer techniques (e.g., see Chapter 31 by Lipschultz, this volume). This method is a sensitive measure of net $^{15}N_2$ uptake into particulate organic nitrogen (PON) (Montoya *et al.*, 1996; Mulholland *et al.*, 2004a,b, 2006), however, because diazotrophs can release fixed N_2 prior to its assimilation into biomass (e.g., Capone *et al.*, 1994; Glibert and Bronk, 1994; Mulholland *et al.*, 2004a,b, 2006), $^{15}N_2$ uptake can underestimate total or gross N_2 fixation (Gallon *et al.*, 2002; Mulholland *et al.*, 2004a,b, 2006). Other assays of nitrogenase activity rely on reduction of other triply bound compounds that serve as N_2 analogs.

Most often, assays of nitrogenase take advantage of the enzymes' ability to reduce acetylene (C_2H_2) (Capone, 1993). The acetylene reduction method is convenient, very sensitive and relatively simple. However, acetylene reduction assays should not be excessively long because nitrogenase preferentially reduces C_2H_2 as opposed to its natural substrate N_2 and thus ''starves'' cells for N. Because C_2H_2 reduction measures just the reduction step, it is a measure of gross N_2 fixation, while movement of $^{15}N_2$ from the dissolved to the particulate pool measures net N assimilation (see Gallon *et al.*, 2002; Mulholland and Bernhardt, 2005; Mulholland *et al.*, 2004a,b). In addition, the stoichiometry of these two reactions is different and a conversion factor is needed to estimate N_2 fixation rates; theoretically a ratio of 3:1 mol C_2H_2:N_2 are reduced (Montoya *et al.*, 1996; Postgate, 1998). However, because nitrogenase reduces H^+ along with N_2, ratios >3:1 are often assumed and observed; for example, if 1 mole of H_2 is also produced for each mole of N_2 reduced, the ratio of C_2H_2:N_2 fixation increases to 4:1. Despite this potential bias, for the marine diazotroph *Trichodesmium*, little net H_2 production has been observed under environmental conditions (Scranton, 1984; Scranton *et al.*, 1987) suggesting either: (1) that hydrogen production from nitrogenase is low in nature (and so a 3:1 conversion factor is justified), or (2) that cells efficiently recoup H_2 produced during N_2 fixation, arguing for higher ratios and the need for a thorough evaluation of hydrogen cycling within cells.

Estimates of acetylene reduction are usually calibrated against $^{15}N_2$ to determine the appropriate ratio extrapolating N_2 fixation from C_2H_2 reduction. However, release of recently reduced N_2 and the difficulty in chemically recovering all possible

dissolved pools into which products of N_2 fixation might be released, may make intercalibration between the two methods impossible (Mulholland, 2007). In numerous paired comparisons, ratios of C_2H_2 reduced to N_2 taken up varied by at least an order of magnitude (see Mulholland, 2007; Mulholland et al., 2006). Even though it may be impossible to intercalibrate the two methods, the difference between N_2 reduction (gross N_2 fixation) and net N_2 assimilation may provide an excellent index of the release of recently fixed N_2 that has proven promising in culture systems (Mulholland, 2007; Mulholland and Bernhardt, 2005; Mulholland et al., 2004a,b, 2006).

More recently, the capacity for N_2 fixation has been evaluated by amplifying *nif* genes (which encode nitrogenase enzymes), from natural samples using appropriate primers (see Chapter 30 by Jenkins and Zehr, this volume). DNA macro- and microarrays have been used to explore the diversity of N_2 fixing organisms (Jenkins et al., 2004) and quantitative PCR approaches have been used to determine the abundance of particular groups of diazotrophs (Church et al., 2005). These molecular methods have high sensitivity but they cannot determine if there is actually nitrogenase activity. Recent studies have coupled gene expression assays with uptake experiments to demonstrate active N_2 fixation in open ocean unicellular diazotroph populations (Montoya et al., 2004).

2.1.1.3. Enzyme regulation and interpretation of activity assays For *Trichodesmium*, probably the best studied marine N_2 fixer, nitrogenase is regulated both at the level of transcription and activity and so gene presence and even the presence of gene products, are not quantitative measures of nitrogenase activity (Zehr and Capone, 1996; Zehr et al., 1993; and see Chapter 30 by Jenkins and Zehr and Chapter 7 by Mulholland and Lomas, this volume). Nitrogenase, like many enzymes involved in N reduction and metabolism, appears to be regulated through negative feedback from intracellular NH_4^+, other downstream metabolites (amino acids), and cellular N:C status (Flores and Herrero, 1994; Guerrero and Lara, 1987; Mulholland et al., 1999). N_2 fixation also appears to be sensitive to or inhibited by other forms of N in the growth medium (Flores and Herrero, 1994; Guerrero and Lara, 1987; Smith and Gallon, 1993). Though it was previously thought that virtually any NH_4^+ or NO_3^- in the growth medium inhibited the synthesis and activity of nitrogenase, recent studies suggest that environmentally-relevant concentrations (e.g., 1 or 2 μM) of these compounds do not inhibit N_2 fixation in the oligotrophic or coastal ocean (Mulholland et al.1999, 2001). In addition to N, nitrogenase activity is dependent on the provision of ATP (and so is tied in with the light cycle in photoautotrophs) and Mg^{2+} and the absence of O_2. As for other N assimilation pathways, further metabolism of NH_4^+ produced through N_2 fixation can also be limited by the provision of carbon skeletons.

In addition to regulation at the level of transcription, at least some nitrogenases can be regulated by covalent modification (inactivation) of the Fe protein (Capone et al., 1990; Gallon, 1992; Zehr et al., 1993). However, regulation of nitrogenase appears to be complex since nitrogenase is also subject to circadian rhythms and may be related to heterocyst formation in heterocystous species (Chen et al., 1996, 1998; Herrero et al., 2001).

2.1.1.4. *Information gained from assays and future potential* Nitrogenase activity assays remain the most important means to estimate rates of N₂ fixation and acetylene reduction assay is a good example of an *in vivo* assay that does not require much manipulation of natural samples. Thus, there should be a good relationship between what the assay measures and the actual rate of N₂ fixation *in situ*. Finding means to convert measures of acetylene reduction to N₂ reduction remains a critical issue, and H₂ production is an important part of the problem. Physiological studies of N₂ fixation and H₂ production catalyzed by nitrogenase in *Trichodesmium* are hampered by the lack of *in vitro* assays.

2.1.2. Nitrate reductase

2.1.2.1. *Overview* Because nitrogen is thought to limit primary productivity in much of the world ocean, and nitrate thought to be the primary form of new N available in most systems (Dugdale and Goering, 1967), nitrate reductase (NR) activity was one of first enzyme measurements to be broadly applied in an oceanographic context (Eppley, 1978; Eppley *et al.*, 1969). Features of the enzyme that made it suitable for assay included high specific activity, relatively easy extraction, and a straightforward, sensitive assay.

In eukaryotes, NR is a complex enzyme containing molybdenum, iron–heme, and flavin adenine dinucleotide that uses NADH, NADPH, or both as a reductant (the basis for division of the enzyme into subclasses: EC 1.7.1.1, 1.7.1.2, or 1.7.1.3). The eukaryotic enzyme is best characterized from higher plants (Campbell, 1999; Fischer *et al.*, 2005), but generalizations hold well in the green algae (Solomonson and Barber, 1990; Song and Ward, 2004; Wang *et al.*, 2003) and diatoms (NR's in *T. pseudonana* and *P. tricornutum* are 50–60% identical to higher plant and green algal gene sequences; Allen *et al.*, 2005) where the genes and proteins have been examined. In most cases, the enzyme appears to be dimeric, with subunits of about 100 kDa in size, however, Iwamoto and Shirawa (2003) have suggested that NR from *Emiliania huxleyi* is different, possibly a hexamer with 85 kDa subunits, and there are reports that NR from dinoflagellates may differ as well (Ramalho *et al.*, 1995). Assimilatory nitrate reductases are quite distinct enzymes in prokaryotes where they are ferredoxin-dependent and contain iron–sulfur clusters (Flores *et al.*, 2005).

2.1.2.2. *Assay techniques* One of the major problems in assaying NR activity has been the stability of the enzyme. Consequently, it has been difficult to measure any NR activity in some field samples (e.g., Hochman *et al.*, 1986), and measured NR activity has often been insufficient to account for observed rates of nitrate incorporation (e.g., Blasco *et al.*, 1984). Attempts to overcome this problem have included: assaying the parts of the enzyme catalyzed reaction that are less affected such as NADH oxidizing (diaphorase) activity, assaying NO_3^- reducing activity in the presence of artificial reducing compounds such as methyl viologen, or developing ''in vivo'' assays (Corzo and Niell, 1991; Hochman *et al.*, 1986). Problems extracting enzyme may be related to the high proteolytic activities observed in phytoplankton, which appear resistant to classical protease inhibitors (see Berges and Falkowski, 1998). Some success in countering this problem has been achieved by adding

''carrier'' proteins like BSA to provide abundant alternative targets for proteases, thereby protecting NR (Berges and Harrison, 1995a). NR activity is also very sensitive to storage conditions. In many cases, there are significant losses of activity when samples are stored frozen at $-80°C$, and preservation at liquid N_2 temperature is essential (Berges and Harrison, 1995a; Vergara et al., 1998; Young et al., 2005).

Typically, NR assays measure production of nitrite, using the sulphanilamide/N-(1-naphyl) ethylenediamine dihydrochloride reaction after a timed incubation period, and correcting for any nitrite present initially in extracts. Continuous spectrophotometric assays that measure NADH oxidation over time are feasible when cell biomass is high (e.g., in cultures), but corrections must be applied because enzymes other than NR present in cell homogenates can also oxidize NADH (Berges and Harrison, 1995a). Complicating things further, high levels of NADH and nitrate inhibit NR activities in some species and so assays need to be optimized on a species–specific basis (Berges and Harrison, 1995a,b). It is equally important to optimize assays for all types of NR because some NR enzymes require flavin adenine nucleotide (FAD) as a cofactor and this is also variable among species (Berges et al., 1995a; Everest et al., 1984).

2.1.2.3. Enzyme regulation and interpretation of activity assays

A diverse range of regulatory mechanisms have been described for NR in higher plants, green algae, and cyanobacteria, and these include induction by nitrate, repression by NH_4^+ or other products of N metabolism, and modulation by light (Campbell, 1999; Herrero et al., 1984; Solomonson and Barber, 1990; Song and Ward, 2004). In addition to feedback inhibition of NR, NH_4^+ can interfere with NO_3^- transport, thereby inhibiting NO_3^- uptake and reduction. The effect of NH_4^+ on NR is rapid in diatoms, indicating a relatively fast turnover of the enzyme (Berges et al., 1995; Vergara et al., 1998). In contrast, in some intertidal macroalgae, additions of ammonium have little effect on NR activity (Young et al., 2007b).

In eukaryotic algae (at least in diatoms), the primary mode of regulation of NR appears to be at the level of synthesis and degradation (Berges, 1997; Vergara et al., 1998). Consequently, the interpretation of NR activity measurements is relatively straightforward, and NR activity and NO_3^- incorporation rates are correlated (Berges and Harrison, 1995a,b; Berges et al., 1995). While posttranslational modification of NR by phosphorylation in response to changes in irradiance is well-described in higher plants (Huber et al., 1992), it has not been demonstrated for algae, and in diatoms, the NR protein appears to lack the key phosphorylation site found in higher plants (Allen et al., 2005) making it unlikely that this is a viable regulatory pathway.

Diel variability in NR activity, and NO_3^- uptake, has been observed in a variety of N–replete marine phytoplankton communities (e.g., Packard et al., 1971). Patterns vary among species (e.g., Lopes et al., 1997; Ramalho et al., 1995). In diatoms, NR activity typically has a peak near the middle of the light period, but there is also an increase in activity just prior to the beginning of the light period (Berges et al., 1995). This periodicity does not appear to be under the control of a circadian oscillator (cf., Chow et al., 2004; Vergara et al., 1998), but is more likely to be

linked to changes in internal carbon pools. The diel variability of NR activity is also affected by N status and N-deplete or starved cells will take up NO_3^- even during the dark (e.g., Cochlan *et al.*, 1991). Interestingly, while macroalgae in the lower intertidal zone show diel variations in NR activity, this is not observed in *Fucus* species in the upper intertidal (Young *et al.* 2007b).

NR activity is also sensitive to temperature, at least in diatom species. (Gao *et al.*, 2000) demonstrated that NR activity in diatoms such as *Skeletonema costatum* tended to lose activity above 16°C, while other eukaryotic enzymes were stable at over 30°C. Lomas and Glibert (1999) combined laboratory and field data to argue that this temperature sensitivity effectively limited diatoms to particular environments. Berges *et al.* (2002) speculated that features of the N-terminus of the NR protein might be responsible for temperature sensitivity, and these appear to be borne out in data from recent sequencing projects (Allen *et al.*, 2005).

In cyanobacteria, transcription of NR seems to be triggered by a high intracellular C:N ratio, as for nitrogenase (see Section 2.1.1.3). Further, regulations of NR and nitrogenase appear to share common features in a variety of organisms (e.g., Flores and Herrero, 1994). We are just beginning to understand many of the complex regulatory systems that control total N acquisition by aquatic microbes.

2.1.2.4. Information gained from assays and future potential   NR activity measurements have not been all that useful in predicting NO_3^- incorporation rates or new production as once hoped (see Blasco *et al.*, 1984), though these earlier assays may have underestimated true NR activities. However, NR assays have proven useful in examining the effects of iron limitation on nitrate metabolism (e.g., Boyd *et al.*, 1998; Timmermans *et al.*, 1994; see also NiR section below). In addition, NR activity measurements have been used extensively to examine patterns of nitrate incorporation in macroalgae (e.g., Corzo and Niell, 1991; Davison and Stewart, 1984; Davison *et al.*, 1984; Hurd *et al.*, 1995; Young *et al.*, 2007a,b) and to assess the contribution of epiphytes to N cycling in seagrass meadows (Young *et al.*, 2005).

Differences among NR enzymes might be exploited to provide taxon–specific information. For example, since cyanobacterial and eukaryotic enzymes differ markedly, there may be potential for using different assays to distinguish eukaryotic and prokaryotic NO_3^- uptake in natural communities (see Berges, 1997). At the protein level, NR from diatom species is remarkably variable in the N-terminal region, so that targeting NR genes could provide community information at a functional level (Allen *et al.*, 2005).

Interestingly, despite the fact that nitrate is one of the largest pools of inorganic N in many marine systems, not all species of phytoplankton can use NO_3^-. For example, some ''brown tide'' organisms are unable to grow on nitrate (DeYoe and Suttle, 1994), and at least some marine *Prochlorococcus* apparently lack nitrate assimilation gene clusters (Rocap *et al.*, 2003). NR activity assays could confirm such findings.

Finally, in addition to playing a key role in nitrate acquisition, NR may play a role in cell signaling. Desikan *et al.* (2002) found that NR mediates nitric oxide (NO) synthesis in *Arabidopsis* guard cells. NO signaling has also been established in *Chlamydomonas* (Sakihama *et al.*, 2002) and quite recently in diatoms (Vardi *et al.*, 2006).

2.1.3. Nitrite reductase

2.1.3.1. Overview   Despite early interest in nitrite reductase (NiR), relatively fewer measurements of the enzyme have been made, compared to NR (e.g., Eppley and Rogers, 1970; Eppley *et al.*, 1971). Traditionally, it has been considered that algae and cyanobacteria have only the ferredoxin-dependent form of the enzyme (Campbell and Kinghorn, 1990; Flores *et al.*, 2005; Luque *et al.*, 1993), but annotation of the *T. pseudonana* genome indicates the presence of both a nuclear-encoded ferredoxin-dependent enzyme, targeted to the chloroplast (homologous to vascular plant/cyanobacterial forms), and a second NiR with homology to a NAD(P)H dependent form found only in heterotrophic bacteria and some fungi (Allen, unpublished data).

2.1.3.2. Assay techniques   NiR is typically measured by following the disappearance of NO_2^- in cell homogenates, when provided with either reduced ferredoxin or alternative electron acceptors such as methyl viologen (Vega *et al.*, 1980). The assay is somewhat more complicated than that for NR. If methyl viologen is used, then sodium dithionite is typically used to reduce it and to maintain reducing conditions throughout the assays, though strictly anaerobic conditions may not be necessary (Milligan and Harrison, 2000; Vega *et al.*, 1980). It seems from the genome annotation data that diatoms, like yeast, may also have NAD(P)H-dependent NiR, but this has not been reported on in the literature.

2.1.3.3. Enzyme regulation and interpretation of activity assays   Available evidence suggests that there is a tight coregulation between NR and NiR in both eukaryotes and prokaryotes. Indeed, in cyanobacteria, N assimilation genes show gene clusters of NiR–permease gene-NaR (nitrate reductase genes) with an operon structure (Flores *et al.*, 2005). In prokaryotes, the two enzymes are very closely linked (Faure *et al.*, 1991), and both probably respond to the presence of NO_3^- (e.g., Galvan *et al.*, 1992; Wray, 1993). Such a regulatory system would presumably prevent the build-up of pools of nitrite that could prove toxic to cells. In many cases, NiR activity is greater than NR activity, which provides additional support that NR is the rate limiting enzyme of the pair (see above). Carbon limitation may also affect NiR, as in the case of NR (Suzuki *et al.*, 1993, 1995).

2.1.3.4. Information gained from assays and future potential   NiR assays have much more seldom been applied in the field, perhaps because of the more complicated assay, but also because it is not the rate limiting step of the NiR–NR pair. However, there may be special cases where NiR does become limiting for NO_3^- acquisition. (Kessler and Czygan1968) provided evidence that because NiR in green algae contains more iron than NR, iron limitation more strongly affects NiR. This also appears to be the case for at least one species of diatom; (Milligan and Harrison, 2000) demonstrated that under iron limitation, NiR was more strongly affected than NR, and speculated that control of nitrate acquisition might shift from NR to NiR under these circumstances.

2.2. Ammonium assimilation pathways

Ammonium is the common denominator in inorganic N assimilation and most forms of N taken up by cells are first reduced to NH_4^+ prior to their assimilation into cellular material. Ammonium assimilation also metabolically links the C and N cycles because C skeletons are required for amino acid synthesis. Consequently, the enzymes involved in N assimilation are extensively regulated in response to a variety of intracellular cues (N and C) and environmental variables. The regulatory mechanisms for NH_4^+ assimilation in microorganisms generally build upon detecting the presence or absence of NH_4^+ in the environment or in the cell or feedback from other downstream metabolites. The regulatory networks of well-studied microorganisms, including bacteria and cyanobacteria, include a transcriptional factor, the global N regulator, *NtcA*, that is involved in regulating transcription of co-regulated genes, and the signal transduction protein P_{II}, which broadcasts and amplifies cellular N status within cells; together, these coordinate N (and to some extent C) metabolism in cells (García-Fernández *et al.*, 2004; Herrero *et al.*, 2001; Tandeau de Marsac *et al.*, 2001). Recently, (Su *et al.* 2006) demonstrated that models of the N assimilation network agreed well with gene expression data from microarray studies for a cyanobacterial system, offering hope that even quite complex metabolic systems may prove tractable to this type of modeling analysis.

The assimilation of NH_4^+ in most marine phytoplankton and bacteria is via a cyclic pathway involving the enzyme pair GS and glutamate synthase (glutamine oxoglutarate aminotransferase [GOGAT]). GS first assimilates ammonium into the amide group of glutamine (gln) and then, GOGAT transfers the amide group to a molecule of α-ketoglutarate (α-kg) to form two molecules of glutamate (glu), one of which is recycled to GS to synthesize another glutamine (gln). While the reaction is fully reversible, in cells it is thought that GS/GOGAT works primarily in the forward or assimilatory direction. However, it does so at the expense of cellular energy since the GS reaction requires one ATP for each molecule of gln produced. More is known about GS because that is generally considered the rate-limiting enzyme in the pair (see above).

Glutamate dehydrogenase offers an alternative pathway by which ammonium can be incorporated through the reductive amination of α-kg. Because of its higher K_m for ammonium (millimolar range for GDH versus micromolar range for GS; Ahmed *et al.*, 1977; Bressler and Ahmed, 1984), GDH is thought to play a minor role in N assimilation in marine microbes (see Muro-Pastor *et al.*, 2005). However, GDH may offer a competitive advantage for cells when energy supplies are limited because it does not require ATP (see below). GDH activity is also common in marine animals, where it appears to operate primarily in the reverse direction resulting in the excretion of NH_4^+ (see Chapter 8 by Steinberg and Bronk, this volume). GS, GOGAT, and GDH are all fully reversible; many assays of their activity take advantage of this and as well as the hydrolysis of an ATP, the oxidation of reduced forms of specific coenzymes such as NADH or NADPH, or the consumption of substrates or production of products, or their analogs, over time.

In addition to their central role in amino acid biosynthesis, as primary products of N assimilation, gln and glu are thought to play an important role in regulating cellular N metabolism and determining cellular N status (Flynn and Gallon, 1990; Flynn et al., 1989, 1994; Mulholland and Capone, 1999; Mulholland et al., 1999). In enteric bacteria, the global N regulatory system (Ntr) is sensitive to intracellular C:N ratios (e.g., α-kg/gln) (Guerrero and Lara, 1987; Magasanik, 1993; Muro-Pastor et al., 2001; Reitzer and Magasanik, 1987). Global N control has also been observed in cyanobacteria but the cellular cues resulting in the expression of the transcriptional activator, NtcA, are unknown (Flores and Herrero, 1994; Lindell et al., 1998).

2.2.1. Glutamine synthetase

2.2.1.1. *Overview* GS (EC 6.3.1.2) catalyzes the condensation of glutamate and ammonium to form glutamine at the expense of ATP. There are three families of GS enzymes that have been identified. GS I, encoded by the glnA gene, is the typical eubacterial GS found in bacteria and archaea (Robertson et al., 1999, 2001), but also occurs in cyanobacteria (Herrero et al., 2001), and possibly some vascular plants (Mathis et al., 2000). It has 12 equally sized subunits. Type II GS, encoded by glnII, is found in eukaryotes and a few bacteria (Robertson et al., 2001). It has eight equally sized subunits. Type III GS, encoded by glnT and glnN, was thought to occur only in limited numbers of bacteria and in nondiazotrophic cyanobacteria (Herrero et al., 2001), but now appears to be more broadly distributed among the algae, e.g., diatoms and haptophytes (Robertson et al., 2001). In cyanobacteria, type III GS (glnN) consists of six identical subunits that are much larger than those of GSI or GSII (Merrick and Edwards, 1995).

The three classes of GSs are structurally related but their sequence similarities vary. Isoenzymes of GS are expressed in different cellular compartments, in microalgae, the cytoplasm and chloroplasts. The evolutionary origin of isoenzymes is the subject of research as are the relationships between different forms of GS (e.g., Robertson et al., 1999, 2001). Recent sequencing projects have provided data that indicate even greater complexities. For example, all of the genomes sequenced for *Prochlorococcus* have glnA and the amino acid sequence, molecular size and kinetic parameters are similar to those of other cyanobacteria (García-Fernández et al., 2004). Unexpectedly, the *T. pseudonana* genome contained all three forms of GS including the prokaryotic GSI type. The function of the different isoenzymes is also unclear. In higher plants, GS1 (cytosolic form) is thought to catalyze an N-recovery pathway and is typically used as a stress marker (e.g., Pageau et al., 2006).

2.2.1.2. *Assay techniques* GS is an excellent example of a reversible enzyme that is highly regulated and modified at multiple levels. GS is assayed in both the forward and reverse directions. In the forward direction, GS assays the biosynthesis of gln in the presence of the divalent cation Mg^{2+} (biosynthetic activity). Because Mg^{2+} appears to inhibit the activity of modified enzyme in the forward reaction, biosynthetic assay is thought to represent *in vivo* potential for gln synthesis via GS (Lee et al., 1988). In the reverse direction, GS assays measure total potential GS activity of both active and inactive enzyme in the presence of Mn^{2+} as the divalent cation (transferase activity) (Lee et al., 1988; Stadtman et al., 1979). In both of these

assays, hydroxylamine can be substituted for NH_4^+ and provides a stable product, γ-glutamylhydroxamate, which is easily measured and does not undergo further metabolism *in vitro* (Mulholland and Capone, 1999). The ratio between the forward and reverse direction assays may also provide an index of enzyme modification and the proportion of the total enzyme that is biosynthetically active in cells (Lee *et al.*, 1988; Mulholland *et al.*, 1999).

A variety of endpoints have been used to estimate GS activity in *in vitro* assays run in both the forward and reverse directions. Colorimetric assays for glutamyl hydroxamate and inorganic phosphate production and the release of NH_4^+ associated with the hydrolysis of gln (Bressler and Ahmed, 1984; Clayton and Ahmed, 1987; Falkowski and Rivkin, 1976; Shapiro and Stadtman, 1970; Stadtman *et al.*, 1979) are commonly employed, as are radioactive assays employing ^{14}C-glutamate (Pahuja and Reid, 1982; Prusiner and Milner, 1970; Rhee *et al.*, 1976; Thomas *et al.*, 1977). The GS/GOGAT pathway was first identified as the primary pathway of N metabolism in cyanobacteria using radioactive $^{13}NH_4^+$ (Meeks *et al.*, 1978); however, this method is impractical for routine assays given the short half life of ^{13}N.

Although this seems to paint a bright picture for GS assays, there are different forms of GS doing different things in different places within cells. *In vitro* assays using cell homogenates mixes these enzyme pools and confounds results measuring total activity in either direction. Currently, it is impossible to distinguish activity among isoforms in bulk enzyme assays. For example, in the diatom, *S. costatum*, GSII appears to play a role in assimilating NH_4^+ derived specifically from NO_3^- reduction (Takabayashi *et al.*, 2005).

Inhibitors have been used not only to identify pathways of N metabolism but also to distinguish between uptake and assimilation of inorganic N. For example, MSX is an irreversible inhibitor of GS and has been used to explore the role of N assimilation products (e.g., gln) and intracellular NH_4^+ on the feedback inhibition of processes such as nitrogenase activity and NO_3^- uptake (Arp and Zumft, 1983). In addition, the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) has been used to distinguish between uptake and assimilation of NH_4^+ in marine algae (Rees *et al.*, 1998).

2.2.1.3. Enzyme regulation and interpretation of activity assays

GS is a highly regulated enzyme and its regulation may preclude the ability to use simple activity assays to derive estimates of N assimilation in marine microbes. Different families of GS are regulated and modified differently. As with many enzymes, GS has characteristics that make interpretation of assay results difficult (e.g., sensitivities to feedback inhibition and divalent cation specificity), regulation occurs at both the level of synthesis and activity, and activity varies with growth conditions. In addition, subunits of GS are differentially and reversibly modified; by adenylylation in bacteria (Silman *et al.*, 1995), covalent modification by ADP-ribosylation (Merrick and Edwards, 1995), phosphorylation (Miflin and Habash, 2002), and by interaction of two inhibitory polypeptides in cyanobacteria (García-Domínguez *et al.*, 1999). The degree of inhibition appears to be governed by the number of modified subunits.

GS is regulated in response to cumulative feedback inhibition by multiple end products of glutamine metabolism (Stadtman, 2001) and the different families of GS are sensitive to different cellular cues (Shatters *et al.*, 1993). Nitrogen regulation of GS occurs under all growth conditions. Under N-replete conditions, nitrogen assimilatory capacity is controlled to coordinate nitrogen and carbon assimilation (balance). Under nitrogen-depleted conditions the cell's assimilatory capacity is modified. Severe starvation can result in expression of alternative N acquisition pathways (Ninfa and Atkinson, 2000).

Modification of GS is mediated through regulatory proteins (e.g., P_{II}) that are themselves highly regulated and reversibly modified (Arcondéguy *et al.*, 2001; Lee *et al.*, 2000; Magasanik, 2000). P_{II} proteins serve as signal processing proteins that integrate cellular carbon and nitrogen status and signal the control of N assimilation via GS in enteric bacteria, archaea, and some cyanobacteria (Arcondéguy *et al.*, 2001). The complexities of these regulatory pathways are beyond the scope of this review but regulation appears to occur in response to environmental factors such as dissolved inorganic nitrogen concentrations and feedback from intracellular metabolites. A *glnB* gene encoding for P_{II} protein has also been identified in marine cyanobacteria including, *Trichodesmium erythraeum, Prochlorococcus, Synechococcus* (García-Fernández *et al.*, 2004) as well as *Synechocystis* sp. and *Synechococcus* sp. (Forchhammer and Tandeau de Marsac, 1995; García-Domínguez and Florencio, 1997; Wyman, 1999).

As for GS, activation of the P_{II} protein is regulated by nitrogen availability and is dependent on photosynthetic electron transport (García-Domínguez and Florencio, 1997; Reyes and Florencio, 1995). P_{II} proteins have been reported so far only for red algae (Reith and Munholland, 1993), and marine cyanobacteria (García-Fernández *et al.*, 2004). However, it has been suggested that the P_{II} protein in *Prochlorococcus marinus*, like that of *Synechocystis* may serve more as a sensor for C acquisition than for N control (Palinska *et al.*, 2002). In addition to P_{II} signal transducer proteins, GlnK and GlnK-like proteins may also contribute to N regulation and signal sensing in bacteria and archaea. These proteins are structurally similar but functionally distinct (Ninfa and Atkinson, 2000).

Transcription of both the GS gene (*glnA*) and the P_{II} gene (*glnB*) are regulated by the transcription activator *NtcA* in enteric bacteria (Magasanik, 2000). However, there appear to be simpler regulatory pathways in some cyanobacteria such as *Prochlorococcus* (Gómez-Baena *et al.*, 2001). We still know little about the regulatory pathways affecting GS across algal taxa or among different GS pools. GS is not the only enzyme that is under N control in cyanobacteria (e.g., nitrogenase and NR above). Other enzymes involved in N uptake and assimilation are also regulated in response to N through common regulatory pathways. Because GS is needed to assimilate all N, GS activity must be maintained for cellular N acquisition. However, it appears that not only are there different GS pools but that they may be regulated differently and are transcribed from different promoters during growth on different forms of N (e.g., N_2; Tumer *et al.*, 1983). For example, nonheterocystous N_2 fixing bacteria and cyanobacteria have been shown to have elevated GS activity when fixing N_2 (Meeks *et al.*, 1987; Rai *et al.*, 1992; Smith and Gallon, 1993). For *Trichodesmium* spp., *glnA* transcript abundance was elevated both during the

pre-dawn period corresponding with the peak *nifH* transcript abundance and another peak late in the afternoon that was not correlated with nitrogenase-associated gene products (e.g., constitutive; Kramer *et al.*, 1996; Mulholland *et al.*, 1999; Wyman *et al.*, 1996). Consistently, the ratio of GS transferase/biosynthetic activity, or the proportion of the enzyme that was biosynthetically active, changed in response to the daily pattern of N_2 fixation in *Trichodesmium* IMS101, consistent with the idea of two separate pools of GS regulated differently, at the level of enzyme synthesis (Mulholland *et al.*, 1999).

2.2.1.4. *Information gained from assays and future potential*   GS activity is critical to our understanding of N assimilation in marine organisms but, is clearly a very complicated enzyme both from the standpoint of activity and regulation. Understanding GS activity and its regulation might offer insights into the marine N cycle but, in practice, it may be beyond our abilities to measure it effectively and to gain useful information from the interpretation of bulk enzyme assays at this time.

2.2.2. Glutamate synthase

2.2.2.1. *Overview*   Glutamate synthase (GOGAT; formerly glutamine: 2-oxogl-utarate amidotransferase) is a complex iron–sulfur flavoprotein that catalyzes the synthesis of two glu molecules from a molecule each of gln and α-kg. The eubacter-ial version of the enzyme is composed of two subunits. The small subunit is a flavin adenine dinucleotide-dependent NADPH oxidoreductase which shows sequence similarity to several other protein domains and enzyme subunits. Indeed, the small subunit of eubacterial glutamate synthase has been proposed to be a prototype of protein domains or enzyme subunits used in many different contexts to transfer electrons from NAD(P)H to an acceptor protein or protein domain. In the case of glutamate synthase, the small subunit provides electrons to the large subunit, which binds L-glutamine and 2-oxoglutarate and forms L-glutamate.

There appear to be three classes of GOGAT based on the source of the electron donor used for the conversion of glutamine and α-kg to glutamate: NADPH-GOGAT (EC 1.4.1.13) found primarily in bacteria and archaea, both NADH-GOGAT (EC 1.4.1.14) and Fd-GOGAT (EC 1.4.7.1) are found in eukaryotes (higher plants and algae), cyanobacteria, and animal cells. Different forms of the enzyme have distinct genes (Suzuki and Knaff, 2005). However, distribution of various forms of the enzyme appear to be complex. All three types of GOGAT were found in the *T. pseudonana* genome; *glsF*, the cyanobacterial ferredoxin dependent version, and two NAD(P) H dependent forms, *gltB* and *glt D*. It had been previously speculated that these two genes were typically fused to form *glnN* in eukaryotes; however, this is clearly not the case in *T. pseudonana* where these genes are separate (Armbrust *et al.*, 2004; Berges, unpublished data).

2.2.2.2. *Assay techniques*   Assays of GOGAT rely on measuring fluorometrically or spectrophotometrically the oxidation of reductant (Ahmed *et al.*, 1977; Avila *et al.*, 1987; Clayton and Ahmed, 1986; Inokuchi *et al.*, 1999), or measuring products of enzyme activity (e.g., ^{14}C-glutamate production; Cullimore and Sims, 1981; Prusiner and Milner, 1970). As for other enzyme assays, these assays are

sensitive to pH, temperature and substrate concentrations. Azaserine, a glutamine analog, inhibits GOGAT and has been used to probe GOGAT activity in nature.

2.2.2.3. Enzyme regulation and interpretation of activity assays

GOGAT activity does not appear to be as heavily regulated as GS activity, or perhaps it is just less studied (Florencio et al., 1987; Muro-Pastor et al., 2005). However, GOGAT does appear to be regulated by light and metabolite sensing systems in higher plants (Suzuki and Knaff, 2005). Cyanobacterial systems are well studied compared to other marine phytoplankton, but no regulation of GOGAT expression by N source has been observed for this group (Herrero et al., 2001). Interestingly, in heterocystous cyanobacteria, GS is present but GOGAT is absent from the heterocysts suggesting that gln or some other amino acid has to be transported from the heterocysts to vegetative cells to be further metabolized (Muro-Pastor et al., 2005).

2.2.2.4. Information gained from assays and future potential

GOGAT may be an excellent example of an enzyme that plays a secondary role in a cyclic enzyme pathway. However, if this is the case, it is unclear why it is so highly regulated in some cases. Additional functions for this enzyme in N metabolism may yet be identified.

2.2.3. Glutamate dehydrogenase

2.2.3.1. Overview

Glutamate dehydrogenase (EC 1.4.1.3) is an alternative pathway for NH_4^+ assimilation and glutamate synthesis in some species of algae, such as green microalgae and macroalgae, but it does not appear to be the dominant pathway of N assimilation in most marine phytoplankton. GDH is thought to function primarily in a catabolic role and likely only plays a role in ammonium assimilation when intracellular ammonium concentrations are very high (Melo-Oliveira et al., 1996; Moorhead and Smith, 2003).

As for GS and GOGAT, there appear to be several families of GDH with many isoenzymes in each family. NADP-GDHs have been found in the chloroplasts of green algae as well as in the mitochondria and cytosol of other species including macroalgae (Inokuchi et al., 1999). Organelle-specific GDH isozymes (chloroplastic, mitochondrial, and cytosolic) and two distinct GDH genes were isolated in the green macroalgae Bryopsis maxima (Inokuchi et al., 1999). NADP-dependent GDH activities were found in all three cellular compartments while NAD-dependent GDH was observed only in the chloroplasts.

Genes encoding GDH are not present in most cyanobacteria; however, they are present in one Prochlorococcus genome and in T. erythraeum (García-Fernández et al., 2004; Muro-Pastor et al., 2005). Interestingly, Trichodesmium appear to release NH_4^+ during active N_2 fixation (Mulholland et al., 2004a,b) and destroy and resynthesize much of their nitrogenase daily (Zehr et al., 1993), suggesting a catabolic role. Both NADH- and NADPH- specific forms of GDH are present in the diatom. T. pseudonana (Armbrust et al., 2004).

In heterotrophs, GDH is involved in the production of NH_4^+ during catabolism of protein (Park et al., 1986). Indeed, zooplankton often account for the majority of

GDH activity in marine systems (Bidigare *et al.*, 1982; Park *et al.*, 1986). Assays of the enzyme in the reverse direction are virtually identical to those discussed below.

2.2.3.2. Assay techniques   GDH utilizes both nicotinamide nucleotide cofactors; NAD^+ in the direction of N liberation (catabolic) and $NADP^+$ for N incorporation (assimilatory). In the forward reaction, GDH catalyzes the synthesis of amino acids from free ammonium and α-kg. The reverse reaction links amino acid metabolism with TCA cycle activity. In the reverse reaction, GDH provides an oxidizable carbon source used for the production of energy as well as a reduced electron carrier, NADH, and production of NH_4^+. As for other enzymes, spectrophotmetric methods have been developed for measuring oxoglutarate and aminotransferase activities by assaying substrates and products of the GDH catalyzed reaction (Ahmad and Hellebust, 1989).

2.2.3.3. Enzyme regulation and interpretation of activity assays   GDH is ubiquitous in both higher and lower organisms where it serves in both biosynthetic and catabolic capacities. Little is known about its regulation in marine microbes because it is not thought to be biosynthetically active in most marine environments and because of low N concentrations. Although it is thought that GDH functions primarily in the reverse direction based on kinetic arguments, activity measurements are sparse as are measurements of intracellular NH_4^+ concentrations. It has recently been argued that in higher plants, GDH may have a role in stress response (Dubois *et al.*, 2003), but this has not been examined in marine algae.

2.2.3.4. Information gained from assays and future potential   This enzyme is easily assayed *in vitro* but results from assays have rarely shown relationships with N assimilation, perhaps because this is not its major function. It might be time to revisit GDH assays and consider relationships with nonassimilatory pathways.

2.3. Organic nitrogen acquisition

Although inorganic N has traditionally been thought to be the primary N source for marine autotrophs, it is now apparent that this is not always true (see Chapter 7 by Mulholland and Lomas, this volume) and that organic N can be a major N source in many marine environments. With that in mind, enzymes involved in the hydrolysis or mobilization of organic N are examined.

2.3.1. Urease
2.3.1.1. Overview   Urease is found in diverse groups of marine bacteria, plants, and animals. In most organisms, urease serves to break down urea to mitigate its toxic effects in the cellular environment. However, urea is the most abundant identifiable form of dissolved organic nitrogen (DON) in most aquatic ecosystems and most organisms can use urea as an N source by taking it up into the cytoplasm where it can be hydrolyzed into two ammonia molecules which can then be assimilated. Intracellular hydrolysis of urea and its ultimate assimilation occurs through one of two enzymatic pathways: urease (urea amidohydrolase, EC 3.5.1.5) or ATP:urea

amidolyase (UALase). The latter has been demonstrated to exist exclusively in the chlorophyte algae (Leftley and Syrett, 1973). UALase complex catalyzes the production of two ammonia and two bicarbonate ions from urea and bicarbonate via a carboxylating reaction and then the produced allophanate, water, and hydroxyl group by hydrolysis (Bekheet and Syrett, 1977).

Urease is widespread in phytoplankton, including diatoms (Lomas, 2004; Milligan and Harrison, 2000; Oliveira and Antia, 1986; Peers et al., 2000), chrysophytes (Leftley and Syrett, 1973), dinoflagellates (Dyhrman and Anderson, 2003; Fan et al., 2003), a pelagophyte (Fan et al., 2003), and cyanobacteria (Herrero et al., 2001); and in bacteria, (Oliveira and Antia, 1986) and other organisms (see Antia et al., 1991; Leftley and Syrett, 1973). Urease activity involves the nickel–dependent hydrolysis of urea into two NH_4^+ molecules and CO_2. Urease is constitutive in many cases for algae but not for bacteria. Urease is generally cytoplasmic (Mobley and Hausinger, 1989).

Urease was the first enzyme whose functional structure was elucidated (see Zimmer, 2000). All together seven polypeptides are needed for functional urease. UreC is the major functional subunit of urease and is large (Collier et al., 1999) relative to the ureA and ureB subunits; all are highly conserved. In addition, four accessory genes, *ureD*, *ureE*, *ureF*, *ureG*, are needed for enzyme assembly.

2.3.1.2. Assay techniques

As for other N compounds, uptake is often estimated using isotopic tracers and urease activity is inferred from incorporation of labeled atoms into biomass. In addition to molecular methods of purifying and characterizing ureases in organisms, *in vitro* colorimetric methods for assaying urease activity in marine microbes have been developed (Peers et al., 2000). Hydrolysis of urea produces two NH_4^+ and a CO_2 molecule, both of which have been used as endpoints to estimate urease activity in marine organisms. $^{14}CO_2$ has been measured as an endpoint of urea hydrolysis using ^{14}C–labeled urea (Bekheet and Syrett, 1977; Ge et al., 1989; Leftley and Syrett, 1973). Assays have also measured NH_4^+ production from urease to estimate activity (Collier et al., 1999; Fan et al., 2003; Jahns et al., 1995; Oliveira and Antia, 1986; Peers et al., 2000). Unfortunately, easily measurable products of urease, NH_4^+ and CO_2, are subsequently released or consumed in both downstream and competing biochemical pathways.

2.3.1.3. Enzyme regulation and interpretation of activity assays

There appear to be species-specific differences in the degree of constitutive expression versus upregulation of urease activity depending upon nutrient prehistory. For example, urease activity has been shown to be upregulated in *T. pseudonana* grown on NO_3^- and urea (Peers et al., 2000), downregulated in *T. weissflogii* grown on NO_3^- (Lomas, 2004), and upregulated in *Synechococcus* WH7805 growing on NO_3^- (Collier et al., 1999). The pelagophyte, *Aureococcus anophagefferens*, and dinoflagellate, *Prorocentrum minimum*, exhibited high urease activities regardless of the N source (Fan et al., 2003a) while the dinoflagellate *Alexandrium fundyense* displayed inducible urease activity (Dyhrman and Anderson, 2003). Constitutive and ammonium–repressible ureases are present in cyanobacteria. However, expression of high affinity urea

uptake activity is subject to N control and has an ABC–type high–affinity transporter (Herrero *et al.*, 2001; Valladares *et al.*, 2002). As for nitrogenase and NR, urease expression in marine *Synechococcus* (WH7805, WH8102) is part of the *NtcA* operon (e.g., therefore, N regulated).

2.3.1.4. *Information gained from assays and future potential*   Urea uptake may be much more important than previously thought in the open ocean (see Chapter 7 by Mulholland and Lomas, this volume). Not only do autotrophs use urea, but bacteria also have ureases, suggesting that they can readily use urea, although the available data suggest that autotrophic phytoplankton have consistently lower K_m values for urea than do bacteria (Fan *et al.*, 2003; Jahns, 1992; Mobley and Hausinger, 1989). Another surprise arising from a genomic analysis of *T. pseudonana* is that this diatom has a fully-functional urea cycle. Such a cycle is typical of animals, where NH_4^+ generated during catabolism is converted into urea for excretion. It is unclear whether diatoms actually excrete "waste" urea since they also possess an apparently constitutively active urease that can hydrolyze urea to NH_4^+ and CO_2 and three urea transporters (Armbrust *et al.*, 2004). The function of the urea cycle in this diatom is still a mystery, but bacteria are known to produce urea and in general, we know little about the production or sources of urea in the marine environment, despite the observation that it can fuel much of the productivity at times (see Chapter 7 by Mulholland and Lomas, this volume). In toad fish, urea synthesis appears to act as part of a N salvage mechanism (Mommsen and Walsh, 1989), and in teleostean fish and elasmobranches, urea serves as an osmolyte. If in fact diatoms produce urea internally in order to turnover amino acids and recycle N, reports of constitutive urease activity indeed make sense.

2.3.2. Extracellular enzymes acting on organic nitrogen

2.3.2.1. *Overview*   In general, less is known about cell surface than intracellular enzymes, particularly for phytoplankton. One of the reasons is that although dissolved organic matter (DOM) is the largest pool of combined N in the ocean, its availability to organisms is unknown because DOM is a complex mixture of compounds, most of which are uncharacterized (see Hansell and Carlson, 2002). Because of this complexity, a variety of different substrate-specific extracellular enzymes are necessary to remineralize DOM in nature (Hoppe, 1991) so that it is available to microorganisms. Most of the DOM is ultimately derived from primary production and proteins typically represent at least 75% of phytoplankton cell N (Dortch *et al.*, 1984; Nguyen and Harvey, 1994) and 80% of bacterial cell N (Kirchman, 2000). Dissolved combined amino acids (DCAA) typically represent between 5 and 20% of the dissolved organic nitrogen (DON) pool (Bronk, 2002; Keil and Kirchman, 1991; Sharp, 1983), and 3–4% of the dissolved organic carbon (DOC) pool in seawater (Benner, 2002). The high molecular weight (HMW) DOM is thought to be the more bioreactive fraction of the bulk DOM pool and it is estimated that 75–85% of the nitrogen in HMW DOM is in amide structures (Benner, 2002). While much of this is resistant to chemical hydrolysis in the laboratory, organisms are clearly able to hydrolyze or use some of this N because the C:N ratio of particulate organic material (POM) and DOM decreases significantly as material is degraded.

Extracellular degradation of HMW DOM into usable components is necessary for microorganisms to take advantage of organically-bound N because most microbes and phytoplankton can take up only inorganic or small organic compounds (Antia *et al.*, 1991; Nikaido and Vaara, 1985) and most HMW DOM is just too big. Therefore, extracellular hydrolysis of proteins and peptides is required before this N can be used for growth. Particularly important are extracellular enzymes that degrade large polymeric biomolecules to small, labile compounds. In addition, some organisms may not be able to use organic compounds at all and require remineralization of DON prior to its uptake. Extracellular amino acid oxidases can facilitate production of dissolved inorganic nitrogen (DIN) from amino acids (Mulholland *et al.*, 1998; Palenik and Morel, 1990a,b; Palenik *et al.*, 1989) and there is close coupling between amino acid oxidation and uptake of NH_4^+ produced from these reactions (Mulholland *et al.*, 2003).

Extracellular enzymes include exoenzymes that occur free in the water or adsorbed to surfaces other than those of their producers whereas ectoenzymes are bound to the cell surface or are found in the periplasmic space (Chròst, 1991). Extracellular enzymes responsible for degrading proteins, peptides, and amino acids have been widely attributed to heterotrophic bacteria (Rosso and Azam, 1987; Sinsabaugh *et al.*, 1997; Hoppe, 1983, 1991; Hoppe *et al.*, 2002), although phytoplankton have been found to possess cell surface enzymes that can oxidize amino acids (Mulholland *et al.*, 1998; Palenik and Morel, 1990a,b; Palenik *et al.*, 1989; Pantoja and Lee, 1994) and hydrolyze peptides (Mulholland *et al.*, 2002, 2003; Stoecker and Gustafson, 2003). LAP activity has also been observed in cyanobacteria (Martinez and Azam, 1993; Stoecker *et al.*, 2005). Other types of plankton may also be responsible for dissolved protease activities. For example, heterotrophic flagellates are a source of protease activity (Karner *et al.*, 1994; Sala and Güde, 1999), and mesozooplankton grazers may also be important (Bochdansky *et al.*, 1995).

2.3.2.2. Assay techniques

Fluorogenic compounds and fluorescent derivatives have been commonly used to evaluate extracellular enzyme activity. Fluorogenic substrates are comprised of an artificial fluorescent molecule bound to a more natural molecule (e.g., amino acids) with a specific linkage (e.g., peptide binding). Fluorescence is observed after enzymatic cleavage of the bond. Assays employing fluorogenic compounds are very sensitive. Extrapolating enzyme activity from assays employing fluorogenic compounds can be complicated, however, by competing or inhibitory compounds naturally occurring in the environment. Two commonly used fluorogenic compounds are 4-methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (AMC). Both L-leucyl-B-naphthylamide and L-leucine-4-methylcoumarinyl-7-amide (Leu–MCA) have been used to assay aminopeptidases in aquatic systems and MUF-*N*-acetyl-glucosaminide has been used to investigate chitinase activity (Berg *et al.*, 2002). These compounds are too large to cross cell membranes.

There are many types of proteases, but an important divison is endo- vs. exopeptidases. Endopeptidases (= proteinases) that cleave within polypeptide chains are likely important in the early stages of protein degradation, while exopeptidases that act on the termini of polypeptide chains (i.e., amino- or carboxypeptidases) are

probably more important during the intermediate and late stages of protein degradation (Barrett, 1986). A number of endo- and exopeptidases with different substrate specificities have been described, some of which are active at alkaline pH (Barrett, 1986). Only a few types have been reported in seawater, probably because of the limited number of artificial substrates available for use in investigating extracellular enzyme activity in aquatic ecosystems. Other extracellular enzymes have been examined in aquatic systems using fluorogenic substrates in natural assemblages or cultures (Berg et al., 2002; Hoppe, 1983; LeCleir and Hollibaugh, 2006; Martinez et al., 1996; Sherr and Sherr, 1999; Sinsabaugh et al., 1997).

There are fewer measurements of endopeptidase activity in aquatic samples (see below). In contrast, there have been numerous measurements of LAP, an exopeptidase, in freshwater and marine environments using artificial fluorogenic substrates, such as Leu-AMC (Martinez et al., 1996; Rosso and Azam, 1987; Sinsabaugh et al., 1997; Stoecker and Gustafson, 2003; Stoecker et al., 2005). LAP hydrolyzes a broad spectrum of substrates with a free amino group, but has a preference for N-terminal leucine and related amino acids (Mahler and Cordes, 1966). Measuring LAP may underestimate peptide utilization not only because dipeptides are directly incorporated (see below), but also because endopeptidases are not the only enzymes that hydrolyze protein-like material.

Alternative methods for measuring rates of amino acid oxidation and peptide hydrolysis in seawater samples are the application of fluorescently labeled amino acids and peptides, Lucifer Yellow Anydride-lysine (LYA-lysine) and Lucifer Yellow Anydride-tetraalanine (LYA-ala4), respectively (Mulholland et al., 1998, 2002, 2003; Pantoja and Lee, 1999; Pantoja et al., 1993, 1994, 1997 Fig. 32.2). These methods have the advantage of using real amino acids and peptides with the availability of several peptide bonds to cleave. Uptake of $^{15}NH_4^+$ from oxidation of ^{15}N-LYA-lysine has been demonstrated (Mulholland et al., 2003). In addition, the primary products of LYA-ala4 hydrolysis appear to be dipeptides (Mulholland et al., 2002, 2003, unpublished data) suggesting that it may measure endopeptidase activity and that endopeptidase activity exceeds exopeptidase activity in the environment. Direct uptake of dipeptides has also been observed suggesting that uptake competes with exopeptidase activity and that LAP assays may underestimate the hydrolysis and utilization of peptides by marine microbes (Mulholland and Lee, in revision).

Assays of extracellular enzyme activity must take care not to disrupt cells and thereby bias results by including intracellular enzyme activity in rate estimates. Berges and Falkowski (1996, 1998) found cell-associated LAP with peak activity between pH 7.5 and 8.5 (the pH range of seawater) in homogenates of marine phytoplankton, however, in this study no attempt was made to distinguish intracellular from ectocellular enzymes.

No molecular methods for cell surface enzymes are yet available, but one study by (Palenik and Koke 1995) found an N-regulated cell surface (membrane or wall) protein, nrp1, present in N-limited cells and during growth on urea. This method located enzyme on the cell surface using a biotinylation process. Derivatizing the cell-surface with a biotin-containing reagent causes the biotin to be transferred to cell-surface proteins which then allows the labeled proteins to be detected on
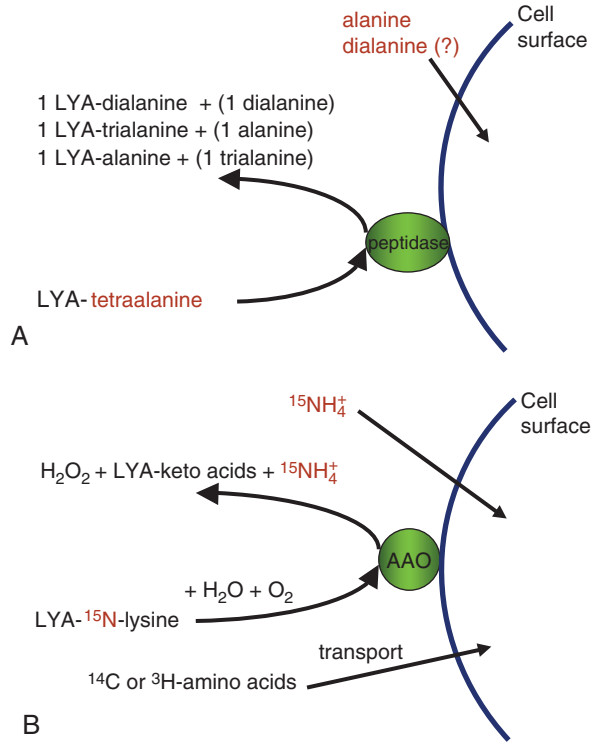
Figure 32.2 Cell surface peptide hydrolysis (A) and amino acid oxidation (B) using the fluorescent compound Lucifer Yellow Anhydride (LYA)–tetraalanine and lysine, respectively. Fluorescent products are measured along with the added tracer by high performance liquid chromatography (HPLC) and products can be further degraded or taken up by cells.

western blots because of the high affinity of the protein avidin to the biotin moiety (Palenik and Koke, 1995). While they were unable to identify the function of nrp1, they were able to rule out a cell surface amino oxidase, but it is possible that nrp1 is another type of degradative enzyme or transporter for organic N.

Other endpoints that have been used to estimate extracellular enzyme activity include monitoring the concentrations of substrates and products of oxidation or hydrolysis reactions (e.g., H_2O_2, α–keto acids, amino acids). These methods are limited however, because there are multiple sources and sinks of these compounds in the environment.

2.3.2.3. Enzyme regulation and interpretation of activity assays   Little to no information is available on the regulation of these enzymes although extracellular peptide hydrolysis and amino acid oxidation appear to be sensitive to the nutrient environment (Mulholland *et al.*, 2004a,b; Mulholland and Lee, in revision).

2.3.2.4. *Information gained from assays and future potential* The contribution of eukaryotic phytoplankton to protease activity in the water has only recently been reconsidered (Sala *et al.*, 2001). Most of the evidence for exo- or endopeptidase activity in phytoplankton is from research with cultures (Stoecker and Gustafson, 2003).

Langheinrich (1995) investigated plasma membrane-associated leucine and alanine aminopeptidase activities in *Chlamydomonas reinhardtii* as well as in several other freshwater microalgae. *C. reinhardtii*, can grow on histidine, thanks to presence of histidinase and urocanase (Hellio *et al.*, 2004). Berg *et al.* (2002) found that axenic cultures of the ''brown tide'' pelagophyte, *A. anophagefferens* had high rates of extracellular hydrolytic enzyme activity and could out-compete several groups of bacteria for organic compounds. Further, these cultures used the hydrolysis products to meet their N demands for growth. Mulholland *et al.* (2002) found high rates of peptide hydrolysis both in nature and in nonaxenic cultures of *A. anophagefferens* and measured rates of peptide hydrolysis varied depending on whether cultures were conditioned on NO_3^-, NH_4^+, or urea as an N source suggesting that enzyme activity is at least partially controlled by nutrients (Mulholland *et al.*, 2004a,b; Mulholland and Lee, in revision). Surveying cultures across a wide range of taxa suggests that the ability to hydrolyze peptides may be common (Fig. 3 from Mulholland and Lee, in revision). Other extracellular enzymes may also be common in the marine environment but far less is known about them.

2.3.3. Digestive proteases

2.3.3.1. *Overview* Heterotrophs represent a major sink for primary production, and thus a critical part of the marine N cycle. In the pelagic realm, there have been attempts to estimate zooplankton grazing (both micro- and macro-zooplankton) using two major enzymatic approaches: activities of digestive enzymes (especially proteases in the case of N) (e.g., Gonzalez *et al.*, 1993) and the activity of GDH, the
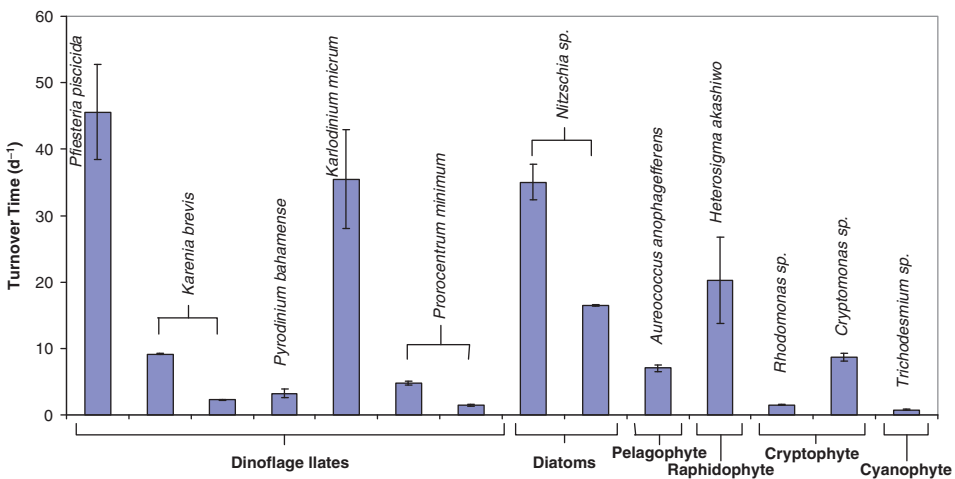
Figure 32.3 Turnover time for the fluorescent compound LYA-tetra-alanine by cultures of different phytoplankton species and taxa (data from Mulholland and Lee, in revision).

key step in the pathway of excretion of NH_4^+ following catabolism of protein (e.g., Bidigare *et al.*, 1982; Mayzaud, 1987; Park *et al.*, 1986). Attention has also been given to the digestive enzymes of benthic heterotrophs, though this has been related more to assessing digestive acclimation and food quality than to quantifying rates. Digestive enzymes have been examined in connection with food supply and feeding rates (e.g., Mayer *et al.*, 1997), and to help determine what materials might be degraded by different species in different environments (Roberts *et al.*, 2001).

2.3.3.2. Assay techniques

Traditional assays for digestive proteases were based on spectrophotometric measurements of substrate hydrolysis, but in some cases these substrates lacked the sensitivity necessary for field work and there may also be problems with specificity. In the past 10 years, due largely to the demands of the emerging proteomics field, a wide range of fluorometric substrates have become available, especially amino methyl-coumarin conjugates (AMC or MCA), which have been discussed above in the section on extracellular enzymes (see also Sarath *et al.*, 1989). A wide range of highly-specific substrates are available (e.g., Suc–Ala–Ala–Phe–AMC for chymotrypsin [http://www.bachem.com/]), as well as corresponding inhibitors).

2.3.3.3. Enzyme regulation and interpretation of activity assays

Because digestive enzymes are released into the guts of multicellular organisms, or contained within structures like food vacuoles of unicellular ones, they are not under the same degree of regulation as are many other enzymes that have been discussed. Control is exerted during synthesis by having inactive forms (proenzymes or zymogens), which are activated just prior to release. As a result, the major difficulty in interpreting digestive enzyme activities is that they may respond to feeding conditions with variable and unpredictable time lags (e.g., Hassett and Landry, 1990; Mayzaud *et al.*, 1992; Roche-Mayzaud *et al.*, 1991). There are also complications in comparing activities among species with differing gut morphology (see Han *et al.*, 2002). In terms of assays, generic substrates have often been used so that the precise identities of the proteases remain unknown; it is likely that intracellular and gut enzymes have often been measured together. For benthic macroorganisms, fitting digestive enzyme activities into N budgets has been challenging because the long recovery times and sharp changes in environment following capture of organisms are not ideal for preserving activities.

Despite the recognition that GDH may be more important in its catabolic role, GDH activities have been difficult to correlate with nitrogen excretion because of the complications of size-scaling; GDH activities scale differently with body size than do N-excretion rates (see Berges *et al.*, 1993). Assays are also potentially complicated by the presence of assimilatory GDH in other organisms in samples.

2.3.3.4. Information gained from assays and future potential

Digestive enzyme activities have proven most useful in indicating the potential for an organism to metabolize a given substrate, and also for inferring that an organism has had the substrate in its diet. In some cases, variation in enzyme activity has proven useful in establishing the presences of diel feeding rhythms (e.g., Head *et al.*, 1984).

Quantitative interpretations of digestive enzyme activities have proven challenging, but newer, more specific assays (involving highly fluorescent substrates) and improvements in image analyses may offer potential solutions, and these measurements are worth revisiting. Digestive enzyme assays have seldom been used for microzooplankton (but see Gonzalez *et al.*, 1993), but they may be more interpretable in these species because the enzyme are intracellular and thus may be under tighter control than those found in the guts of metazoans. Very few molecular characterizations of digestive enzymes have been conducted in any group of zooplankton.

2.4. Other enzymes connected with nitrogen cycling

There are a variety of other enzymes that cannot be excluded in our discussion of N assimilation.

2.4.1. Enzymes associated with Carbon metabolism: Carbon-fixation and respiratory pathways

2.4.1.1. Overview   While enzymes associated with C metabolism are not directly involved in marine N cycling, they are significant for two reasons. First, N is inextricably linked with C metabolism; without C skeletons, N cannot be assimilated by autotrophs (Huppe and Turpin, 1994; Young and Beardall, 2003), and virtually all N degradation also results in respiration of C (Collos *et al.*, 1992). Second, there is some evidence that algal species, like higher plants, may use the enzymes (especially Rubisco) as forms of N storage. This is clearest in the green algae where the pyrenoid of the chloroplast can contain the greater part of the Rubisco pool in crystalline form. Although it is not yet known how widespread such a strategy might be (see Ekman *et al.*, 1989 for an example in red macroalgae), starvation does result in degradation of Rubisco in several species (Falkowski *et al.*, 1989; Garcia–Ferris and Moreno, 1994). Carboxylase activities have been measured in marine systems for some time (e.g., Fontugne *et al.*, 1991; MacIntyre *et al.*, 1996) and more recently, species-specific measurements of gene expression of Rubisco in natural communities have become feasible (e.g., Pichard *et al.*, 1997; Wyman, 1999).

Because of the links between rates of N acquisition and C metabolism (e.g., Grobbelaar *et al.*, 1991), there have been attempts relate the two using the activities of respiratory enzymes and metabolic modeling (see Packard *et al.*, 2004). Metabolic rate has been estimated using specific enzymes (succinate dehydrogenase, isocitrate dehydrogenase, cytochrome *c* oxidase, citrate synthase), but also by using a multienzyme assay known as ETS activity (see Packard, 1985), or bulk reduction of tetrazolium substrates (Bamstedt, 2000).

2.4.1.2. Assay techniques   For Rubisco and other carboxylases, while some spectrophotometric methods exists (e.g., Gerard and Driscoll, 1996), the sensitivity necessary for measurements in the field makes radiochemical methods based on ^{14}C most useful (Ashton *et al.*, 1990; Keys and Parry, 1990). Most respiratory enzyme assays are spectrophotometric in nature, based on use of NADH or tetrazolium salts (e.g., Packard, 1985; Thuesen and Childress, 1993).

2.4.1.3. Enzyme regulation and interpretation of activity assays   Carboxylases and respiratory enzymes are heavily regulated *in vivo*, making interpretation of activity assays challenging. In the case of Rubisco, for example, there is a second enzyme system involved in posttranslational activation (Rubisco activase, Macintyre *et al.*, 1997; Pierce *et al.*, 1982). For respiratory enzymes, as well, control of fluxes through pathways may not rest with a single enzyme and may involve posttranslational regulation, so individual enzyme activities may be of limited use in predicting overall fluxes (Arnold and Kadenbach, 1997). As is the case for GDH (see above), there are issues involving metabolic size scaling of respiratory enzymes, as well (see Berges *et al.*, 1993). Moreover, enzymes involved in carbon metabolism are linked to those involved in nitrogen metabolism on a number of levels. The regulatory networks of well-studied microorganisms including bacteria and cyanobacteria include a transcriptional factor, the global nitrogen regulator, *NtcA*, and signal transduction proteins (e.g., P_{II}, glnK see above) which coordinate the metabolism of N and C in the cell (García-Fernández *et al.*, 2004; Herrero *et al.*, 2001; Tandeau de Marsac *et al.*, 2001).

2.4.1.4. Information gained from assays and future potential   Because of the high degree of regulation, C cycle enzyme activities may prove difficult to interpret by themselves. The potential for combining such measurements with activities of N cycle enzymes has been discussed (see Packard *et al.*, 2004) but not yet realized.

2.4.2. Esterases
2.4.2.1. Overview   The presence of esterase activity in seawater has been used as evidence for lysis of cells, which represents a potentially important pathway from PON to DON in marine environments (e.g., Brussaard *et al.*, 1995; van Boekel *et al.*, 1992). Esterases represent a large group of enzymes (EC 3.1.x.x) that catalyze the hydrolytic cleavage of an ester bond. These enzymes have very broad specificity, and a variety of other enzymes including proteinases have "esterolytic" action. Esterase methods are based on the assumptions that these enzymes are solely intracellular, and that they do not retain activity for very long outside the cell. Additional assumptions permit estimates of lysis rate to be calculated (e.g., Agusti *et al.*, 1998). Criticisms of the lysis rate methods have led to a number of modifications (see Agusti and Duarte, 2002; Riegman *et al.*, 2002), and the accuracy of the quantitative method is in some doubt, however, the basic measurements have been correlated with independent measurements of cell losses in at least some cases (e.g., Brussaard *et al.*, 1995). There is evidence to suggest that phytoplankton are the main source of dissolved esterase activity, and that grazing does not result in significant release of esterases.

2.4.2.2. Assay techniques   Esterases are measured using the substrate fluorescence diacetate (FDA). FDA cleavage leads to an increase in fluorescence, which can be measured with high sensitivity in a fluorometer. A number of controls for spontaneous degradation of substrate are necessary (Agusti and Duarte, 2002; Agusti *et al.*, 1998; Riegman *et al.*, 2002; van Boekel *et al.*, 1992).

2.4.2.3. Enzyme regulation and interpretation of activity assays   A major uncertainty in using esterases to estimate cell lysis is the very broad nature of the assay. It is quite clear that FDA can be hydrolyzed by a number of different enzymes, including some of the extracellular enzymes describe above (e.g., Schnurer and Rosswall, 1982); obviously this is not compatible with the assumption that they are solely intracellular. Within cells, esterases are typically regulated by compartmentalization rather than other mechanisms, and so regulatory features are not likely to complicate interpretations of activity assays.

2.4.2.4. Information gained from assays and future potential   The application of esterases assays have provided the first direct evidence that cell lysis (as distinguished from loss terms such as grazing and sedimentation) may be an important factor in marine systems (see Kirchman, 1999). While the meaning of cell lysis, its incidence and ecological importance remain unclear (see Franklin *et al.*, 2006), and there are concerns about the assay, we currently lack objective, independent methods with which to measure lysis (cf., Agusti and Duarte, 2002). In future, it may be possible to identify particular esterases that are truly found only within cells and choose substrates that are more specific for them.

2.4.3. Cell death-associated proteases
2.4.3.1. Overview   The esterase assays described above provide evidence for cell lysis, but they do not give information about the causes of cell lysis. One type of cell lysis that has received attention recently involves mortality driven by internal factors (e.g., nutrient or energy stress), often referred to as ''cell death.'' This process is poorly characterized, but it applies to both eukaryotic (Berges and Falkowski, 1998; Bidle and Bender, 2008; Segovia *et al.*, 2003; Vardi *et al.*, 1999, 2007) and prokaryotic cells (Berman-Frank *et al.*, 2004). There is considerable speculation about the origins and function of cell death pathways. Hypotheses about origins from transfer of genes following viral infections have been put forward, and suggestion of roles in preventing mass viral lysis, allowing survival of subpopulations of cells under stress, and facilitating formation of resting stages have been advanced (e.g., Bidle and Falkowski, 2004; Franklin *et al.*, 2006; Segovia *et al.*, 2003; Vardi *et al.*, 1999). Recently, activation of cell death–associated proteases have been associated with viral lysis in the coccolithophore, *Emiliania huxleyi* (Bidle *et al.*, 2007).

Cell mortality of this type appears to be related to the process of apoptosis described in multicellular systems. Apoptosis is mediated by a group of cysteine endopeptidases (EC 3.4.22.x), termed caspases, that cleave after aspartate residues. Thus, caspase activity (or what is better termed ''caspase-like activity,'' see Section 2.4.3.2) may prove to be a good marker of this form of cell mortality.

2.4.3.2. Assay techniques   Because cleavage after aspartate residues is uncommon among proteases, caspase substrates are highly specific. In animal systems, over a dozen caspases have been described, and specific substrates have been developed. Relatively little work has been done exploring caspase activities in other organisms, but gene sequence comparisons reveal that algae and bacteria appear to have variant forms of the animal caspases, termed ''metacaspases''; metacaspases are also the form found in higher plants (Aravind *et al.*, 2001; Bidle *et al.* 2007; Uren *et al.*, 2000). Although sequence

homologies between caspases and metacaspases are striking (especially in the active site regions), information about metacaspase proteins and the specificities of these enzymes are largely lacking. For this reason, the conservative term "caspase-like" has often been used to describe enzymes in algae and cyanobacteria that cleave caspase substrates (but note that use of "metacaspase" is becoming more common, e.g. Bidle and Bender, 2008).

As for the proteases described above, a number of spectrophotometric and fluorescent methods are available, but typically AMC conjugates are used (Stennicke and Salvesen, 2000). Aldehyde inhibitors can be used in order to verify specificity of the assays (Bedner et al., 2000), and labelled inhibitor can also be used for direct cell staining (Bidle and Bender, 2008).

2.4.3.3. Enzyme regulation and interpretation of activity assays Caspases are best characterized in animal species where a complex system of enzymes, proenzymes, and activation cascades exist (see Aravind et al., 2001). There is evidence suggesting that some components of these systems exist in algae (e.g., Segovia et al., 2003), but the nature of the regulation remains unknown. Some level of caspase activity is likely to be constitutive (Berman-Frank et al., 2004; Segovia et al., 2003), and it is also possible that algae and cyanobacteria contain non-caspase proteases that are able to cleave caspase substrates. Work in culture has correlated higher caspase-like activities with cell lysis events (e.g., Berman-Frank et al., 2004; Segovia et al., 2003), but relatively little can be deduced from activities measured using different substrates that are specific for mammalian caspases (see Segovia et al., 2003).

2.4.3.4. Information gained from assays and future potential So far, few field measurements have been made of caspase-like activities (Berman-Frank et al., 2004; Vardi et al., 1999), but the assay methods appear to be sensitive enough to allow use in natural communities. As work using cultures proceeds, and our understanding of cell death processes improves, assays of capase-like activity may offer an important means to distinguish different forms of cell mortality. Aside from bulk in vitro assays, the availability of cell-permeable substrates, coupled with flow cytometry will provide improved resolution and specificity (e.g. Bidle and Bender, 2008).

Interestingly, there is evidence that cell death processes in phytoplankton may be driven by allelopathy (e.g., Casotti et al., 2005), and that similar processes may also occur in zooplankton (e.g., Romano et al., 2003). So far, caspase activities have not been detected in these situations, but the potential usefulness of the assays should be assessed.

3. SUMMARY

For many enzymes, the period surrounding publication of the first edition of this volume was a peak in the application of activity assays to marine environments. Problems with interpretation of such measurements and inadequate characterization of the enzymes in marine organisms led to a period of decline in their use. In the years that have followed, we have made considerable progress in understanding

enzymes, the genes that encode them, and their regulatory pathways. Studies of gene expression in marine environments are now feasible and have provided new understanding. Now, as many areas of biological research move from genomics to proteomics and ''metabolomics,'' it seems timely to revisit enzyme activity measurements in marine systems using our new knowledge and technical advances in substrates and equipment. There are still very few integrated studies that examine relationships between *in situ* uptake rates, enzyme activities, and transcription and translation, yet such work is critical to understanding regulation of marine N cycling. It is also humbling that many of the problems identified in 1983 remain exactly the same as today. We are still not much closer to reconciling the species-specific measurements in cultures with the sort of bulk assays that can be made in the field. We now know that in many marine systems, bacteria compete for the same resources as phytoplankton (Chapter 7 by Mulholland and Lomas, this volume), but we still cannot effectively determine which organisms are responsible for enzyme activities in natural samples.

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Ocean Nitrogen Cycle Modeling

Raleigh R. Hood *and* James R. Christian

Contents

1. Introduction

There has been a marked increase over the last decade in ocean biogeochemical modeling research, largely in response to the need for prognostic models to predict how ocean nutrient cycling and carbon export and sequestration will respond to global warming. Although much of this work is focused on the carbon cycle, it has stimulated research on nutrient cycling in general (Hood *et al.*, 2006; Le Quéré *et al.*, 2005) because carbon fixation and export are controlled by the availability of nutrients (N, P, Si, Fe), as well as by food web interactions, and physical and biological processes that control the sinking speed of particulate organic matter and remineralization rate. Understanding the N cycle and its connections to other elemental cycles is particularly important to understanding the current state of

the ocean and for predicting its future response to climate change because N is an essential and often a limiting element in organic matter production. Gaining such understanding will critically depend on further development and application of N cycle and multi–element models.

Historically, phosphorus has been the preferred currency of global box modelers who often address longer time scales over which the oceanic N inventory is variable, i.e., the full–ocean residence time of P is about 30,000 years—an order of magnitude longer than the residence time of N within the "fixed" N pool. This difference in residence time exists because the fixed nitrogen pool in the ocean does not derive primarily from terrestrial sources, but is instead regulated by N_2 fixation and denitrification. In contrast, phosphorus is mostly supplied from weathering. N is usually the preferred currency in studies with shorter time scales, such as simulations of the spring bloom (e.g., Steele and Frost, 1977) or episodic upwelling (e.g., Moloney and Field, 1991; Wroblewski, 1977), because it is the primary limiting nutrient for phytoplankton in these circumstances. When modelers began to simulate the full seasonal cycle of open-ocean ecosystems, N was used as the primary currency (e.g., Evans and Parslow, 1985; Fasham *et al.*, 1990) for the same reason. Nevertheless, the assumption of N limitation in open ocean environments is no longer considered robust. Over the last decade it has been shown that iron limits primary production in many ocean environments (e.g., Boyd *et al.*, 2000; Coale *et al.*, 1996; Martin *et al.*, 1991). More recent studies indicate the potential for significant phosphorus limitation or co-limitation in some regions (e.g., Ammerman *et al.*, 2003; Christian, 2005; Karl *et al.*, 2001; Wu *et al.*, 2000), and silica limitation is a very important factor that controls diatom growth (e.g., Dugdale and Wilkerson 1998; Whitney *et al.*, 2005) (see Hutchins and Fu, Chapter 38, this volume).

The importance (or not) of the distinction between N and P as a model currency is closely related to the concept of the Redfield ratio (Redfield *et al.* 1963). Because many models implicitly or explicitly enforce fixed elemental ratios, the distinction between N and P limitation is often of little importance in models. In most cases a fixed ratio (e.g., "Redfield") model will behave almost identically in terms of primary production, plankton abundance, and carbon fluxes, whether N or P is specified to be the primary limiting nutrient (the exception is when initial or boundary conditions are drawn from observations in which deviations from the Redfield N:P ratio are present).

Nonetheless, the N cycle is still the primary focus (and currency) in many oceanic modeling studies. The N cycle is intrinsically interesting because it is complex, with the oxidation state of N mediated by a wide range of biologically catalyzed reactions. Moreover, the oceanic N cycle is not fully understood. New reduction-oxidation reactions and the organisms that catalyze them are still being discovered (e.g., the "anammox" reaction (Codispoti *et al.*, 2001, see Devol, Chapter 6, this volume) and "microbial diazotrophs" (Montoya *et al.*, 2004; Zehr *et al.*, 2001; see Carpenter and Capone, Chapter 4, this volume). Furthermore, the global ocean nitrogen budget is still highly uncertain. Uncertainties in global estimates of nitrogen fixation and denitrification are so large that we don't know for sure whether or not the oceans are gaining or losing N (Codispoti *et al.*, 2001; Codispoti, 2007).

Models of the N cycle are still under active development with new pathways and processes being added all the time.

In the pages that follow we provide a broad overview of open-ocean N-cycle modeling. In this review we focus primarily on pelagic N-cycle research. Although we discuss a few studies from coastal waters and inland seas, we have made no attempt to systematically review nutrient cycle modeling work from these systems. Nor do we consider estuarine models, benthic environments, or paleo-oceanographic studies. This is not intended to be an exhaustive review. Rather, our goal here is to provide a general overview, citing some examples and seminal papers along the way (Table 33.1 provides a list of most of the N-cycle models that are discussed in this paper). Additional information on N-cycle modeling can be found in reviews by Ducklow (1994), Franks (2002), and Fennel and Neumann (2004).

2. Evolution of Nitrogen-cycle Modeling

The first mechanistic plankton and nutrient dynamics models were developed by Gordon Riley (Riley, 1946; Riley *et al.*, 1949) and shortly thereafter, John Steele (Steele, 1958). These models did not use nitrogen as a currency because, at that time, phosphorus measurements were more widely available. Two of the earliest N-cycle models were published by Walsh and Dugdale (1971) and Steele (1974). Steele's model, for example, included state variables for dissolved inorganic nitrogen (N), phytoplankton (P) and zooplankton (Z), where P and Z were represented in terms of carbon assuming a fixed C/N ratio. Nitrogen uptake by phytoplankton was formulated using a hyperbolic saturation (Monod) function and remineralization was by zooplankton excretion. This model was essentially similar to modern "NPZ" models (Franks, 2002). As with other NPZ-type formulations, it did not take into account different forms of inorganic nitrogen (i.e., NO_3 and NH_4) or the role of the "microbial loop" (small phytoplankton, bacteria, protozoa, and dissolved organic nitrogen) in the nitrogen cycle. (Throughout this chapter we will refer to nitrate and ammonium as NO_3 and NH_4, for simplicity ignoring the charges.) During this same period Caperon (1968) showed that the growth rate of an N (nitrate) limited continuous culture of *Isochrysis galbana* was not directly related to the ambient nitrate concentration but related to the calculated cell N quota. Droop (1968) independently arrived at essentially the same result using vitamin B_{12}-limited *Monochrysis* (now *Pavlova*) *lutheri*, and derived the model that is often called the "Droop cell-quota" model (see details below in section 3.1).

Early N-cycle models were incorporated into dynamic, spatially-explicit frameworks in the mid-1970s (e.g., Walsh, 1975; Wroblewski, 1977). For example, Wroblewski (1977) incorporated an N-cycle model into a two-dimensional upwelling model. This coupled system allowed simulation of primary production and ecosystem response in an idealized, wind-forced coastal upwelling circulation with NO_3 infusion from depth and offshore surface Ekman transport (Fig. 33.1).

Table 33.1 General characteristics of N-based ecosystem models

Reference		Biota	DIN pools	Other elements	DON	PON	HBAC
Steele, 1974	0D	P, Z	N	C			
Jamart et al., 1977	1D	P	N, A				
Wroblewski, 1977	2D	P, Z	N, A			Y	
Hofmann and Ambler, 1988	3D	2P, nZ	N, A			Y	
Anderson and Nival, 1988	1D	P, 2Z	N			Y	
Fasham et al., 1990	0D	P, Z, B	N, A		Y	Y	Y
Moloney and Field, 1991	0D	P, H	N, A	C	★	Y	★
St. John et al., 1993	3D	P	N				
Kawamiya et al., 1995	1D	P, Z	N, A		Y	Y	
Doney et al., 1996	1D	P, Z	N			Y	
Franks and Chen, 1996	3D	P, Z	N				
Anderson & Williams 1998	0D	P, Z, B	N, A	C	Y	Y	Y
Levy et al., 1998	1D	P, 2Z, B	N, A		Y	Y	Y
Tusseau-Vuillemin et al., 1998	3D	2P,B	N, A	Si	Y		Y
Bissett et al., 1999	1D	4P, B	N, A	C	Y	Y	Y
Leonard et al., 1999	1D	2P, 2Z	N, A	Fe		Y	
McClain et al., 1999	1D	P, Z	N, A				
Walsh et al., 1999	3D	P, B	N, A	C	★	Y	Y
Denman and Pena, 1999	1D	P, Z	N	Fe★		Y	
Smith and Tett, 2000	1D	P, H	N, A	C		Y	
Tian et al., 2000	1D	2P, 2Z, B	N, A	C	★	Y	Y
Vallino, 2000	0D	P, Z, B	N	C	Y	Y	Y
Azumaya et al., 2001	3D	2P, Z	N	Si		Y	
Dadou et al., 2001	1D	P, Z, B	N	O_2	Y	Y	
Hood et al., 2001	1D	2P, H	N		Y	Y	★
Moore et al., (2002a,b)	2D	4P, Z	N, A	C, Fe, P, Si		Y	
Fennel et al., 2002	1D	P, Z	N	P		Y	
Chai et al., 2002	1D	2P, 2Z	N, A	C, Si		Y	
Christian, 2005	1D	P, Z	N	C, P, O_2	Y		
Fennel et al., 2006	3D	P, Z	N, A			Y	

Biota defines the biomass pools included as state variables: P=phytoplankton, Z=zooplankton, B=bacterioplankton. H indicates nonspecific heterotroph population including both grazers and bacteria; nZ indicates multiple stages within a single zooplankton population. A indicates ammonium; N implies nitrate+nitrite if ammonium is included and total DIN otherwise. DON, PON, HBAC columns indicate which models have explicit pools of dissolved organic nitrogen, particulate organic nitrogen (detritus or fecal matter), and bacteria. A ★ under "DON" implies that the model has DOC but not DON. A ★ attached to Fe implies parameterized Fe limitation but no explicit Fe pools. A ★ under HBAC means that bacteria are subsumed within a generalized heterotroph group. In Dadou et al. (2001) bacterial biomass appears in the DON equations but is a specified model parameter.
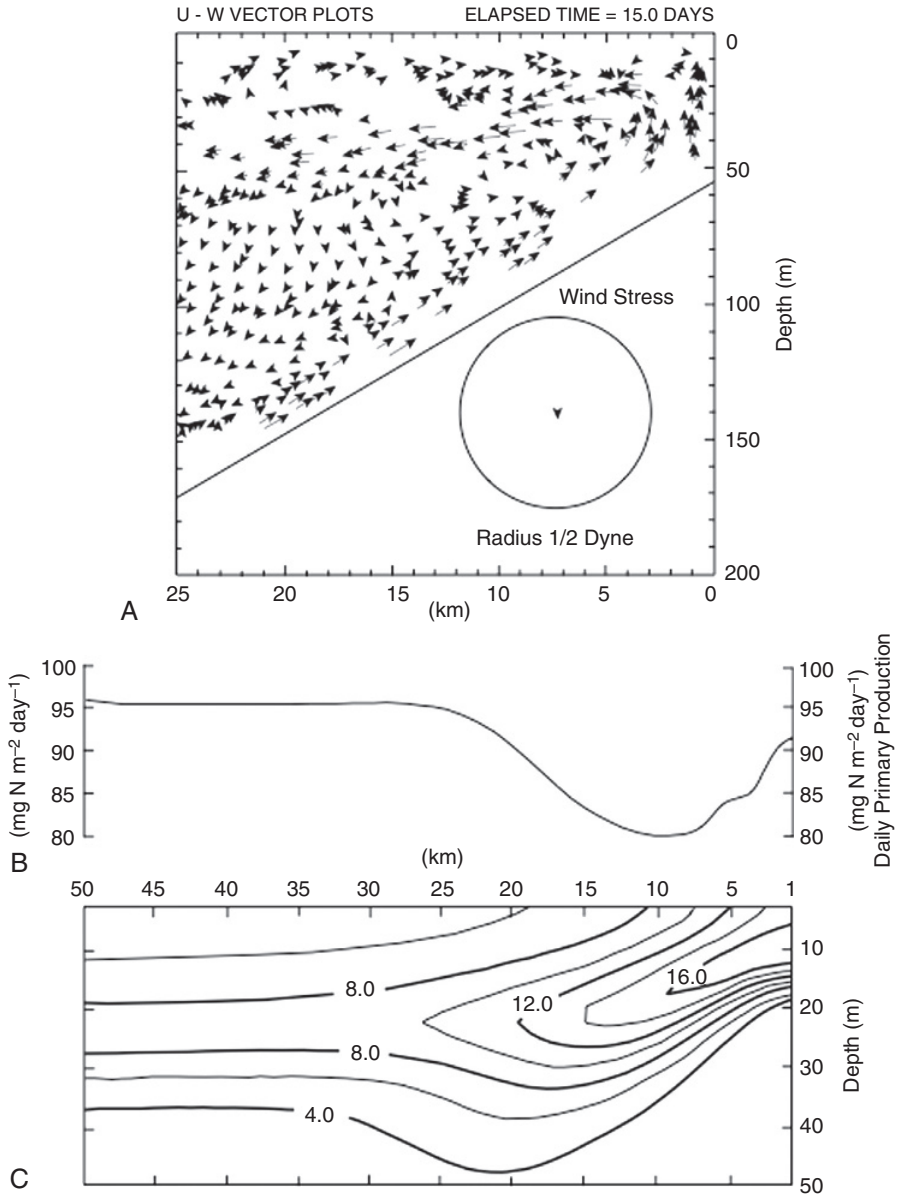
Figure 33.1 Physical and biological upwelling response simulated by the Wroblewski (1977) 2-dimensional coastal upwelling model: (A) The circulation in the transverse plane normal to the coast, the bottom topography, and the wind stress. The maximum u and w velocities in the field are -2.9 cm s^{-1} and 1.4×10^{-2} cm s^{-1}, respectively. (B) The daily gross primary production of the water column. (C) The distribution of phytoplankton. Contour intervals are 1 µmol N l^{-1}. Redrawn with permission from Wroblewski (1977).

The N-cycle model included an explicit representation of detritus–N production, transport, sinking and remineralization. Moreover, this model carried both NH_4 and NO_3 with a representation of NH_4 inhibition of NO_3 uptake by phytoplankton. Although the inhibition approach is now generally considered to lack a convincing mechanistic basis (see Section 3.2), both the N-cycle model and the achievement of coupling it to a dynamic circulation model represent significant advances in ocean N-cycle modeling.

In the mid-1980s, simple NPZ-type N-cycle models were applied to address a variety of oceanographic and ecological questions using climatological or idealized forcing (e.g., Evans and Parslow, 1985; Frost, 1987). Although these models did not advance the state-of-the-art in N-cycle modeling per se, some of the earliest basin-wide N-cycle model applications were carried out using NPZ models (Wroblewski, 1989; Wroblewski *et al.*, 1988). Using climatological mixed layer depth, solar irradiance at the sea surface, and basin-scale distribution of nitrate, Wroblewski (1989) simulated phytoplankton bloom progression in both space and time from mid to high latitudes in the North Atlantic (Fig. 33.2). To our knowledge, this was the first time-dependent, basin-wide application of an N-cycle model.

Another significant advance during this time period was the development of N-cycle models for simulating plankton dynamics that included more detail in both the P and Z compartments than previous N-cycle models (e.g., Hoffman, 1988;
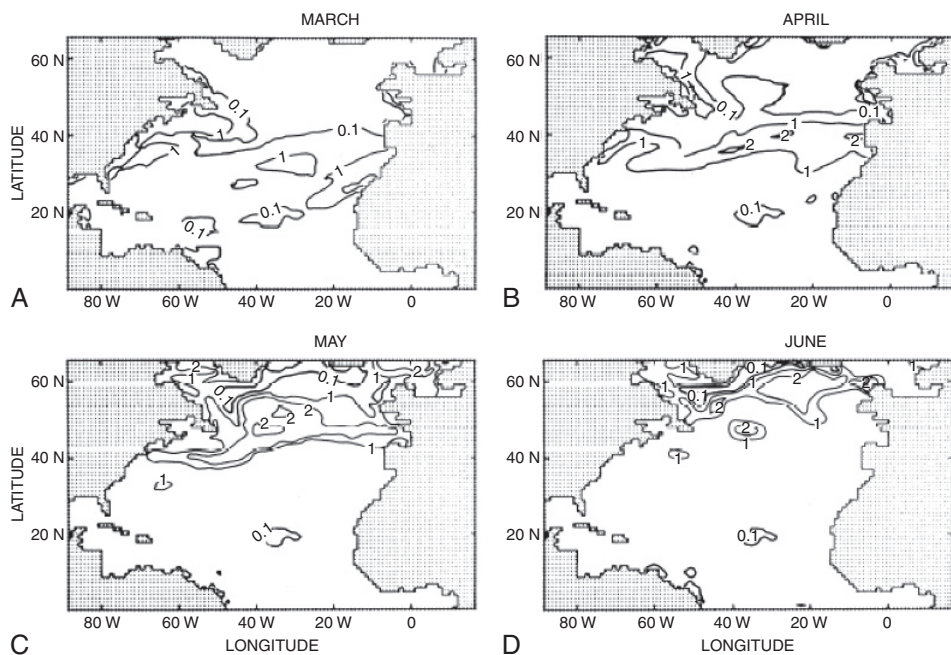
Figure 33.2 Phytoplankton fields simulated by Wroblewski (1989) for the end of March (A), end of April (B), end of May (C), and mid-June (D). Concentrations >1 mmol m^{-3} were considered to be a phytoplankton bloom. Reproduced with permission from Wroblewski (1989).
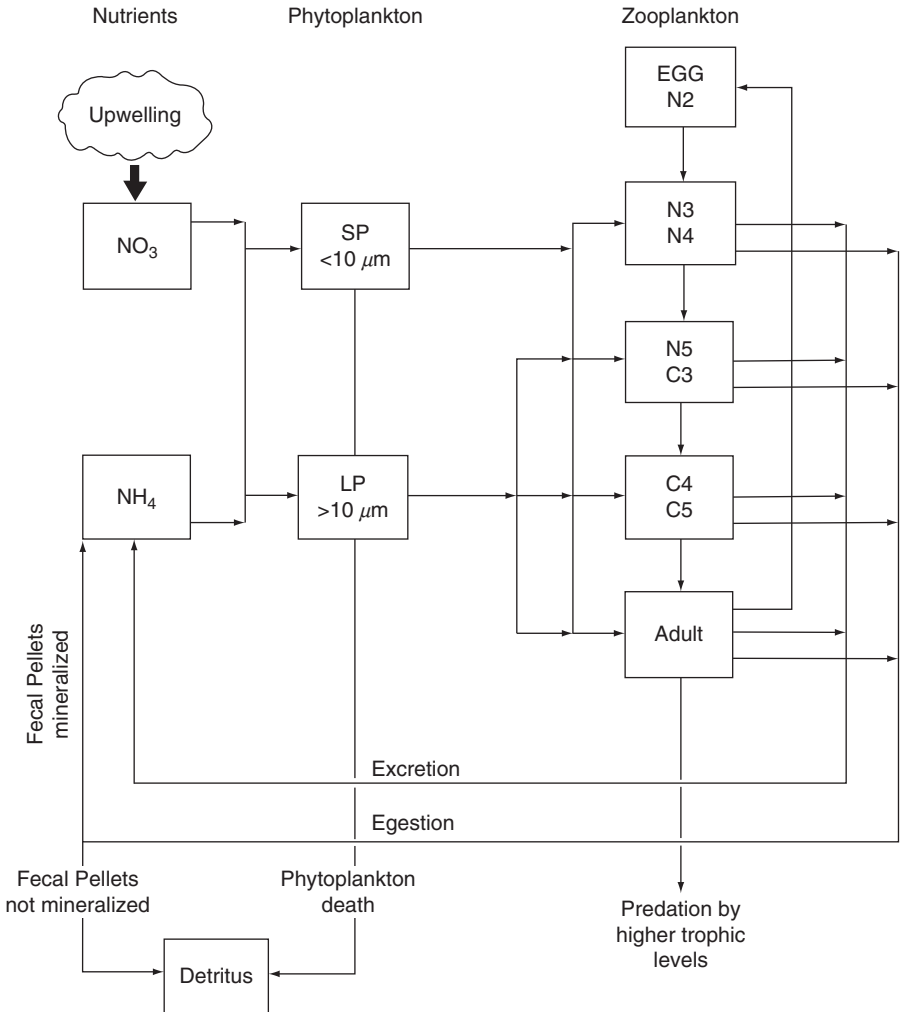
Figure 33.3 Schematic diagram of the components and biological interactions included in the Hofmann and Ambler (1988) time-dependent ecosystem model. Reproduced with permission from Hofman and Ambler (1988).

Hofmann and Ambler, 1988). The Hofmann and Ambler (1988) model had 10 compartments which included NO_3 and NH_4, large and small phytoplankton size classes and a copepod submodel (Fig. 33.3). The latter included parameterizations for N assimilation, excretion, egg production, molting and predation. Steele and Frost (1977) incorporated comparable levels of detail more than 10 years earlier. Although not, strictly speaking, an N-based model, it specified N as the limiting inorganic nutrient and assumed a fixed C/N ratio for biota defined in carbon units, so the distinction is to some degree semantic.
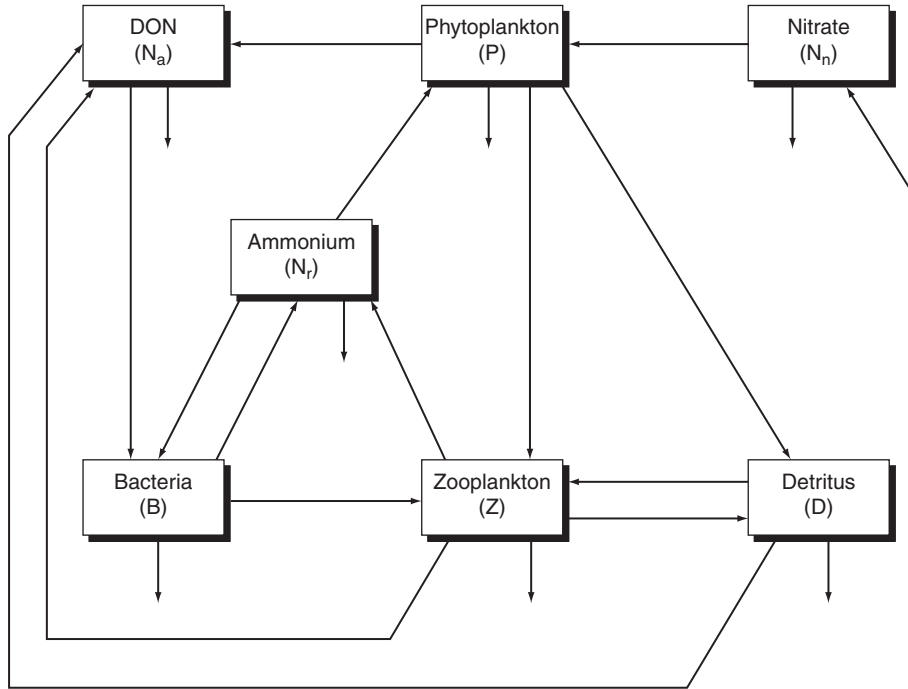
Figure 33.4 Schematic representation of the Fasham *et al.* (1990) model showing the compartments and the modeled nitrogen flows between compartments and to the deep ocean. Redrawn with permission from Fasham *et al.* (1990).

The Fasham *et al.* (1990) model was a major milestone in N-cycle model development and validation. This model had seven state variables: nitrate, ammonium, phytoplankton, zooplankton, detritus, bacteria and dissolved organic nitrogen (DON) (Fig. 33.4), and it included several important innovations. For example, in order to incorporate bacteria into the model the authors had to devise a means of determining how much nitrogen was taken up by the bacteria in the form of DON versus NH_4 (for details see section 3.3 below). The Fasham *et al.* (1990) model also introduced a formulation for specifying different zooplankton grazing rates on multiple food sources using preference terms, which has been widely adopted in subsequent models (e.g., Hood *et al.*, 2001). In addition, the model was applied and validated using an extensive biological data set that included, among other things, nitrate concentration, primary production and bacterial biomass measurements (Fig. 33.5).

In retrospect the Fasham *et al.* (1990) model stands out for two primary reasons. Firstly, it was one of the first N-based marine ecosystem models that included explicit compartments for DON and bacteria. Secondly, and perhaps even more importantly, the model's source code was "open", i.e., the code was readily available to any one who wanted it. As a result of the latter, the Fasham *et al.* (1990) model was subsequently applied to a wide variety of modeling problems by numerous
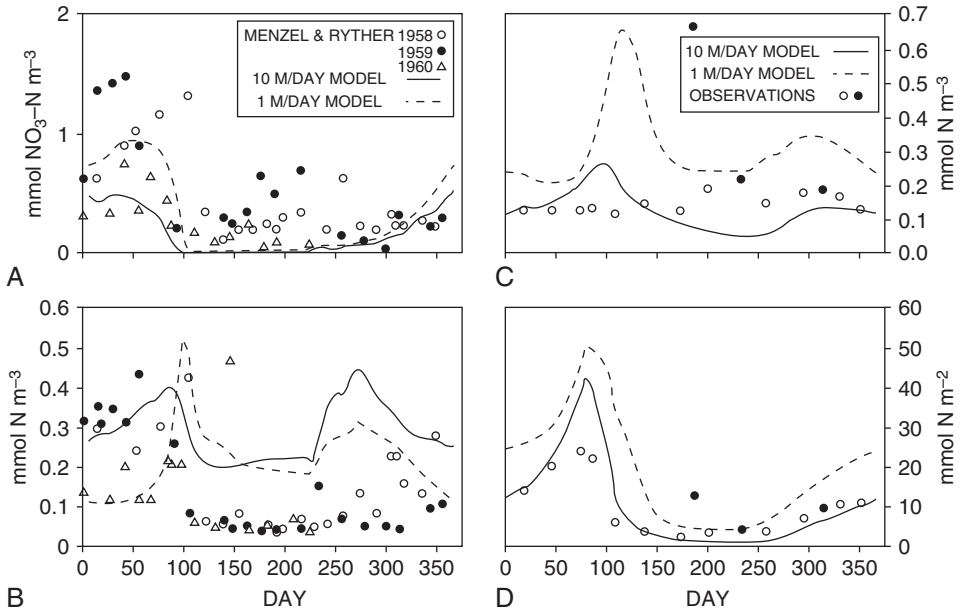
Figure 33.5 Comparison of observed data near Bermuda with the Fasham *et al.* (1990) model output for (A) nitrate concentration, (B) phytoplankton N biomass, (C) bacterial N biomass, and (D) integrated bacterial N biomass. Reproduced with permission from Fasham *et al.* (1990).

investigators, and it became the starting point for the development of a new generation of marine N-cycle models that explicitly considered bacteria and DON (e.g., Anderson and Williams, 1998).

At about this same time, another new modeling approach was introduced by Moloney and Field (1991). They developed a model of both nitrogen (Fig. 33.6) and carbon cycling with three autotrophic size classes (pico-, nano- and net-phytoplankton) and four heterotrophic size classes (bacteria, heterotrophic micro-flagellates, and micro- and meso-zooplankton) where all model parameters were determined by body size, using empirically determined allometric relationships (Moloney and Field, 1989). In theory, because the model was based upon general ecological principles and not on a specific ecosystem or data set, it could be used to simulate N and C cycling within plankton communities of any ecosystem. In a companion publication, this model was applied to three contrasting southern Benguela food webs (Moloney *et al.*, 1991). It is interesting to note that in spite of the fact that this modeling approach included microbial cycling and was, in many respects, more novel and universally applicable than the Fasham *et al.* (1990) model, it was not as widely adopted and applied by the scientific community. This is, perhaps, attributable to the simple fact that the source code was not as easy to get.

We conclude this section with a brief overview of some of the 3-dimensional, dynamic coupled physical-biological models that were developed in the 1990s. There is a historic split in the practice of ocean biogeochemical modeling, i.e., the field has been divided into large-scale global box and later circulation models with
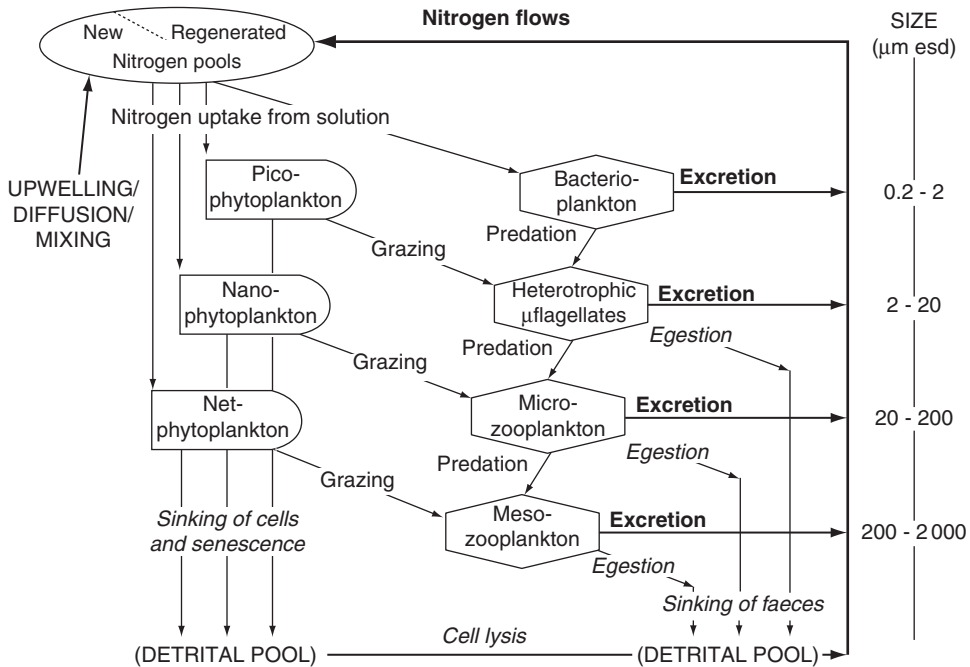
Figure 33.6 Schematic representation of the N-cycle component of the Moloney and Field (1991) model. Bullets represent the size classes of autotrophs, and hexagons the size classes of heterotrophs. Reproduced with permission from Moloney and Field (1991).

highly parameterized biology (e.g., Maier–Reimer, 1993; Najjar *et al.*, 1992; Sarmiento and Toggweiler, 1984; Siegenthaler and Wenk, 1984) and "ecosystem" models with a simplified and often highly localized spatial frame of reference (e.g., Evans and Parslow, 1985; Fasham *et al.*, 1990; Wroblewski, 1977). These are often described as "geochemical" versus "ecological" modeling approaches, although these terms probably derive as much from the scientific backgrounds of their respective practitioners as from any real scientific meaning. It was only in the 1990's that the two approaches began to merge, with ecosystem models with fully explicit biology being embedded in general circulation models. As noted above, phosphorus has been the preferred currency of global box modelers. But as explicit ecosystem models have been increasingly implemented in three-dimensional frameworks, there has been a shift towards N as the primary model currency, although P-based models also exist (e.g., Six and Maier–Reimer, 1996).

Fasham *et al.* (1993) and Sarmiento *et al.* (1993) describe one of the first efforts to couple a more complex N-cycle model to an ocean general circulation model. To represent the N cycle they used the 7 component Fasham *et al.* (1990) model (Fig. 33.4), which they coupled to a general circulation model running at 2° horizontal resolution in the North Atlantic with 25 vertical levels. Among other things, this model was used to estimate basin-wide phytoplankton biomass and nitrate concentrations (Fig. 33.7) and it suggested that, with the exception of some
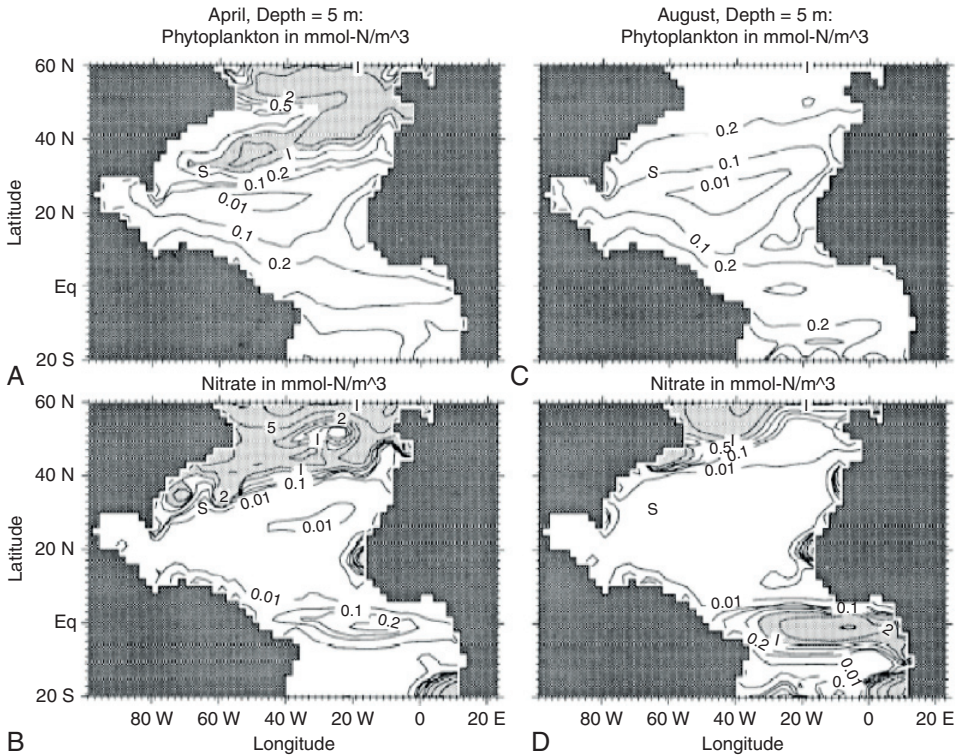
Figure 33.7 Basin-wide contour plots of the Fasham *et al.* (1993) model solution of (A) modeled average phytoplankton concentration (mmol N m^{-3}) at 5 m during April, (B) modeled average nitrate concentration (mmol N m^{-3}) at 5 m during April, (C) and (D) as for Figures (a) and (b) but for August. Concentrations greater than 1 mmol N m^{-3} are shaded. The positions of Bermuda Station "S" and Ocean Weather Station "India" are indicated by the letters S and I, respectively. Reproduced with permission from Fasham *et al.*, 1993.

upwelling regions and high latitudes during winter, almost the entire Atlantic is NO$_3$ depleted (see section 4.1). Using a similar approach, Chai *et al.* (1996) coupled a 5 compartment N–cycle model to the modular ocean model (MOM), running at $1 \times 1/3°$ horizontal resolution to study the origin and maintenance of high nitrate conditions in the equatorial Pacific. McCreary *et al.* (1996) coupled an NPZD model to a reduced gravity circulation model, and used it to study the N cycle and bloom dynamics in the Arabian Sea. Reduced gravity models have the advantage of not having to include deep ocean layers in their calculations and so they can be run at somewhat higher resolution (the McCreary *et al.* (1996) model had only two active vertical layers and so could be run at 0.5° horizontal resolution over the entire Indian Ocean).

One of the more revolutionary aspects of these early coupled–modeling studies is that they could be used to determine the degree to which different physical processes were responsible for supplying new nitrogen to surface waters. For example, McCreary *et al.* (1996) showed that in their model, vertical mixing and entrainment

from the nitracline is the primary nutrient source for the offshore bloom in the central Arabian Sea during the Southwest Monsoon, but horizontal advection of nutrients from coastal upwelling areas also makes significant contributions to the nutrient flux. However, most of these early studies were subject to constraints imposed by computation speed, and so were forced to run at relatively low horizontal resolution (0.5°–2.0°). As a result, they could not fully resolve some important processes that control the supply of new nitrogen, e.g., coastal upwelling and filament formation (Hood *et al.*, 2003) and eddy pumping in the open ocean (McGillicuddy *et al.*, 1998). These issues are discussed in more detail in Section 4.

3. STATE OF THE ART AND EVOLUTION OF BIOLOGICAL PROCESS MODELS

3.1. Modeling phytoplankton growth and cell quota

Current phytoplankton growth models can be roughly divided into those that model intracellular N concentration (cell quota) and those that do not. When internal N is not modeled, growth rate is directly dependent on the ambient dissolved inorganic nitrogen (DIN) concentration. This has the advantage of minimizing the number of model pools that cannot be validated against observation, as intracellular concentration is rarely measured. Cell quota models, however, retain the observed (ambient) pool as well as the unmeasured (internal) pool, and model validation against other fields such as chlorophyll or total particulate N may be improved by incorporating a more mechanistic representation of processes relating these to DIN. At steady-state, the two models are more or less equivalent, as the N cell quota is constant.

The work of Droop (1968) has led to the use in many subsequent studies of the following equation to express the relationship between growth and cellular nutrient concentration:

$$\mu = \mu_{\max}\left(\frac{Q - k_{\mathrm{q}}}{Q}\right) \tag{33.1}$$

where μ is the realized specific growth rate, $\mu_{\max}$ is the maximum specific growth rate, Q is the cell quota and k_{q} is the minimum concentration of limiting nutrient per cell required for growth to occur. Although Droop's model was originally developed using vitamin B_{12} uptake measurements, it has since been shown to account for "luxury consumption" and variations in cell N:P ratio reported in the literature. However, the model fits some culture data poorly, especially under N-limitation, and it has been suggested that it works best for nutrients that constitute a small fraction of total biomass, e.g., P or vitamin B_{12} (Fogg and Thake, 1987).

More recently, models with varying cell N quotas have been developed by Geider *et al.* (1998) and Flynn (2001). Here we will discuss Geider's model in depth because it contains some important details specific to the ecophysiology of

N and has also been incorporated into several other N-cycle models. The model of Geider *et al.* (1998, hereafter GMK98) contains separate state equations for phytoplankton C, N and chlorophyll. It has a variable N cell quota with specified maximum and minimum values (Q_{max}, Q_{min}). N uptake is weakly regulated by cell N status at N/C less than about 0.2 g/g but declines precipitously as the maximum N quota is approached. The light-saturated photosynthetic rate is proportional to $(Q - Q_{min})/(Q_{max} - Q_{min})$. Chlorophyll synthesis is regulated by N only indirectly, via the photosynthetic rate and the cell C/N; there is a maximal chlorophyll-to-nitrogen ratio but no specified limits on the chlorophyll-to-carbon ratio. The basic model functional relationships are illustrated in Fig. 33.8. This model was initially tested only against culture data, but variants have subsequently
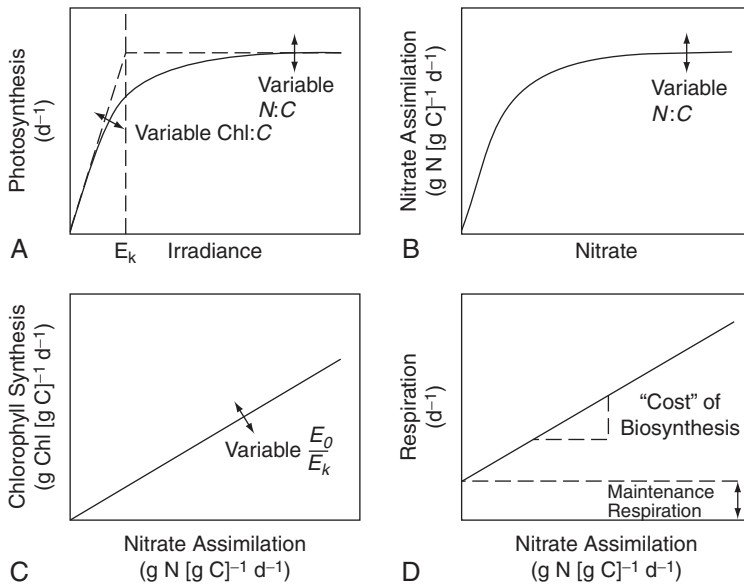
Figure 33.8 Graphical summary of the Geider *et al.* (1998) model showing dependencies of Photosynthesis , nitrate assimilation, Chla synthesis, and respiration on environmental variables. (A) Photosynthesis is a saturating function of irradiance where the initial slope increases with increasing Chl:C and the light-saturated rate increases with increasing N:C. The light-saturation parameter (E_k) is given by the irradiance at which the initial slope intercepts the light-saturated rate. (B) The carbon-specific nitrate assimilation rate is a saturating function of nitrate concentration where the maximum uptake rate is downregulated at high values of N:C. (C) The rate of Chla synthesis is obligately coupled to protein synthesis and thus to nitrate assimilation. However, the magnitude of the coupling depends on the ratio of irradiance to the light-saturation parameter (E_o/E_k). At a given rate of nitrate assimilation the carbon-specific rate of Chla synthesis declines as E_o/E_k increases. (D) The carbon-specific respiration rate is a linear function of the rate of nitrate assimilation. It is assumed that there is no lag between nitrate assimilation and protein synthesis. Major respiratory costs are associated with reduction of nitrate to ammonium, incorporation of ammonium into amino acids, and polymerization of amino acids into proteins. Other respiratory costs are assumed to scale with the rate of protein synthesis. Figure and caption reproduced with permission from Geider *et al.* (1998).

been applied to field data by Moore *et al.* (2002a), Lefêvre *et al.* (2003), and Christian (2005). Models like GMK98 that decouple photosynthesis from cell production better reproduce carbon-based primary productivity measurements in nutrient-depleted low-latitude ocean areas where high rates of photosynthesis are observed in the absence of significant inorganic nutrients (Christian, 2005).

The GMK98 model has been questioned by Pahlow (2005) and Armstrong (2006) on the grounds that it fails to account for independent regulation of cellular C, N, and chlorophyll under both light- and N-limitation, and makes incorrect predictions about the maximum growth rate under low light. In GMK98 the growth rate is maximized when the cell N quota is at its maximum value (which is a specified model parameter), and the N-limited growth rate does not depend on the Chl/C ratio. However, because chlorophyll and its associated proteins contain N, the maximal cell N quota is itself light dependent, and the nitrogen-limited growth rate depends on the demand for N in chlorophyll synthesis. The models of Pahlow (2005) and Armstrong (2006) are based on optimal allocation of N to different cellular functions, and are better able to explain continuous culture data that suggest that the cell N quota is inversely related to growth rate under light limitation, which can not be easily reconciled with GMK98 or other earlier models.

3.2. Modeling new production, NO_3, and NH_4

The concept of ''new production'' (Dugdale and Goering, 1967; Eppley and Peterson, 1979) has received a great deal of attention in the last 2–3 decades, as a useful proxy for the fraction of primary production that is exported from the euphotic zone on seasonal to annual time scales. Knauer (1993) wrote that the equation of new production with carbon export had bestowed upon new production ''an almost mystical importance''. New production, defined in terms of measured rates of NO_3 and NH_4 uptake, can be a useful proxy for export production or net community production (NCP), which are difficult to measure directly. In models, however, NCP is known exactly. The question of whether the parameters for NO_3 and NH_4 actually differ sufficiently to justify separation is a complicated one. There are several possible rationales for separating NO_3 and NH_4 in ocean models: (1) NO_3 is measured much more frequently and therefore separation makes sense from a validation standpoint, (2) the model is to be validated against measured rates of NO_3 and NH_4 uptake, (3) the parameters of NO_3 and NH_4 uptake are sufficiently different that a single aggregated DIN variable cannot give an accurate growth rate, (4) the model contains distinct phytoplankton species that use only one or the other form of N, (5) the modeler wishes to account for the different metabolic costs of biosynthesis from NO_3 and NH_4, and (6) an explicit model of nitrification is required for some other purpose such as modeling production of N_2O or validation against measured nitrification rates. In the end, whether or not NO_3 and NH_4 are different enough relative to other model errors and uncertainties to justify introduction of additional state variables and parameters will depend on the model and the questions asked.

There is a relatively extensive literature on phytoplankton N uptake and particularly on the question of NH_4 inhibition of NO_3 uptake. In the early expression

introduced by Wroblewski (1977) the NH_4 inhibition is parameterized by multiplying the NO_3 uptake function by an exponential suppression term based on the NH_4 concentration:

$$V = V_m \left[\frac{NO_3}{K_{NO_3} + NO_3} e^{-\Psi NH_4} + \frac{NH_4}{K_{NH_4} + NH_4} \right] \qquad (33.2)$$

where V and V_m are the realized and maximum phytoplankton nitrogen uptake rate, respectively, the K_{NO_3} and K_{NH_4} values are half-saturation constants, and Ψ is an NH_4 inhibition intensity factor.

This function has been widely applied in ocean models (e.g., Chai et al., 1996; Fasham et al., 1990; Hofmann and Ambler, 1988; Leonard et al., 1999), but is generally considered to lack a convincing mechanistic basis, and has some unusual properties that the user should be aware of (Frost and Franzen, 1992; Yajnik and Sharada, 2003). In particular, the total N ($NO_3 + NH_4$) uptake rate or the N-limited growth rate under steady-state conditions, does not increase monotonically with [NH_4] for NO_3 concentrations greater than about 1 μM. It is also important to note that this expression can take on values greater than unity and therefore cannot be treated as a ''nondimensional'' nutrient-limitation term, which is multiplied by a light and/or temperature dependent growth rate.

Some formulations of jointly NO_3/NH_4 limited growth have not relied on inhibition. For example, Jamart et al. (1977) defined preference functions that divided the N ''sink'' between NH_4 and NO_3, while the two jointly regulate the phytoplankton growth rate by a single Monod function of their aggregate concentration. Similarly, Frost (1993) defined a Monod function for NH_4 uptake, but assumed that N is not limiting to phytoplankton growth, and that any additional N requirements would be met by NO_3.

Other inhibition models of NH_4 and NO_3 uptake have been based on enzyme kinetics (e.g., noncompetitive inhibition; Frost and Franzen (1992)). Whether or not the analogy is strictly applicable, this would appear to have a more sound biological basis than the Wroblewski (1977) exponential function. Yajnik and Sharada (2003) defined general equations for a two-nutrient interaction that can be reduced to hyperbolic inhibition such as that employed by Frost and Franzen (1992) and Parker (1993).

The generic uptake equations are:

$$\rho_1 = P V_1 \frac{N_1}{k_1 + N_1} \frac{1 + a_{12} N_2}{1 + b_{12} N_2} \qquad (33.3a)$$

$$\rho_2 = P V_2 \frac{1 + a_{21} N_2}{1 + b_{21} N_2} \frac{N_2}{k_2 + N_2} \qquad (33.3b)$$

where P is phytoplankton biomass, N_i is concentration of nutrient i, k_i the half-saturation coefficient for uptake of N_i, V is the asymptotic specific uptake rate of nutrient i as N_i approaches infinity in the absence of other nutrients, and a and b are

kinetic coefficients describing the nutrient interaction. These can be shown to reduce to the familiar coefficients of noncompetitive inhibition. For a, $b \geq 0$ there are four possible cases:

Case 1: $a_{12}/b_{12} = 0$. ρ_1 asymptotically approaches zero as N_2 approaches infinity.

Case 2: $0 < a_{12}/b_{12} < 1$. ρ_1 asymptotically approaches some positive "threshold" value.

Case 3: $a_{12}/b_{12} = 1$, in which case ρ_1 does not depend on N_2.

Case 4: $a_{12}/b_{12} > 1$, in which case ρ_1 increases with increasing N_2.

The literature on NO_3/NH_4 interactions generally assigns the effect of NO_3 on NH_4 to Case 3 (no effect) and the effect of NH_4 on NO_3 to Case 1. Yajnik and Sharada argue for Case 2, to which point we will return. Obviously, if $a = b$, Eq. (33.3) reduces to the familiar hyperbolic uptake without inhibition (Case 3). Case 1 is equivalent to the hyperbolic inhibition employed by Frost and Franzen (1992), Parker (1993), Loukos *et al.* (1997), and Christian *et al.* (2002a). Yajnik and Sharada (2003) further defined coefficients $k = 1/b$ and $c = 1-a/b$ such that Eq. (33. 3a) becomes

$$\rho_1 = PV_1 \frac{N_1}{k_1 + N_1} \left(1 - \frac{cN_2}{k + N_2} \right) \tag{33.4}$$

This is identical to the equation derived by Harrison *et al.* (1996) and to the models cited above, for the special case where $c = 1$ (which is Case 1, because if $c = 1$ a must be 0). Harrison *et al.* (1996) showed that NO_3 uptake converged towards a nonzero asymptotic value as NH_4 increased. However, this pattern is not universal (Armstrong, 1999).

A more detailed, mechanistic approach to NH_4 inhibition was taken by Flynn *et al.* (1997). In this model intracellular pools of NO_3 and NH_4 are explicitly simulated, as well as the concentration of glutamine, which is assumed to be the molecule responsible for feedback regulation of NO_3 transport and reduction (Fig. 33.9). In addition to these intracellular substrate pools, the model includes variable activities of the enzymes nitrate reductase (NR) and glutamine synthetase (GS) (for a description of the underlying biochemical pathways see Falkowski, 1983 and Mulholland and Lomas, Chapter 7, this volume). NR synthesis, as well as transport of both NO_3 and NH_4 into the cell, are repressed by high levels of glutamine, but there is no direct regulation of NO_3 reduction by glutamine or other forms of reduced N. An important consequence is that there is a "training period" for substrate switching, i.e., cells grown on NH_4 are not immediately able to take up NO_3 even when N-starved. No other model discussed here can simulate this effect, i.e., all of the others have instantaneous feedback. This model has also been condensed to simplified versions (Flynn and Fasham, 1997) and expanded to include interactions with other elements such as P, Fe, and Si (Flynn and Hipkin, 1999; Flynn, 2001).

Armstrong (1999) approached NH_4 inhibition from the perspective of iron–light–nitrogen co-limitation. In this model, iron is allocated among different cell functions so as to maximize growth rate (Fig. 33.10). Iron is allocated to nitrate reduction only if this will maximize growth given the availability of light and NH_4.
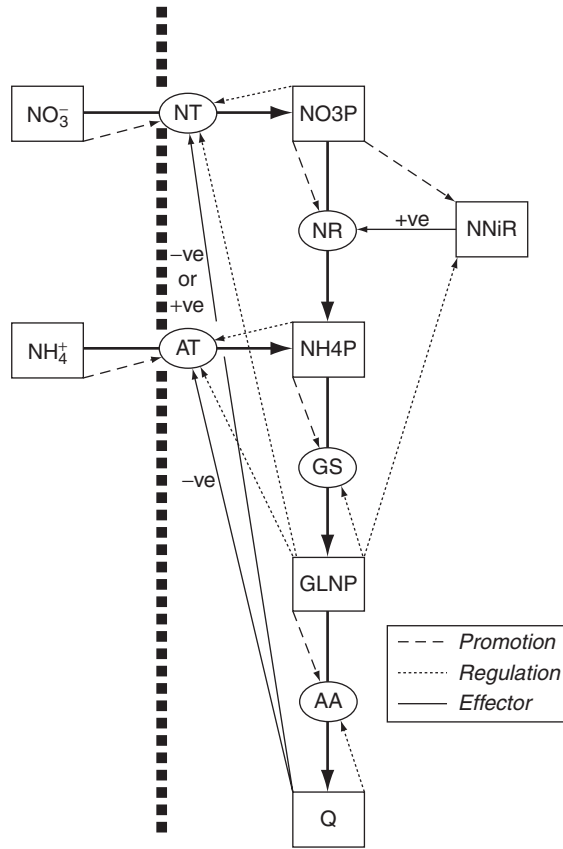
Figure 33.9 Schematic diagram of the Flynn *et al.* (1997) model. NO3P, NH4P, GLNP and Q are internal pools of nitrate, ammonium, glutamine and other organic cellular N, respectively. NNiR is nitrate–nitrite reductase, and GS glutamine synthetase activities. NT and AT are nitrate and ammonium transporters, respectively, NR describes the process of nitrate reduction through to ammonium, and AA the synthesis of amino acids and all other nitrogenous compounds from GLN. "Promotion," "regulation" and "effector" are used in general terms, with no specific biochemical meaning, indicating positive, negative or complex feedbacks, respectively. Figure and caption reproduced from Flynn *et al.* (1997) with permission.

Armstrong (1999) used this model to explain differences among various NH_4-inhibition experiments that showed distinct patterns of repression of NO_3 uptake (Fig. 33.11). When iron is not limiting, NO_3 uptake declines asymptotically with increasing NH_4, whereas under Fe limitation nitrate uptake declines approximately linearly to a value near 0, sometimes at quite low concentrations of NH_4. The model permits five possible limitations: light-limitation, nitrogen-limitation, iron–light co-limitation, nitrogen–light co-limitation, and nitrogen–iron–light co-limitation. As NH_4 concentration increases, iron is reallocated to functions other than nitrate reduction, but the replacement is not one-for-one. The relative reduction in NO_3 uptake depends on light because NO_3 reduction requires energy as well as iron.
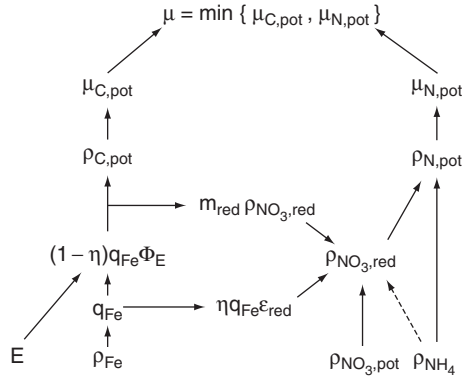
Figure 33.10 A schematic flow diagram of the Armstrong (1999) model. In this model it is assumed that a fraction η of iron uptake is allocated to reducing nitrate and that a fraction determines the maximum flux of reduced nitrate, $\rho_{NO_3,red}$, that is available for growth. When added to the ammonium uptake rate, ρ_{NH_4}, which itself influences the optimal value of η, the flux of reduced nitrate, $\rho_{NO_3,red}$, determines the potential nitrogen uptake rate, $\rho_{N,pot}$, and hence the potential nitrogen limited growth rate, $\mu_{N,pot}$. It is also assumed that a fraction $1 - \eta$ of iron is reserved for energy (carbon)–related transport, and that this fraction determines a potential carbon-limited growth rate, $\mu_{C,pot}$. Finally, the model assumes that η is dynamically altered to make the best use of limited iron by maximizing overall growth rate, $\mu = \min\{\mu_{C,pot}, \mu_{N,pot}\}$. Schematic and figure caption reproduced from Armstrong (1999) with permission.

Although there are now several different formulations to choose from for representing NO_3/NH_4 interactions, we are not aware that these differences in the treatment of NH_4 inhibition etc. has had much impact on the overall behavior of N–cycle models. In contrast, as we discuss in the next section, it is clear that other aspects of these model formulations, such as the form of the grazing functions, can have a profound impact on model behavior.

3.3. Loss terms, remineralization and the microbial loop

The loss terms in N–cycle models that transform particulate and dissolved organic nitrogen into other forms can include a variety of processes (e.g., phytoplankton exudation, zooplankton grazing, sloppy feeding, phytoplankton and zooplankton mortality, bacterial remineralization, etc.). Different models may differ substantially in terms of which of these are included and their formulation (Christian and Anderson, 2002). Many N–cycle models now include significant "phytoplankton exudation" loss terms. This is often parameterized by simply specifying that some fixed fraction of the DIN uptake by phytoplankton is shunted directly to the DON pool (e.g., Anderson and Williams, 1998). "Sloppy feeding" by zooplankton can be similarly accounted for. Many models also include linear loss terms in the phytoplankton equation that represent either "natural mortality" or phytoplankton respiration (e.g., Hood *et al.*, 2001).

However, in most N–cycle models the primary balance between autotrophic formation of PON and the recycling of that PON back to the DON and DIN pools
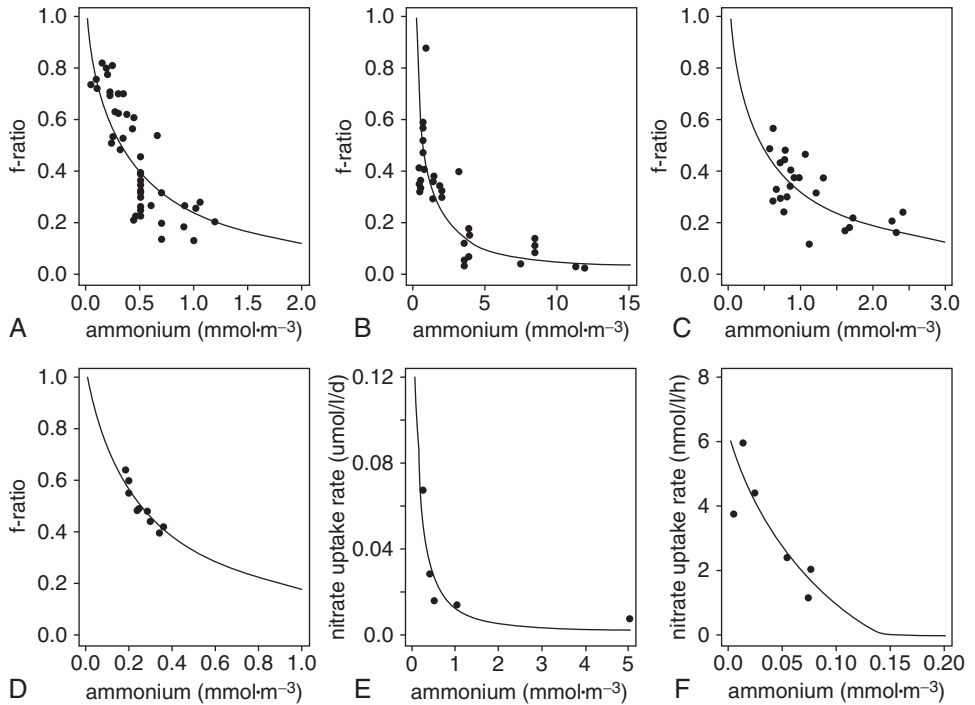
Figure 33.11 Fits of the Armstrong (1999) model to six data sets: (A) Cochlan (1986), (B) Garside (1981), (C) Glibert *et al.* (1982), (D) Olson (1980), (E) Price *et al.* (1994), (F) Wheeler and Kokkinakis (1990). Figure reproduced with permission from Armstrong (1999).

is maintained by zooplankton grazing. In simple NPZ and NPZD models, all of the grazing is represented by a single Z compartment, whereas in more complex models this grazing may be represented by multiple zooplankton size classes. This grazing is typically specified using classical functional forms, e.g., Holling's type I, II, III functional responses. One (or some combination) of these functions is also usually used to specify the zooplankton mortality, or ''closure'' in the model. It is well known that these different grazing formulations have a profound impact on N-recycling rates and also model stability (Gentleman *et al.*, 2003; Kemp *et al.*, 2001; Steele and Henderson, 1992). Yet, remarkably little attention has been focused on the grazing formulations in N-cycle models (Gentleman *et al.*, 2003). Suffice it to say that the representation of grazing losses in all N-cycle models is extremely crude compared to reality.

Remineralization of PON and DON to DIN is clearly a key process in models, and also provides some useful illustrations of the classification and evolution of models. The earliest NPZ models represented loss terms from phytoplankton and zooplankton as fluxes directly to the DIN pool. An NPZD model differs from an NPZ model in having an explicit compartment for ''detritus,'' or nonliving organic matter (Edwards, 2001; Franks, 2002). Remineralization from this pool is most simply expressed as

$$\frac{\partial N}{\partial t} = -\frac{\partial D}{\partial t} = eD \tag{33.5}$$

where e can be constant, or an exponential or Arrhenius function of temperature. This basic expression is commonly used, but models differ widely in the exact representation of remineralization. Some models have explicit compartments for bacteria and/or DON, some partition DIN into different species (e.g., NO_3 and NH_4 as discussed in the previous section), and some have fluxes to DIN directly from phytoplankton and zooplankton as well as from detritus.

In the absence of an explicit detrital compartment, the vertical flux needs to be parameterized, i.e., the fraction of production or losses that is exported to greater depths must be defined in some fashion (see section 3.5 below). Steele (1998) devised a scheme for parameterizing microbial recycling based on a priori knowledge of how the partitioning of production between recycling and export varies with nutrient influx. This simple NPZ model does not include detritus, which its author believed is "very poorly defined ecologically, and normally has no basis in data." The basic hypothesis is that because large inputs of inorganic nutrients favor large phytoplankton, metazoan grazers, and export, the exported fraction (f- or e-ratio) can be described as a hyperbolic function of nutrient concentration

$$G(N) = G(NO_3) = \frac{NO_3}{K_N + NO_3} \tag{33.6}$$

where K_N is the equivalent of a half-saturation constant. The relative rates of uptake of NO_3 and NH_4, and partitioning of grazing losses between recycling and export, are described by hyperbolic functions

$$N_{\text{tot}} = \frac{e^{-\phi NH_4} NO_3 + ANH_4}{K_N + NO_3 + ANH_4} \tag{33.7}$$

$$f = \frac{NO_3}{NO_3 + e^{-\phi NH_4} NO_3 ANH_4} \tag{33.8}$$

where N_{tot} is the total N uptake by phytoplankton and A, ϕ, and K_N are constants. So this model makes export directly dependent on the relative availability of NO_3 and NH_4. The overall objective here is to parameterize nutrient recycling by the microbial community so that the Z in the NPZ model is unambiguously associated with macro- rather than microzooplankton, and to represent the effects of community structure on the export ratio in a simple and computationally efficient model. The simulations with this model conducted by Steele (1998) were for a single layer, so the vertical redistribution of nutrients would need to be formulated before it could be used in a full ocean model. Nonetheless, it offers some promise for representing within an NPZ model processes for which single-species models are generally considered unsuitable (Armstrong, 1994).

Other models have represented the "microbial loop" explicitly to varying degrees, incorporating bacteria, micrograzers, and DON, and considering the remineralization pathways in different ways. As discussed above, the Fasham *et al.* (1990) model (Fig. 33.4) included bacteria, DON, NO_3, and NH_4. These authors assumed that under balanced growth, the ratio of bacterial NH_4 uptake to DON uptake should be constant in order to ensure that bacterial biomass of the required C:N ratio (R_b) is produced from DON with a given C:N ratio (R_d). The rates of DON (U_1) and NH_4 (U_2) uptake are

$$U_1 = \frac{V_b B [\text{DON}]}{K_4 + S + [\text{DON}]} \tag{33.9}$$

$$U_2 = \frac{V_b B S}{K_4 + S + [\text{DON}]} \tag{33.10}$$

where B is bacterial biomass, V_b is the maximum bacterial uptake rate, K_4 is the half-saturation coefficient for uptake, and $S = \min(NH_4, \eta \text{DON})$. η defines the ratio of bacterial uptake of NH_4 and DON based on R_b and R_d and the bacterial growth efficiencies for carbon (g_c) and nitrogen (g_n):

$$\eta = \frac{g_c R_d}{g_n R_b} \tag{33.11}$$

This formulation ensures that the uptake of NH_4 will be η times the DON uptake, as required by balanced growth, as long as there is sufficient NH_4 present to meet this demand. If there is insufficient NH_4, then the uptake rate of both DON and NH_4 are reduced accordingly. So the model permits both N and energy limitation of bacteria, or co-limitation, without having an explicit DOC pool. However, by treating DON as a proxy for DOC it implicitly treats the C/N ratio as fixed.

Anderson and Williams (1998, hereafter AW98) modified this model to include both DON and DOC (including "fast" and "slow" pools of each), for a more explicitly stoichiometric treatment of bacterial metabolism. In AW98 the C/N of dissolved organic matter (DOM) is variable, and the controlling parameters are the C/N ratio of bacteria (5.5 mol/mol) and the (carbon) growth efficiency. By specifying these two quantities as constant, bacteria can utilize DIN or remineralize DON, depending on the substrates available. In FDM90, remineralization of DON can occur, but the specific rate of respiration is fixed, i.e., respiration is subsumed within a constant biomass-specific rate of "excretion" of NH_4. Respiration is, strictly speaking, the process of deriving energy from C compounds, although it is clearly related to catabolism of protein or RNA that can generate inorganic N as a byproduct. Ideally, respiration should be represented as a process fed by the supply of C, and so that heterotroph biomass is not lost to the DIN pool if the supply of C is adequate. This approach was employed for microzooplankton grazers by Christian (2005), who employed a fixed C/N for grazers but a variable ratio for their phytoplankton prey.

The stoichiometry of remineralization by grazers as a function of prey C/N was derived in detail by Anderson (1992) and Landry (1993). The latter argues that microzooplankton, not bacteria, are primarily responsible for remineralization of organic N to NH_4 in the ocean, as bacteria need substrates that are more N-rich than are generally found in the ocean DON pool to be net remineralizers. Anderson and Pondaven (2003) allowed grazers to graze on phytoplankton (C/N = 7.5), bacteria (C/N = 5.1), and detritus (variable C/N), so that grazers will be N-limited if the C/N of the total prey ingested exceeds the threshold value

$$\theta_f^* = \frac{\beta_N \theta_Z}{\beta_C k_c} \qquad (33.12)$$

where β_X is the assimilation efficiency for element X, θ_Z is the grazer C/N ratio and k_C is the C net growth efficiency.

Bacteria also provide a potential pathway for transferring DON and detritus N to higher trophic levels, i.e., bacteria constitute a potential ''link'' for trophic transfer up the food web as well as a ''sink'' in terms of remineralization (Sherr et al., 1987). This link is represented in all N-cycle models that include explicit representations of bacteria that consume DON and/or detritus N, and are in turn grazed by zooplankton. The Fasham et al. (1990) model, for example, includes this link by including bacteria that consume DON, which provide an additional food source to a generic zooplankton compartment (Fig. 33.4). According to the model, this link constitutes a small fraction of the total annual zooplankton food supply at the Bermuda Atlantic Time-series (BATS) station, i.e., an N flux of 0.021 mol m^{-2} year^{-1} from bacteria to zooplankton compared to 0.126 mol m^{-2} year^{-1} from phytoplankton in the 10 m day^{-1} sinking rate case. In contrast, the AW98 model, which is based on the Fasham et al. (1990) model, indicates that the food supply from DOM via bacteria can provide up to 50% of the needs of the zooplankton during winter in the English Channel (Anderson and Williams, 1998).

3.4. Modeling nitrification

Most models that include both NH_4 and NO_3 consider nitrification. In general, nitrification has been treated as a relatively simple process that converts NH_4 to NO_3 with a fixed rate or a first-order dependence on [NH_4], and a time scale of days to weeks. Nitrification is inhibited by light (see Ward, Chapter 5, this volume), so generally it is only applied below the euphotic zone. For example, Christian et al. (2002a) set a cutoff of 120 m (Table 33.2), which has since been modified to a light level (daily mean) of 5 µmol m^{-2} s^{-1} (Wang et al., 2005). The daily mean is used so that nitrification does not occur in the surface layer during the day. Some authors do not specify a nitrification function because the term is considered small compared to fluxes of nitrate and detritus across the open lower boundary. For example, in Fasham et al. (1990) the ocean below the mixed layer consists of a single infinitely deep layer where [NO_3] is fixed and [NH_4] is zero, so any PON that sinks into this layer is implicitly remineralized to NO_3. When this ecosystem model was

Table 33.2 Nitrification model descriptions

Reference	Nitrification model
Wroblewski, 1977	1st-order $f(NH_4)$
Sarmiento *et al.*, 1993	1st-order $f(NH_4)$, $z > 123$ m
Kawamiya *et al.*, 1995	1st-order $f(NH_4)$ with T-dependent coefficient
Chai *et al.*, 1996	1st-order $f(NH_4)$, $z > 120$ m
Levy *et al.*, 1998	1st-order $f(NH_4)$ with T-dependent coefficient
Bissett *et al.*, 1999	hyperbolic $f(NH_4)$, $E < 1$ µmol m^{-2} s^{-1}
McClain *et al.*, 1999	Hyperbolic $f(E)$
Walsh *et al.*, 1999	fixed rate, $E < 0.1 E_o$, $[NH_4] \geq 0.1$ µM
Tian *et al.*, 2000	1st-order $f(NH_4)$, hyperbolic $f(E)$, $E < 0.1 E_o$
Christian *et al.*, 2002a	1st-order $f(NH_4)$, $z > 120$ m
Denman, 2003	1st-order $f(NH_4) \times$ nonlinear z profile

E – irradiance; E_o – surface irradiance; T – temperature; z – depth.

embedded in a 3D GCM, nitrification was included as a first-order process at depths greater than 123 m (Sarmiento *et al.*, 1993, see Table 33.2).

Wroblewski (1977) modeled nitrification as a first-order process, but did not include light or depth-dependence because it was not demonstrated until the early 1980s that light was the controlling factor (see Ward, Chapter 5, this volume and Mills (1989) for an extensive discussion of early hypotheses and investigations). Even some relatively recent models have permitted nitrification throughout the euphotic zone (e.g., Jiang *et al.*, 2003; Kawamiya *et al.*, 1995; Levy *et al.*, 1998). A few models have taken more explicit approaches to the light-dependence of nitrification. McClain *et al.* (1999) included a spectral irradiance model from 280–700 nm, with nitrification dependent on the integrated irradiance from 300–470 nm. Denman (2003) defined a nitrification profile using the function $z^n / (z_{ox}^n + z^n)$ where $z_{ox} = 60$ m and $n = 10$. This function specifies nitrification increasing at depth with higher values of the exponent n giving a sharper cutoff around z_{ox}^n rather than a gradual decline. Tian *et al.* (2000) created a very similar profile using a hyperbolic term $(E^\star - E)/E^\star$, where E is irradiance and $E^\star$ is 10% of the maximum daily value at the sea surface (there is no nitrification when $E > E^\star$). Bissett *et al.* (1999) included a hyperbolic dependence on NH_4 concentration with a half-saturation of 0.1 µM, for total irradiance <1 µmol m^{-2} s^{-1}. The Bissett *et al.* (1999) and McClain *et al.* (1999) models also specified a maximum rate of nitrification rather than a rate constant. Their values differ substantially: 2 vs 40 nmol L^{-1} day^{-1} for McClain *et al.* and Bissett *et al.*, respectively. Perhaps uniquely, Oguz *et al.* (2000) made the rate of nitrification a function of oxygen concentration (see Section 3.6.2). Oxygen is obviously a necessary substrate, but most open-ocean nitrification occurs in the upper thermocline where oxygen is often near saturation, so its role in regulating nitrification rates is ignored. In the Black Sea, however, the oxic-anoxic interface occurs immediately below the euphotic zone.

3.5. Modeling export

Export in N–cycle models is typically handled in one of two ways, i.e., it is either parameterized using a "Martin curve" type of approach which distributes the N flux according to an empirically defined profile (Martin et al., 1987) or it is modeled explicitly through the generation of detritus N particles that sink and remineralize. The advantage of the former is simplicity: with a Martin curve, export and remineralization at depth can be modeled by simply specifying the particulate N export flux from the euphotic zone (F_0) and then redistributing that flux over depth $F(z)$ instantaneously using an empirical function of the form:

$$F(z) = F_0(z/z_0)^{-b} \qquad (33.13)$$

where z represents depth in meters and b is a dimensionless parameter. The obvious disadvantage of using this approach in a dynamic 3D model is that it cannot account for changes in the remineralization profile that may occur due to physical controls (e.g., changes in temperature) or changes in the quality of organic N that is formed and exported, unless these are specified by changing the exponent b as a function of space and/or time. Nor does this parameterization account for the time it takes for particles to sink and thus the potential impact of horizontal advection on particle export and deposition (Siegel and Deuser, 1997).

Other N–cycle models simulate detritus N formation, sinking and remineralization explicitly (e.g., Hood et al., 2001; McCreary et al., 1996; Sarmiento et al., 1993). In its simplest form this involves specification of a detritus (D) pool that sinks with a constant sinking rate w_s and remineralization rate e. If detritus is not produced or consumed by any other processes, then the detritus equation has the form

$$\frac{\mathrm{d}D}{\mathrm{d}t} = w_s D - eD \qquad (33.14)$$

At steady state this equation simplifies to $D_z = (e/w)D$, which has the solution

$$D(z) = D_0 e^{z(e/w_s)} \qquad (33.15)$$

where D_0 is the detritus concentration at some initial depth. Thus, this simple explicit formulation generates an exponential remineralization/detritus flux profile with a decay length scale e/w_s. Elaborations on this approach may include addition of temperature controlled remineralization rate, multiple size classes that sink at different rates and/or additional biological terms (e.g., consumption by bacteria or zooplankton).

Most modern N–cycle models simulate N export using one or the other of these approaches, but details vary and some models combine aspects of explicit sinking and remineralization with Martin curve parameterizations. For example, Chai et al. (1996) modeled a 5-component N-based ecosystem including a detrital N compartment with explicit sinking, but did not permit detrital remineralization within the euphotic zone. In contrast, Christian et al (2002a) used a Martin curve to redistribute detrital N vertically, but rather than remineralizing at the specified depth, it is returned to the

detrital pool where it is subject to all of the processes in the detritus state equation including advection, mixing, and further sinking. Christian (2005) employed parameterized sedimentation, with instantaneous downward transport and solubilization to DON, but no direct remineralization from the "sinking" flux.

Particle aggregation effects can also be parameterized in explicit formulations by making the sinking rate of the detritus a function of detritus concentration. For example, Hood *et al.* (2003) applied this kind of simple parameterization in an NPZD model to account for aggregation that can occur in diatom blooms. In their model phytoplankton sink at the rate

$$
w_p = \begin{cases} w_a, & P \le P_a \\ \dfrac{w_a + (P - P_a)(w_b - w_a)}{P_b - P_a}, & P_a < P < P_b \\ w_b, & P \ge P_b \end{cases} \tag{33.16}
$$

where P_a and P_b are threshold phytoplankton (P) concentrations. When $P \le P_a$ the phytoplankton sink at the rate w_a, i.e., very slowly or not at all, whereas when $P \ge P_b$ the phytoplankton sink very rapidly at the rate w_b. In the concentration range $P_a < P < P_b$, the sinking rate is linearly ramped up from w_a to w_b. This simple formulation crudely reproduces the observed phenomenon of diatom aggregation/flocculation that can occur under bloom conditions and lead to rapid sinking and export (Alldredge and Gotschalk, 1989). It significantly improved the NPZD model's simulation of chlorophyll concentrations in coastal upwelling regions in the Arabian Sea. Much more sophisticated approaches for modeling the effects of aggregation have also been formulated. For example, Jackson (2001) incorporated a mechanistic coagulation dynamics model into the Fasham *et al.* (1990) food web model and showed that coagulation can have an important effect on particle flux even in low particle concentration oligotrophic environments because it increases average particle settling speed (for a review of coagulation theory and models of oceanic plankton aggregation see Jackson (2005)). Thus, a wide range of formulations are available for accounting for particle aggregation effects in N cycle models. In addition, studies have shown that the presence of mineral ballast (i.e., calcite or silica) can have a very significant impact on the remineralization profiles and deep fluxes of particulate organic carbon (e.g., Armstrong *et al.*, 2002), and presumably nitrogen.

3.6. Modeling reduction-oxidation reactions that impact total fixed nitrogen in the oceans

3.6.1. N$_2$-fixation

Another area where there has been significant progress in recent years has been the development of models that account for oxidation-reduction reactions that impact the nitrogen inventory of the oceans, i.e., N$_2$-fixation and denitrification. N$_2$-fixation has been considered in several open-ocean modeling studies. Tyrrell (1999) developed a simple two-box global model with an explicit representation of diazotrophs with both nitrogen and phosphorus cycling. In this model the

N$_2$-fixation rate is a function of the supply ratio of inorganic nitrogen and phosphorus. The underlying assumption is that N$_2$-fixation increases when reactive nitrogen concentrations become low relative to Redfield N:P. Neumann (2000) included N$_2$-fixation in a mechanistic 3-dimensional N-cycle model of the Baltic Sea that includes cycling of both nitrogen and phosphorus, but without an explicit representation of diazotrophs. The N$_2$-fixation rate in this model depends on phosphorus supply, temperature and irradiance. Bissett *et al.* (1999) similarly included N$_2$-fixation implicitly, i.e., as an N flux to the dissolved organic matter pool using a simple parameterization that assumes a nitrogen source that is proportional to temperature and irradiance. Christian (2005) took a similar approach but without temperature-dependence and divided new N equally between DIN and DON.

Several more recent efforts have provided models that can be used to incorporate dynamic representations of diazotrophs and N$_2$-fixation in N-cycle models, although all of these models have deficiencies that potential users should be aware of. Hood *et al.* (2001) explicitly included a state variable for *Trichodesmium* in a 6 compartment N-cycle model (Fig. 33.12). In this model, growth of phytoplankton (G_P) and *Trichodesmium* (G_T) are specified by the following equations:
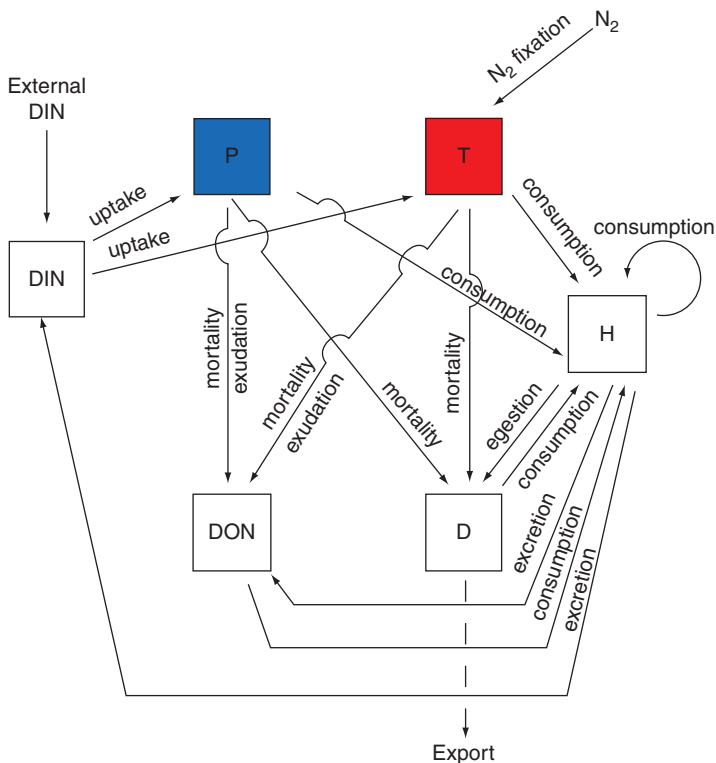
Figure 33.12 A schematic box diagram of the Hood *et al.* (2001, 2004) ecosystem model. The state variables are dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON), phytoplankton (P), *Trichodesmium* (T), heterotrophs (H), and detritus (D). Redrawn and modified with permission from Hood *et al.* (2004).

$$G_P = \mu_p \left(1 - e^{-I/I_p}\right) e^{-I/I_{\beta P}} \, DIN/(DIN + PKS) \qquad (33.17)$$

$$G_T = \mu_T \left(1 - e^{-I/I_T}\right) \qquad (33.18)$$

where μ_P and μ_T are the maximum growth rates for phytoplankton (P) and *Trichodesmium* (T) and I is irradiance. The terms I_P and I_T are the light saturation parameters for P and T, $I_{\beta P}$ is the photoinhibition parameter for phytoplankton (Platt *et al.*, 1980), and PKS is the half-saturation coefficient for N limitation of phytoplankton. Thus, in the Hood *et al.* (2001) model P and T growth differ in that P is subject to photoinhibition and nitrogen limitation whereas T is not. In addition, μ_T is substantially lower than μ_P. Nitrogen fixation is implicit, i.e., when *Trichodesmium* grows in the absence of DIN uptake it introduces N into the system. The N$_2$-fixation rate in this model is assumed to depend entirely upon light, which is primarily regulated by mixed-layer depth/stratification. In subsequent studies, Hood *et al.* (2004) and Coles *et al.* (2004) coupled this model to a 3-dimensional circulation model of the tropical and subtropical Atlantic and, among other things, attempted to simulate basin-wide temporal and spatial variability in *Trichodesmium* biomass and N$_2$-fixation rate (Fig. 33.13). The obvious deficiency in this model is that it does not account for the effects of either P or Fe limitations.
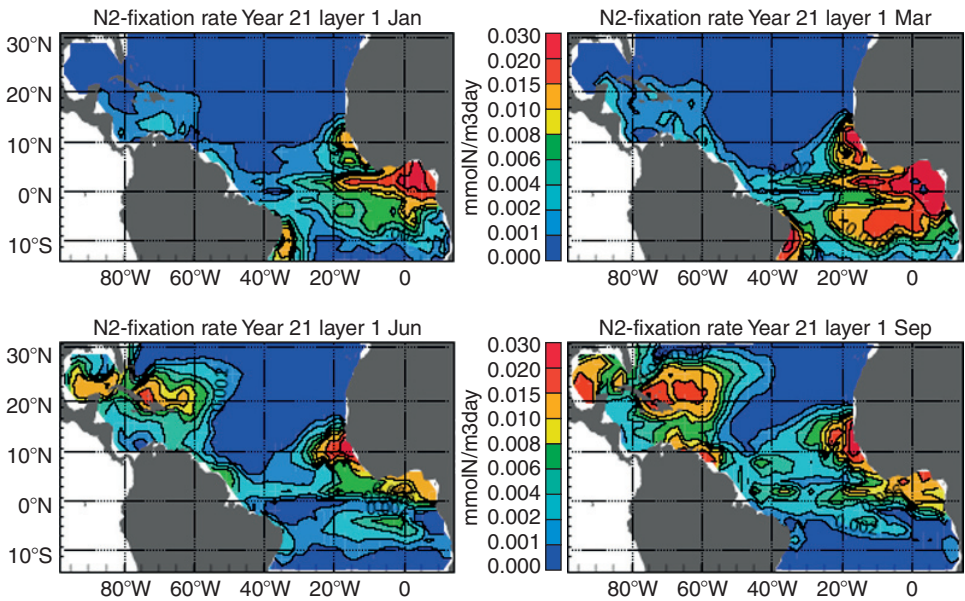
Figure 33.13 Hood *et al.* (2004) model–estimated nitrogen fixation rate in the mixed layer as a function of season. The fields are synoptic, from the middle of the specified month. The rates are expressed in mmol N m^{-3} day^{-1}. Reproduced with permission from Hood *et al.* (2004).
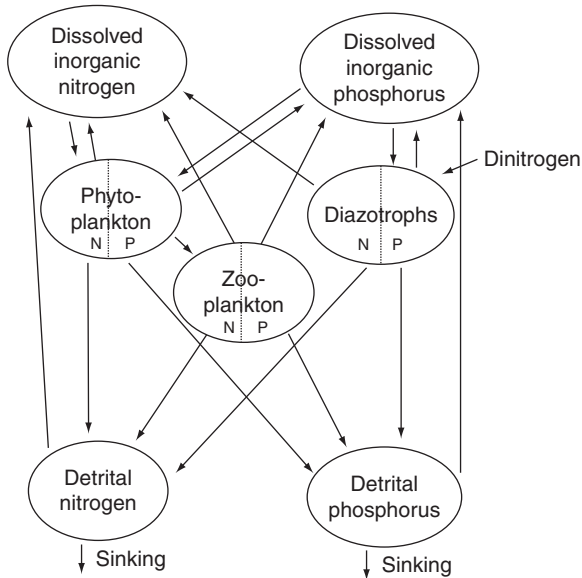
Figure 33.14 Schematic diagram of the Fennel *et al.* (2002) ecosystem model. Reproduced from Fennel *et al.*, 2002 with permission.

Fennel *et al.* (2002) developed a seven compartment model (Fig. 33.14) with temperature-controlled N_2-fixation and P limitation (Fig. 33.15). This model specifies different stoichiometry for different taxa (high N:P ratio in diazotrophs) and permits differential export of nitrogen and phosphorus from the upper ocean. This model also includes a term that applies direct wind speed modulation of diazotroph growth rate, i.e., the observed association of N_2-fixation with calm conditions is directly incorporated into the model in a non-mechanistic fashion. An obvious deficiency in this model is that it does not include a mechanism for supplying additional phosphorus to the surface waters where diazotroph populations and nitrogen fixation rates are typically highest (Capone *et al.*, 1997). As a result the model generates subsurface diazotroph/N_2-fixation maxima that, at least for *Trichodesmium*, are probably unrealistic. One potential mechanism for supplying additional phosphorus to the surface is ''phosphorus mining,'' i.e., the idea that large diazotrophs like *Trichodesmium* can migrate down to the phosphocline periodically to satisfy their phosphorus requirement (Karl *et al.*, 1992). Another, perhaps more likely, mechanism is that phosphorus availability can be maintained at the surface to support diazotrophic growth by simply recycling it faster than nitrogen (Coles and Hood, 2007; Hood *et al.*, 2006; Wu *et al.*, 2000).

Finally Moore *et al.* (2002a; 2002b; 2004) included explicit representations of diazotrophs and N_2-fixation in their multi-species, multi-element biogeochemical model. In this model the growth rate of diazotrophs is subject to light, nitrogen, phosphorus, iron and temperature control. These global simulations generate spatial patterns in N_2-fixation in the Atlantic that are similar to those reported in Hood *et al.* (2004) suggesting that diazotrophic growth and N_2-fixation is, to a large extent,
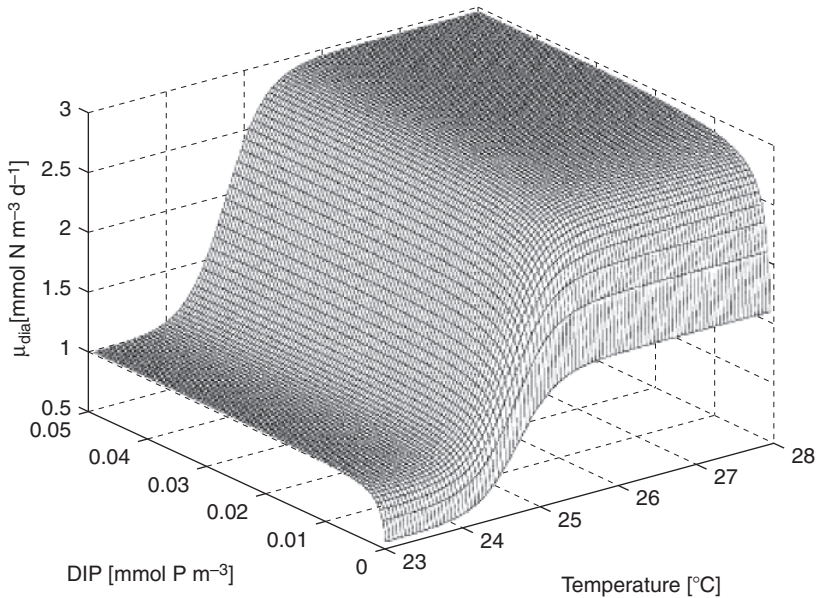
Figure 33.15 The growth rate of diazotrophs as a function of temperature and dissolved inorganic phosphorus concentration in the Fennel *et al.* (2002) model. Reproduced from Fennel *et al.* (2002) with permission.

controlled by mixed layer depth and light. However, Moore *et al.* (2002b) and Moore *et al.* (2004) also report widespread phosphorus limitation of diazotrophs in the Atlantic, particularly in Moore *et al.* (2004), where the N/P ratio is fixed. Both Fennel *et al.* (2002) and Moore *et al.* (2004) include a P requirement for diazotrophy but do not include the (admittedly speculative) mechanisms, such as P mining or enhanced P recycling, that permit diazotrophs to grow in P-depleted subtropical surface waters. Until such mechanisms are better understood, model-derived inferences of P-limitation must be considered tentative.

3.6.2. Denitrification

In contrast to N_2-fixation and nitrification, examples of open ocean N-cycle models that include dynamic models of denitrification are much more limited. This is not due to an absence of a chemical, mathematical or numerical foundation for modeling denitrification (Fennel and Neumann, 2004). Numerous models that include denitrification have been developed and applied in coastal/benthic systems (e.g., Fennel *et al.*, 2006). Denitrification has not been included in many open ocean N-cycle models because in many regions, and over shorter time scales, its impact on the N inventory can be assumed to be negligible. But another likely factor is the difficulty inherent in simulating organic matter loading and oxygen concentrations in hypoxic/anoxic mesopelagic regions (i.e., oxygen minimum zones or OMZs), which are pre-requisites for making a truly prognostic denitrification rate calculation. And there is also the practical constraint that any attempt to model all of the

denitrification in a particular basin must also account for it in shelf/slope waters and sediments. Most GCMs applied at these scales do not resolve the continental shelves.

Anderson *et al.* (1982) published an early modeling study investigating nitrate deficits and the secondary nitrite maxima that result from denitrification regions within OMZs in the Arabian Sea, the eastern tropical North Pacific, and coastal waters off Peru. They integrated the 1-D form of an advective-diffusion-reaction equation set that tracked nitrate and nitrite. Rates were specified for nitrification and the consumption of nitrate and nitrite by denitrification, with nitrite being reduced directly to N_2. An additional simplification consisted of characterizing the OMZ as a two-layer system with separate nitrifying and denitrifying layers.

In addition, several important modeling efforts have been focused on the Black Sea low oxygen environment that include denitrification. For example, Yakushev and Neretin (1997) developed a 1-D advection-diffusion-reaction model to study chemical transformations in the low oxygen mid-water regions of the Arabian Sea and the Black Sea. Oxygen-dependent cycling of nitrogen in the marine environment was included in their model, along with denitrification. In this model the transition from denitrification to nitrification between the anoxic core and the surrounding oxic waters is determined by linear relations that modify the rate of each process, with O_2 thresholds taken from the available literature.

A subsequent effort in the Black Sea consisted of coupling models of biological production and export, nitrogen cycling and redox cycling to a turbulence closure mixed layer model (Oguz *et al.*, 2000). This study represents a significant advance in simulating the linkage between nitrifying and denitrifying regimes because both mixing and export production from the overlying euphotic zone were dynamic components of the modeling scheme. Nitrification and denitrification were explicitly included in this model with nitrite as an intermediate byproduct. Changes in NO_2 and NO_3 concentrations were specified as follows:

$$\frac{dNO_2}{dt} = k_1 f_n(O_2)NH_4 - k_2 f_n(O_2)NO_2 \\ + k_3 f_d(O_2)NO_3 - k_4 f_d(O_2)NO_2 \tag{33.19}$$

and

$$\frac{dNO_3}{dt} = k_2 f_n(O_2)NO_2 - k_3 f_d(O_2)NO_3 \tag{33.20}$$

where the k_x values are reaction rate constants. In (33.19) the first two terms represent the ammonium to nitrite and nitrite to nitrate oxidation reactions in oxygenated waters, and the third and fourth terms represent denitrification under oxygen deficient conditions, i.e., the third term models nitrate to nitrite reduction, and the fourth term represents the nitrite reduction to N_2 (the second stage of denitrification). Changes in nitrate in (33.20) involve increases from the second stage of nitrification (the first term) and decreases from the first stage of denitrification (the second term).

In these equations oxidation reaction rates are determined using a hyperbolic saturation function:

$$f_n(O_2) = \left[\frac{O_2}{O_2 + R_O}\right] \quad \text{for } O_2 \geq 3\mu M \qquad (33.21)$$

where R_O = 10 μM. This equation specifies rates that decrease with decreasing oxygen concentrations and vanish when $[O_2] < 3$ μM in the water column. In contrast, reduction reaction rates are determined using a complementary function of oxygen:

$$f_d(O_2) = \left[\frac{K_O}{K_O + O_2}\right] \quad \text{for } O_2 < 3\mu M \qquad (33.22)$$

where K_O = 2.5 μM. This function specifies that the maximum rate occurs at zero oxygen concentration and decreases gradually as the oxygen concentration increases. When $O_2 \geq 3$ μM, the rate is set to zero.

More recently, Konovalov et al. (1999) and Murray et al. (2005) built upon these previous achievements in modeling biogeochemical cycles in the Black Sea by incorporating a more realistic model of physical exchange (Ivanov and Samodurov, 2001) and including additional biogeochemical constituents. Specifically, transformations of O_2, PON, DON, NO_3, NO_2, N_2, NH_4, H_2S, S°, and dissolved and particulate Mn(II) and Mn(IV) and Fe(II) and Fe(III) were included in their model. Equations that parameterize biogeochemical transformations were written to follow either chemical or biological uptake (e.g., Michaelis-Menten) kinetics; biogeochemical processes are stoichiometrically coupled; and all of the biogeochemical components are conserved. This model generates remarkably detailed and realistic vertical profiles of all of the above constituents (Fig. 33.16).

We are not aware of any basin or global scale coupled N-cycle models that have attempted to include a dynamic, prognostic model of denitrification that is anywhere near the level of sophistication and complexity of the Oguz et al. (2000) or the Konovalov et al. (1999)/Murray et al. (2005) models. Moore and Doney (2007) recently incorporated a prognostic denitrification module into the Moore et al. (2004) global biogeochemical model. In this formulation oxygen is dynamically determined and NO_3 is used in place of O_2 in decomposition of organic carbon when $[O_2] < 4$ μM. For the ocean N inventory to remain in a quasi–steady-state there must be stabilizing feedbacks between denitrification and N_2 fixation. In the model N_2 fixation is stimulated when water that is depleted in N (relative to P) by denitrification is upwelled to the surface.

The authors found strong stabilizing feedbacks in the northern Indian Ocean and in the North Atlantic that act to minimize changes in the marine N inventory over relatively short timescales (~30–200 years) and concluded that rates of N_2 fixation are tightly linked to N:P ratios in surface waters and thus denitrification, locally for the Indian Ocean and more remotely for the Atlantic Ocean. However, the strength of these stabilizing feedbacks was weaker in other regions mainly due to Fe
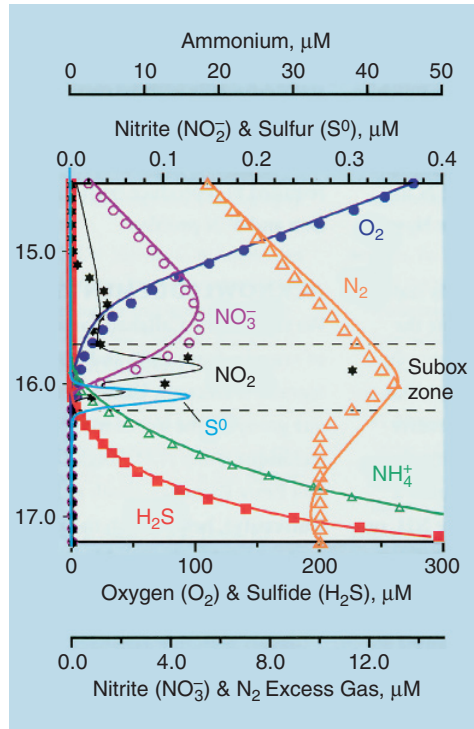
Figure 33.16 Model simulations of biogeochemical constituents in the Black Sea generated by the Konovalov *et al.* (1999)/Murray *et al.* (2005) model. Figure reproduced with permission from Murray *et al.* (2005).

limitation of diazotrophs and also due to spatial separation between areas of N_2 fixation and denitrification, i.e., the feedbacks occur regionally, but globally they aren't strong enough to balance the N budget over the time scales examined. In their base model run the global N inventory goes down by 7% over 200 years. In addition, the authors found that maintenance of the OMZs is dependent, in part, on N_2 fixation driven export production and they concluded that Fe limitation of diazotrophs significantly hampers the ability of N_2 fixation to compensate for N losses due to denitrification in the current climate, leading the oceans to lose fixed nitrogen.

Perhaps the most significant caveat in the Moore and Doney (2007) study is that their model does not include shelf denitrification, which would suggest that they are missing more than half of the global marine denitrification signal and its associated N sink (Codispoti *et al.*, 2001), or they are producing this sink in the wrong place, i.e., in the open ocean rather than on the shelves. If the former is true, then the imbalance (loss) of N from the oceans in their model is even greater. Regardless, the Moore and Doney (2007) study represents an important step forward in large-scale N cycle modeling. It is one of the first to incorporate dynamic representations of both denitrification and N_2 fixation and feedbacks between them. The study also provides

important insights into the potential linkages between Fe supply, N_2 fixation, denitrification and the maintenance of open ocean OMZ regions.

Nitrous oxide (N_2O) is both an important greenhouse gas and a byproduct of both nitrification (see Bange, Chapter 2, this volume and Ward, Chapter 5, this volume) and denitrification (see Devol, Chapter 6, this volume), and has been the subject of several modeling studies. Suntharalingam and Sarmiento (2000) and Suntharalingam et al. (2000) coupled a simple N_2O production model to a coarse resolution ocean general circulation model, with N_2O production given as a function of oxygen consumption, i.e., it was assumed to be proportional to remineralization of organic matter, and therefore to nitrification. In the latter paper, a dependence of N_2O yield on O_2 concentration was introduced, based on studies of ammonia-oxidizing bacteria in culture. These data suggest that yields are extremely high at the O_2 concentrations at which denitrification occurs. So by replacing oxygen consumption with a C- or N-based estimate of remineralization, this model could be extended to include a parameterization of N_2O production from denitrification as well as nitrification. In a follow on study, Jin and Gruber (2003) further elaborated the Suntharalingam et al. (2000) model and used it to assess the potential impacts of iron fertilization on ocean N_2O emissions. They found that increased N_2O production could significantly offset the radiative benefit from fertilization-induced atmospheric CO_2 reduction.

At present, the relative importance of nitrification and denitrification as sources of N_2O remains unknown, and models linking all of these processes to environmental conditions remain tentative. As noted above, the efforts of Yakushev and Neretin (1997) and Oguz et al. (2000), respectively, employ linear and hyperbolic forms of O_2-dependent relations to capture the transition between the two processes, and while the former effort provides for an overlap regime, the latter does not. It should also be noted that, although Redfield's stoichiometry for denitrification (e.g., Richards, 1965) has been assumed to be applicable for all the denitrification modeling efforts discussed above, there is growing evidence that this is not the case (Codispoti et al., 2001; Van Mooy et al., 2002). Codispoti et al. (2001) point out that there are a number of factors that can alter the apparent stoichiometry of denitrification. These include variations in the C:N composition of the organic matter substrate (Van Mooy et al., 2002) as well as the influence of more exotic processes that produce dinitrogen gas, such as the ''anammox'' reaction and transformations involving Mn as a ''pseudo-catalyst'' (Luther et al., 1997). None of these reactions have been considered in N-cycle modeling studies. These and many other challenges remain.

4. Nitrogen in the Ocean (Modern Coupled Models)

4.1. Nitrogen fluxes and ocean circulation

The supply of new nitrogen (mostly NO_3) to the euphotic zone has been a primary focus for oceanographers for the past two decades. As noted above, models can provide estimates of quantities that are not directly observable, such as fluxes of nutrients by different physical processes. For example, Sarmiento et al. (1993) used a

basin-scale model of the North Atlantic (20°S-60°N) to estimate fluxes of nitrate by different physical processes and examine controls on surface nitrate concentrations. For the full model domain, they found that flux of nitrate to the euphotic zone was 64% from upwelling, 29% from convective overturning, and 7% from mixing. The largest N flux was from upwelling in the subpolar gyre and the equatorial zone associated with large scale Ekman transport. Horizontal advection as a local source of N was a "mirror image" of vertical transport, i.e., highest in regions of down-welling. The degree to which N can be advected from upwelling to downwelling regions depends strongly on the rate of export and therefore on the ecosystem model used. In Sarmiento *et al.* (1993) downward transport was predominantly by sedimentation, but downward advection and mixing of particulate and dissolved N (NH_4, DON) accounted for 15%. The basin-averaged *f*-ratio was 0.43.

The companion paper by Fasham *et al.* (1993) focused on two specific sites: Ocean Weather Station India (OWSI) in the subarctic and BATS in the subtropics. At BATS, vertical nitrate supply to the euphotic zone was overwhelmingly from winter convection (more than 25 times larger than vertical mixing). More than half of this was removed by vertical advection (Ekman pumping). They also found that a significant fraction of the nitrate at this location was supplied by lateral advection. Subsequent studies with eddy-resolving models suggest that some of these results are artifacts of the coarse-resolution ($2 \times 2°$) model used (McGillicuddy *et al.*, 2003; Oschlies, 2002).

In this model, nitrate concentration remained above 1 μM in summer over large areas of the subpolar gyre. Sarmiento *et al.* (1993) discuss the difference between transport-dominated and uptake-dominated regimes. In a transport-dominated regime, uptake does not keep up with the physical supply of nitrate and concentrations rise above the biologically-regulated concentration, which is the half-saturation point for phytoplankton uptake (in this case 0.5 μM). This occurs in the equatorial Atlantic in boreal summer when the strongest upwelling-favorable winds are present (see also Frost and Franzen, 1992), and in the subpolar gyre in winter when phytoplankton are light-limited. Over most of the model domain, the fraction of surface NO_3 removed by the biota (relative to accumulation in an abiotic run) exceeds 90%. However, there is a region of the subpolar North Atlantic where nitrate concentration remains high even in summer, which is attributed primarily to grazing control of phytoplankton (see also Armstrong, 1994). As discussed above, physical transport of nutrients depends in part on the ecosystem model structure.

A subsequent study using the same ecosystem model was carried out in the equatorial Pacific by Toggweiler and Carson (1995). They calculated N fluxes in the equatorial *x-z* plane (integrated to ±5° latitude), showing that the primary source of N to the surface waters of the equatorial upwelling is upwelling from depths >100 m, the approximate depth of the equatorial undercurrent (EUC). In the 90–144 m depth stratum there is an eastward advective flux of 6.9 Tmol N year^{-1} at 135°W, of which about 15% is advected east of 90°W, 10% advected poleward of 5°, and the remainder upwelled to the surface layers. In the surface layer (0–40 m) the upwelling flux between 90–135°W is 10.2 Tmol year^{-1}. About half of this N is exported as particulates, and the remainder divided equally between zonal and meridional advection. A subsequent study using a different ecosystem/circulation

model (Jiang et $al.$, 2003) used the same geographic boundaries, although their vertical levels differ. Jiang et $al.$ found that upwelling across 112 m transported 5.6 Tmol N year^{-1} between 90–135°W, in between Toggweiler and Carson's values for 90 and 144 m, but nearer to the low end (based on linear interpolation of the depth levels). Losses were divided between particulate export ($\sim$2/3) and meridional advection ($\sim$1/3), with a negligible contribution from zonal advection when integrated from 0–112 m.

An important conclusion of the Toggweiler and Carson (1995) study is that the EUC is the most important source of N to the equatorial zone; paradoxically, NO_3 concentration in the EUC is lower than in the surrounding waters. The resolution of this apparent paradox lies in the nonconservative nature of bioactive elements such as N. The primary mass balance for N is between zonal advection by the EUC and sedimentation of particulate N, while the mass balance for water is between zonal advection and poleward advection of surface waters that have been depleted of N by the biota. This is not intended to minimize the importance of vertical advection, which is of course very important for equatorial ecosystems, but to understand sources to the region as a whole, by calculating fluxes in and out of a "box" encompassing the upper equatorial ocean down to the base of the thermocline.

A similar analysis of the equatorial Atlantic, using a model with 16 vertical layers and a simple N-based biogeochemical model was conducted for the years 1983 and 1984 by Loukos and Memery (1999). In their model, vertical advection along the equator shows a distinct maximum that follows the slope of the thermocline, with maximal values at 40–80 m depending on longitude, while the vertical mixing flux is maximal in the surface layer in the central basin, coincident with the maximal biological uptake. Loukos and Memery emphasize the importance of mixing for vertical N fluxes: upwelling accounts for most of the flux to the euphotic zone, but mixing brings it to the surface layer, where most of the biological uptake occurs. This may differ between the Atlantic and the Pacific because in the Pacific vertical gradients of N concentration are less steep due to iron and/or silicate limitation. Although the discussion here has focused primarily on DIN fluxes and specifically NO_3, it should also be noted that upwelling can transport significant amounts of NH_4 and organic N from the aphotic zone to the euphotic zone in the coastal zone (Gruber et $al.$, 2006), and probably also in intense open ocean upwelling regions (e.g., Christian et $al.$, 2002b).

As we have variously discussed in previous sections, there are several other sources that contribute to the new nitrogen supply to the euphotic zone in the open ocean, i.e., in addition to the supply of new nitrogen (mostly NO_3) due to physical processes. Atmospheric inputs include NO_x and NH_3 deposition derived from anthropogenic (e.g., fossil fuel combustion and domestic animal waste) and natural (e.g., lightning) sources. Although rates of N_2-fixation in open ocean waters are still not well constrained, it is generally thought that this process provides the most significant atmospheric source of new nitrogen (Galloway et $al.$, 2004; Michaels et $al.$, 1996), about 120 Tg N year^{-1} globally, as compared to $\sim$25 Tg N year^{-1} from the other atmospheric sources. It is believed that this influx of N from N_2-fixation is restricted primarily to tropical and subtropical waters where it can be comparable to the supply of new NO_3 from depth (Capone et $al.$, 1997; 2005; Karl

et al., 1997). Yet most open ocean N–cycle models do not account for any of these atmospheric N sources, although some now include N_2-fixation as discussed above. The model results of Hood *et al.*, (2004) and Moore *et al.*, (2002a; 2002b) suggest that new nitrogen inputs from N_2-fixation significantly alter phytoplankton production patterns in tropical and subtropical waters.

4.2. Mesoscale processes

The pioneering studies of Sarmiento *et al.* (1993) and Fasham et al (1993) illustrate both the strengths and weaknesses of basin-scale coarse-resolution models. Despite some unrealistic aspects of the modeled circulation, these allowed oceanographers to examine the time-dependent dynamics of basin-scale processes. The weakness is that low model resolution can result in important processes being entirely overlooked. For example, Fasham *et al.* (1993) found that vertical advection (Ekman pumping) is a sink for NO_3 at BATS and horizontal advection a significant source. Eddy-resolving models, by contrast, suggest that both of these results are artifacts of low resolution (McGillicuddy *et al.*, 2003).

There is a long-standing controversy over levels of new production in the Sargasso Sea. Estimates from integrative geochemical tracer methods cannot be sustained by the traditionally accepted mechanisms of vertical nutrient supply (Jenkins and Goldman, 1985; Shulenberger and Reid, 1981). McGillicuddy and Robinson (1997) used a high-resolution coupled physical-biological model to show that mesoscale upwelling could supply the required amount of NO_3. In a later study, McGillicuddy *et al.* (2003) simulated the entire North Atlantic at eddy-resolving resolution ($0.1° \times 0.1°\cos\phi$, ϕ = latitude). They compared N fluxes with both the coarse resolution model of Fasham *et al.* (1993) and an eddy-permitting, but not eddy-resolving, model by Oschlies *et al.* (2000). The eddy-permitting model had a much lower input of NO_3 from horizontal advection at BATS than the 2° model, but had a similar loss to vertical advection (0.13 mol m^{-2} year^{-1}) and relied on winter convection as the dominant source. Convection is an important source in McGillicuddy *et al.* (2003) as well, but the 0.13 mol m^{-2} year^{-1} N sink from vertical advection becomes a 0.12 mol m^{-2} year^{-1} source. However, another eddy-resolving simulation by Oschlies (2002) found a relatively modest eddy-induced N flux of 0.05 mol m^{-2} year^{-1} and concluded that mesoscale upwelling does not by itself provide a solution to the conundrum.

An important difference between these two models is that Oschlies (2002) incorporated an NPZD ecosystem model, whereas McGillicuddy *et al.* (2003) parameterized biological uptake, and continually relaxed NO_3 concentrations back to a climatological nitrate-density regression immediately below the euphotic zone. McGillicuddy *et al.* (2003) argue that the model of Oschlies (2002) underestimates NO_3 concentrations in precisely the depth/density range where it has a large effect on fluxes to the euphotic zone. Whatever the reasons for the discrepancies between these two models, this example provides a useful illustration of the importance of the choice of the biological model even for estimates of N fluxes determined primarily by physical processes. Furthermore, it indicates that sedimentation and remineralization are very important aspects of the model that remain poorly understood (see Sections 3.3 and 3.5).

4.3. Modern nitrogen-cycle model structures

As we discussed at the end of section 2, the modern era of basin–scale N–cycle modeling began with the seminal work of Fasham *et al.* (1993) and Sarmiento *et al.* (1993). Current models encompass a wide range of biological complexity and spatial resolution. Although simple N–cycle models (e.g., NPZD) are still being used to address a variety of important questions (e.g., Hood *et al.*, 2003; Kawamiya and Oschlies, 2003; Oschlies, 2002), there has been a general movement toward the application of N–cycle models that include more detail in the food web (e.g., Christian *et al.*, 2002a), that incorporate important reduction-oxidation reactions such as N_2–fixation (e.g., Fennel *et al.*, 2002; Hood *et al.*, 2001; 2004), and that consider limiting nutrients other than nitrogen (e.g., Aumont *et al.*, 2003; Christian *et al.*, 2002a,b; Gregg *et al.*, 2003; Moore *et al.*, 2002a,b, 2004).

Several N–cycle models have been developed in recent years that have explicit representations of both the small (slow sinking) and large (fast sinking) components of the planktonic food web. For example, Fig. 33.17 shows a schematic diagram of a 9-component N–cycle model that was originally developed by Leonard *et al.* (1999) and subsequently applied in three-dimensional studies in the tropical Pacific, Atlantic and Indian Oceans (Christian *et al.*, 2002a; Christian and Murtugudde, 2003; Wang *et al.*, 2005; Wiggert *et al.*, 2006). This model includes state variables for large and small autotrophs (i.e., net–phytoplankton and pico–phytoplankton) along with large and small grazers (i.e., macrozooplankton and microzooplankton), which then produce fast and slow sinking detrital components (large and small detritus). NO_3 and NH_4 are included along with Fe, and photoacclimation of the carbon-to-chlorophyll ratio. This model was intended to include aspects of the N–cycle that are essential for partitioning between export and recycling, but does not include explicit compartments for bacteria or DON. The implication of the latter is that bacteria and DON are *not* essential for representing export and recycling in N–cycle models; this is discussed further below.
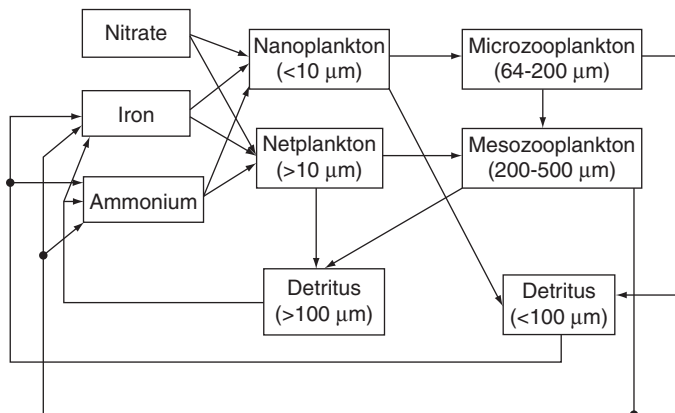
Figure 33.17 Schematic box diagram of the Leonard *et al.* (1999) model.
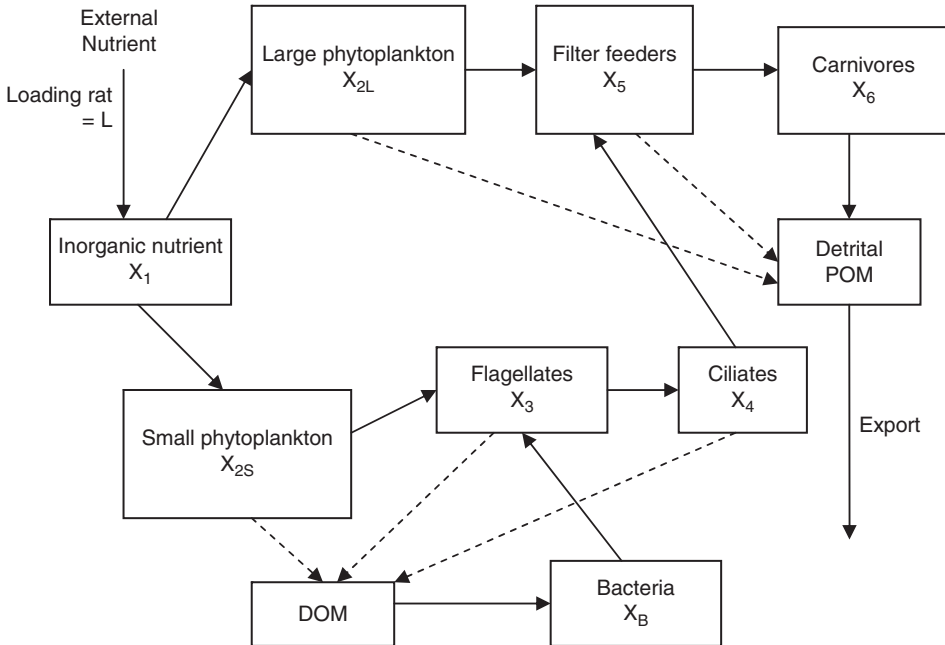
Figure 33.18 Schematic box diagram of the Laws *et al.* (2000) model. Redrawn with permission from Laws *et al.* (2000).

Additional variations on this basic model form are the Laws *et al.* (2000) and the Fennel *et al.* (2006) models. The Laws *et al.* (2000) model (Fig. 33.18) has 10 state variables and includes bacteria and DOM plus additional higher trophic level grazers, but only one fast-sinking detritus compartment and one inorganic nitrogen compartment. This model is structured so that consumers of large phytoplankton produce detritus that sinks, whereas consumers of small phytoplankton produce DOM that does not sink, thereby partitioning production into export and recycling. In contrast, the Fennel *et al.* (2006) model (Fig. 33.19), which has only 6 state variables, accomplishes this partitioning by explicitly including fast (large) and slow (small) sinking detritus compartments. However, rather than generating different size classes of detritus via different size classes of phytoplankton and zooplankton, instead this model produces large detritus through aggregation of small detritus and phytoplankton. The Fennel *et al.* (2006) model also includes nitrification and an explicit representation of shelf/sediment biogeochemical processes, i.e., a simple parameterization of nitrogen losses associated with sediment denitrification following Seitzinger and Giblin (1996).

As discussed in the previous section, an alternative approach that has been advocated by Steele (1998) and more recently by Denman and Pena (2002) and Denman (2003), is to stick with simple NPZ and NPZD formulations and, instead, try to "parameterize" additional food web complexity (see also Pahlow and Vezina, 2003). In his review of these various simplifying approaches, Denman (2003)
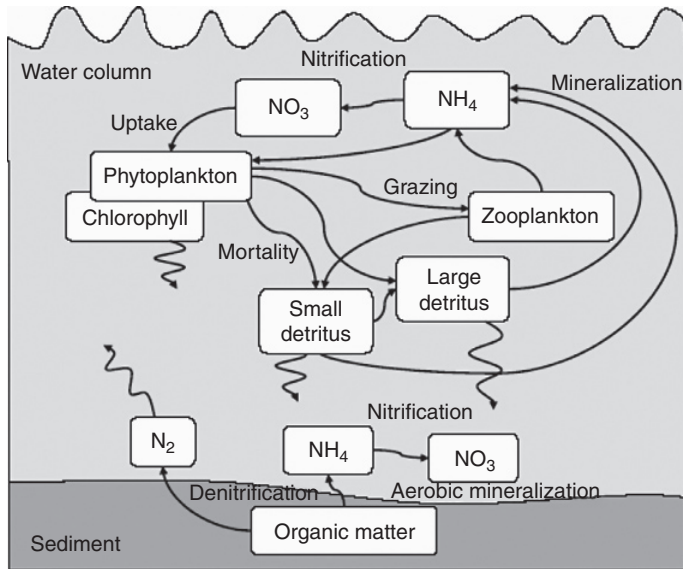
Figure 33.19 A schematic diagram of the Fennel *et al.* (2006) N-cycle model. In addition to the pelagic components, this model also includes benthic N-cycle processes, i.e., sediment denitrification. Reproduced from Fennel *et al.* (2006) with permission.

concluded that there are compelling reasons to consider adopting relatively simple N-cycle model formulations that represent increasing complexity in compact forms. Among other things, he points out that more complex model formations tend to exhibit more unstable behavior, and adding compartments usually leads to a non-linear increase in the number of parameters in the model that often cannot be adequately constrained. However, recent model intercomparison studies suggest that some of the more complex N-cycle models can provide better predictive skill and can be more readily ported, without reparameterization, from one ocean basin to another (Friedrichs *et al.*, 2007). It appears that the latter is a consequence of increased flexibility in more complicated model structures which allows them to "adapt" to different forcing conditions, for example, by automatically altering the balance between N recycling and N export. However, these improvements in skill and portability appear to be highly model specific, i.e., some complex model formulations perform better than simple ones while others do not. The reasons for these differences in model performance are not yet fully understood.

5. Future Challenges and Conclusions

In this paper we have provided a brief overview of the history and state-of-the-art of marine N-cycle modeling. Our emphasis has been on prognostic open-ocean models and coupled physical–biological model systems. There has been significant

progress in the development of these models over the past 30 years, i.e., we have progressively and substantially improved the degree to which they represent the relevant biological and chemical constituents and processes that are important for N cycling in marine systems. There have also been significant advances in the correctness and completeness of the functions and formulations that are used in these models.

However, most of the N–cycle models employed today are fundamentally similar to the ones that were developed in the early 1970s, i.e., relatively simple models composed of a fixed number of state variables representing a relatively small fraction of the dissolved and particulate constituents in the marine N cycle compared to those that are present in nature. Simple NPZD models are still widely employed in large scale modeling studies in spite of the fact that these models do not explicitly represent some of the most important agents that drive N-cycling in marine systems such as bacteria, protozoa and the microbial loop, and they aggregate all autotrophs and all grazers into single ''P'' and ''Z'' boxes. The use of these models has persisted because they are simple and computationally efficient, and because they appear to reproduce to first order the variability of the modeled N pools. These models can also be made to represent different aspects of the marine ecosystem by changing the parameters to represent, for example, diatoms in one context and *Prochlorococcus* in another. Although a wide variety of new models and modeling approaches have been developed over the last three decades, there is still no consensus in the ocean modeling community as to which are best, and what model compartments are crucial or not (but see also Friedrichs *et al.*, 2006; 2007, as examples of recent efforts to quantitatively assess the performance of different N–cycle model formulations).

A prime example of this lack of consensus is the question of whether or not it is necessary to include explicit representation of bacteria in N cycle models. The short answer is obviously no, because there are numerous modern N–cycle model formulations that seem to work quite well without them (e.g., Christian *et al.*, 2002a; Gregg *et al.*, 2003; Hood *et al.*, 2003; Moore *et al.*, 2002a; see Table 33.1). Rather, these models have simple parameterizations of the net effects of bacteria on the N cycle, where dissolved and particulate organic matter are remineralized directly to inorganic nutrient pools. The omission of bacteria is often justified on the grounds that bacteria are ubiquitous, and their biomass and activity tends to co–vary with primary production and organic matter concentrations. Hence they can be parameterized as a component of the detritus and/or DON pool. But these assumptions are gross oversimplifications. While bacterial biomass and production are positively correlated at large scales to phytoplankton biomass and production, respectively, there is significant variability in the ratios of these parameters among various ocean systems (Cole *et al.*, 1988; Ducklow, 1999). Thus, assuming a constant relationship between bacterioplankton and phytoplankton and detritus properties in models is not appropriate for all oceanic regimes and may be a poor assumption for global applications. However, we are not aware of any quantitative studies of model performance that definitively show that including explicit bacteria substantially improves representation of the marine N cycle (for additional discussion on this topic see Hood *et al.*, 2006).

Numerous marine N–cycle models have been constructed that include representations of DON production and cycling (e.g., Anderson and Williams, 1998;

Fasham *et al.*, 1990; Kawamiya *et al.*, 1995; Levy *et al.*, 1998). These models include, at most, two state variables representing rapidly cycling DON constituents (i.e., "labile" DON with turnover rates on the order of hours to days), and slower cycling DON constituents (i.e., "semi–labile" DON with turnover rates on the order of weeks to months). The typical sources of DON in these models include direct DON production from phytoplankton exudation and/or lysis, excretion and sloppy feeding from zooplankton, and DON production associated with bacterial degradation of organic detritus. Typical sinks for DON include uptake by phytoplankton and bacteria. Photochemical degradation of DON is also potentially important but has received little consideration from modelers to date. To our knowledge few, if any, models have been constructed that represent DON production, composition and cycling at a significantly higher level of detail.

It is important to note that DON may provide an important vector for transport of organic N in the ocean. It has been hypothesized that some of the more refractory constituents of the DOM pool may get transported over long distances horizontally and/or vertically before they are remineralized, providing the potential for linkages between fixation of inorganic nutrients into organic matter at one place, and release of this nutrient at some other place in the ocean (e.g., Roussenov *et al.*, 2006). Obviously, models that represent the DON pool with only one labile component cannot account for this transport vector. If it is ultimately determined that long-distance transport of DON is a quantitatively important component of the N cycle in the ocean, then N-cycle models will need to include *at least* two state variables representing both rapid and slower cycling DON constituents.

Perhaps the greatest challenge we face in terms of modeling DON is presented by the uncertainty in its composition, i.e., the majority of the compounds that comprise DON are still not characterized (Bronk, 2002). As a result, we are constrained to model bulk properties, i.e., binning a very complex mixture of compounds into a small number of boxes. Another significant challenge in DON modeling is presented by the uncertainty in the relative importance of different DON sources and the relative proportions of labile and refractory compounds produced by these different sources. Finally, the processes by which labile forms of DON are transformed into more refractory constituents, i.e., via microbial consumption and recycling and photodegradation, are also not well understood and so are difficult to incorporate into models in a meaningful way. The reader is referred to Christian and Anderson (2002) for additional details on how DOM cycling is represented in models. Suffice it to say here, that the current state-of-the-art in DON modeling reflects, to a large extent, the current state of our knowledge of DON composition and cycling, which is still rudimentary.

The relatively recent discovery of large numbers of viruses (Bergh *et al.*, 1989), especially bacteriophages, in all marine waters has opened up an entirely new area of modeling exploration in recent years. Maintenance of these high viral abundances may involve high incidences of host (bacteria) mortality, which could have a profound effect on the microbial loop (Section 3.2) and the nitrogen cycle (Proctor *et al.*, 1988; Suttle, 1994).

One of the first models that included an explicit representation of viruses in order to explore their role in nutrient cycling was a steady-state food web model by

Murray and Eldridge (1994), which was designed to show how a range of virus-induced bacterial mortalities affects the amount of production that reaches meso-zooplankton. Thingstad (2000) created a model, based on earlier work by Thingstad and Lignell (1997), that included multiple strains of bacteria with a common predator and host-specific viruses. To date, most prognostic "biogeochemical" models that have accounted for the influences of viruses on nutrient cycling have done so via a generic mortality term to account for loss processes including viral lysis (Christian and Anderson, 2002).

Despite these early developments, models with explicit representations of viruses have not yet been widely applied or tested in natural marine systems. The major question that needs to be addressed is, do we need to account for the impact of viruses in pelagic N–cycle models and if so, at what level of realism? Do viruses need to be explicitly modeled or can their effects be parameterized as a component of the generic death terms that we already have in our models?

As we pointed out at the beginning of this section and elsewhere in the paper, very simple N–cycle models are still being employed in large scale, coupled physical–biological modeling studies (e.g., Hood *et al.*, 2003; McGillicuddy *et al.*, 2003; Oschlies, 2002). However, there has been a general trend towards increasing model complexity. This trend has been motivated by our ever-increasing appreciation of the details and complexity of microbial foodwebs, and it has been permitted by the ever increasing power of computers. This raises an important question: Is our ability to simulate and predict N–cycle variability in marine systems increasing in proportion to the complexity of our models? Models become more flexible as you increase the number of degrees of freedom. So we can expect that this trend will result in models that are able to fit available data sets progressively better. But better fits may not reflect increased predictive skill or even increased levels of understanding. As the order of a polynomial regression model increases, the fit to data improves, but this in no way reflects an increase in understanding. Moreover, if this increase in degrees of freedom allows the model to fit noise in the data, then it will also give rise to a model with lower predictive skill. The same principles apply to mechanistic N–cycle models, i.e., the degree to which a model can fit a particular data set says little about how well the model can predict observations that were not used to constrain it, and complex model formulations do not necessarily give rise to improved understanding because they are more difficult to diagnose. Clearly, our ability to judge the efficacy of the current trend toward increasingly complex models is going to be dictated by the availability of the data to constrain and judge their skill, and the ability of modelers to devise effective means to determine why more complex models behave the way they do.

The focus of this review has been on the history and state-of-the-art of N–cycle models. One can legitimately ask how relevant these models are given recent advances in our understanding of the factors that control primary production and particulate matter export in the ocean. Fifteen to twenty years ago, when the Fasham *et al.* (1990) model was still under development, the potential importance of iron limitation was only beginning to be appreciated. The emphasis at that time was on N limitation, the intricacies of the food web and the potential importance of bacteria and the microbial loop. We now know that iron limits phytoplankton growth over

vast areas. We also know that the availability of other nutrients, like silica and phosphorus, has a strong influence on the export of both nitrogen and carbon. Moreover, there is reason to believe that on longer time scales phosphorus ultimately controls primary production and export in the ocean, with nitrogen availability alternating from limitation to excess in response to shorter time-scale changes in the nitrogen inventory, i.e., the balance of nitrogen fixation and denitrification (Gruber, 2004; Moore and Doney, 2007; Tyrrell, 1999).

Nonetheless, N-cycle models are still relevant and useful tools for several important reasons. Although phosphorus may be an appropriate model currency for longer time-scale studies, in our contemporary oceans, N *is* one of the primary limiting nutrients and so is a natural choice as a currency for a variety of applications. Fe is also a primary limiting nutrient in the ocean, but our current understanding of Fe chemistry and cycling, and regulation of biological Fe requirements, is still too rudimentary to use it as a model currency. Moreover, we don't have nearly enough *in situ* measurements of the different phases and forms of Fe to constrain Fe-based biogeochemical models. One of the major challenges we face is dealing with co-limitation and switching between N and Fe limitations, and also accounting for the effects of low P and Si concentrations when they arise. N-based models provide a logical foundation for carrying out studies along these lines and for developing the multi-element models of the future. As these multi-element models become more complete the distinction of an element as the primary currency will become progressively more obsolete, i.e., when the models get to the point where all of the elements are represented as separate state variables in all of the model compartments, then each element can be regarded as a model currency as much as any other.

Finally, the N cycle is important because understanding it is essential to understanding the C-cycle in the ocean. The N cycle is also of great interest in its own right for both modeling and observational studies because there are still many aspects of it that are not fully understood or adequately captured in models. Given recent advances in molecular/genetic methods that can be used to identify and quantify new genes, enzymes and their biogeochemical expression, we can expect to learn many new things about the marine N cycle in the near future. The challenge for N-cycle modelers will be to figure out how to incorporate these into our models, and use these models to help understand their significance.

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NITROGEN IN PAST MARINE ENVIRONMENTS

Eric D. Galbraith, Daniel M. Sigman, Rebecca S. Robinson,
and Thomas. F. Pedersen

Contents

1. INTRODUCTION

Many reservoirs of biologically important elements turn over so rapidly at the Earth surface that modest differences between input and output could either remove them completely or double their concentrations in the geologically short span of thousands to millions of years. Despite this precarious situation, evidence indicates that the Earth has been remarkably stable in its habitability, allowing the chain of multicellular life to persist for more than a half billion years. Such continuity requires a conspiracy of feedbacks within the Earth system that stabilizes the availability of life's key ingredients. Yet we know little about these feedbacks, how they develop, or the constraints that they impose on the environment and life. Beyond these

fundamental scientific questions, an understanding of the regulatory mechanisms is the key to predicting how the system will respond to anthropogenic climate change.

The marine nitrogen budget provides an important test case in the broader effort to understand the stabilizing environmental feedbacks on the Earth surface. Because both the principal source (N_2 fixation) and sink (denitrification) of fixed nitrogen in the oceans are mediated entirely by marine organisms, the marine nitrogen (N) cycle is particularly well-suited to the development of biological homeostasis, providing an important contrast in this regard to nutrients such as phosphorus, silicon, and iron. However, it is difficult to quantify, on the basis of temporally and spatially limited modern observations, the sensitivities of the N budget to environmental parameters. Fortunately, geological and glaciological archives have recorded past events that approximate large-scale experiments in which the oceanic N budget responds to naturally imposed forcings.

Although some studies have explored the nitrogen cycle of the distant past (e.g., Fennel *et al.*, 2005; Jenkyns *et al.*, 2001; Kuypers *et al.*, 2004; Rau *et al.*, 1987; Saltzman, 2005), we focus on the cycles of ice ages and interglacial periods that have dominated the last 2 million years of Earth history. Several aspects of these cycles make them well suited to this application. First, they are relatively well recorded in sediment and ice core records. Second, the glacial and interglacial periods are each at least three times longer than the residence time of oceanic fixed N (≤ 3 kyr, (Codispoti *et al.*, 2001)). Thus, a given glacial/interglacial transition (such as the last deglaciation) approximates a discrete natural experiment, with the ocean N budget beginning near steady state, responding to the climate-driven forcing and then potentially reaching a new steady state condition. It has been suggested that the marine N budget during the last glacial maximum was markedly different from today (Altabet *et al.*, 1995; Falkowski, 1997; Ganeshram *et al.*, 1995), which may have impacted atmospheric concentrations of the greenhouse gases CO_2 (Broecker, 1982; McElroy, 1983) and N_2O (Gruber, 2004; Suthhof *et al.*, 2001). By comparing the modern nitrogen budget to this alternate state from the recent geological past, we gain insight into the potential for future change.

While the budget of fixed N is unique among the nutrients, the internal cycling of nitrogen and phosphorus appear to be tightly coupled through the stoichiometry of algal uptake and subsequent remineralization in the modern ocean (Redfield, 1934). Thus, if we can understand the controls on the oceanic distribution of nitrate, we would gain similar insight into the phosphate distribution. It has been posited that coupled changes in polar ocean overturning and algal nutrient consumption drive the observed variation in atmospheric CO_2 over glacial/interglacial cycles (see Sigman and Boyle (2000) for a review). If the relationship of high latitude nitrogen cycling with climate over past glacial/interglacial cycles can be determined, it would also shed light on the oceanic control of $p\text{CO}_2$.

In this chapter, we review the ongoing efforts to use sediment and ice core records to understand the dynamics of oceanic fixed N, focusing on recent glacial–interglacial cycles. Research has, up to this point, followed the reductionist approach of trying to reconstruct either changes in the ocean N budget (largely through low latitude records) or changes in the internal cycling and distribution of N (largely through high latitude records). However, evidence is building for a coupling of high

and low latitude changes, connecting the processes that control the distribution of N in the ocean with those that control the size of the whole ocean N inventory. We begin by considering how the nitrogen cycle of times past may have differed from that of today, followed by an overview of how nitrogen cycle features are recorded in marine sediments and, finally, summarize the observations and interpretations made from available records.

2. POTENTIAL CHANGES IN THE MARINE NITROGEN CYCLE DURING THE PAST 2 MILLION YEARS

We divide the potential changes frequently considered by paleoceanographers into two broad categories: those that most directly involve changes in the ocean nitrogen inventory, that is, the total mass of fixed (non-gaseous) nitrogen in the oceans, and those that most directly involve changes in the distribution and cycling of fixed nitrogen within the ocean.

2.1. Inventory changes

Fixed nitrogen is continuously added to the ocean surface and removed from its interior by the processes depicted schematically in Fig. 34.1. As discussed elsewhere in this volume, the emerging view of the Holocene marine nitrogen budget is largely independent of terrestrial nitrogen inputs and is dominated by the single
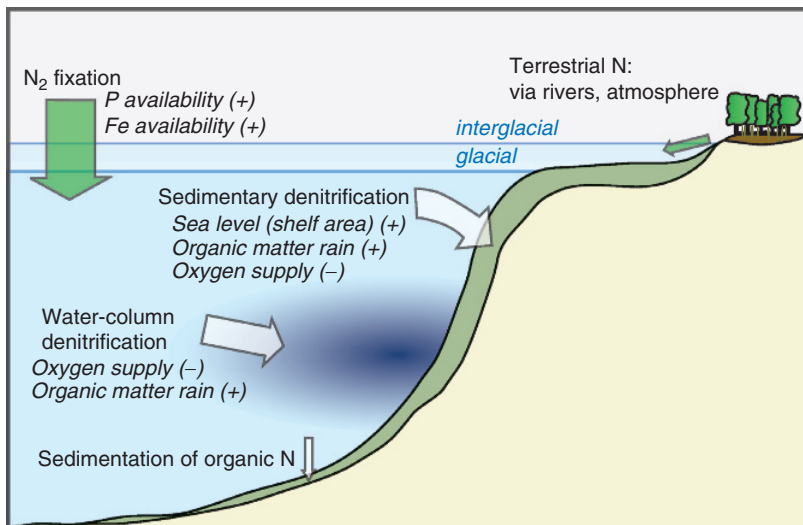
Figure 34.1 Schematic view of the major sources (green arrows) and sinks (white arrows) of fixed N in the marine environment. Important factors for controlling the variation of each source or sink term over time are given in italics, with (+) and (−) indicating direct and indirect proportionality, respectively.

input term of marine N_2 fixation and by the output terms of sedimentary and water column denitrification (where ''denitrification'' refers to the total conversion of fixed N to gaseous products by any pathway, including anammox). Given their dominance with respect to mass flux, the potential for inventory changes is determined by the temporal variability of these three terms. As described below, it has been suggested that the N inventory was larger during the last glacial maximum (LGM), which would have allowed for greater biological sequestration of CO_2 in the deep sea, helping to explain the glacial pCO_2 decrease.

2.1.1. Marine nitrogen fixation

Fixed nitrogen is supplied to the global ocean by specialized microbes, termed diazotrophs, near the ocean surface (see Chapter 4, Carpenter and Capone this volume). It has been proposed that the low supply rate of iron to the present-day ocean hampers the growth rates of diazotrophs, exacerbating nitrogen limitation among marine ecosystems (Falkowski, 1997). If the degree of global Fe-limitation varies over geological time, then the rate of N_2 fixation might be expected to follow. It is believed that greater dust fluxes during glacial times (Petit *et al.*, 1999; Winckler *et al.*, 2008) probably would have led to higher trace metal concentrations in the oceans, though it is not clear by what degree (Parekh *et al.*, 2004). If true, this could have allowed diazotrophs to fix N at greater rates during ice ages, and the resulting increase in N_2 fixation rates could have increased the whole ocean N inventory (Falkowski, 1997). Comparison of ice core records of atmospheric pCO_2 and dust led Broecker and Henderson (1998) to promote this idea.

2.1.2. Sedimentary denitrification

Organic matter is rapidly delivered to continental shelves of the modern ocean, producing sediments that are generally anoxic very close to the sediment-water interface. The physical proximity of oxygen-bearing bottom waters to anoxic sediments with high concentrations of labile organic matter allows diffusion and bioturbation to effectively supply oxygen to nitrifying organisms and nitrate to nitrate-reducing organisms in the sediment, leading to high rates of denitrification in shelf sediments (see Chapter 6, Devol, this volume). During ice ages, sea level dropped by up to 120 m, exposing ~75% of global shelf area (Hay and Southam, 1977). As a result, organic matter from highly productive coastal ecosystems would have been shunted to the deep sea. Because this organic matter must settle through a greater height of water column, more organic matter oxidation would have been accomplished there instead of in sediments. One would expect, therefore, that the lowering of sea level would have significantly reduced the global rate of sedimentary denitrification, as proposed by Christensen *et al.* (1987). To obtain a rough maximal estimate of this effect, we can assume that the area-normalized rates of sedimentary denitrification on slopes and in the deep sea remained constant during glacial maxima, despite the fact that they would be the primary depositional sites for coastally produced sinking organic matter during low sea level. In this extreme case, a reduction in global shelf area to 25% of the modern area would have produced a decrease in sedimentary denitrification of 75 Tg N/year (Middelburg *et al.*, 1996), representing a ~30% decrease in the global rate.

Hypsometry is not the only parameter affecting global sedimentary denitrification rates. A higher flux of organic matter to the sediment strongly increases sedimentary denitrification (see Section 2.2.2). The oxygen content of bottom waters has a secondary and less certain effect. In some cases, higher denitrification rates would be expected under more oxygen-poor conditions (Middelburg *et al.*, 1996), though this may be reversed if nitrification becomes limited by oxygen (Devol and Christensen, 1993).

2.1.3. Water column denitrification

The other large sink of marine nitrogen is by denitrification in suboxic regions of the water column. This process only occurs in the most poorly ventilated regions of the modern ocean, where dissolved oxygen has been depleted through aerobic respiration, to below 2–5 μM O_2 ((Codispoti *et al.*, 2005) see Chapter 6, Devol, this volume). In the modern ocean, this is limited to less than 0.2% of the ocean volume, almost entirely within the upper 1000 m of the Eastern Tropical Pacific and the Arabian Sea. These subsurface regions are driven to suboxia by the coincidence of rapid organic matter delivery with a slow re-supply of dissolved oxygen (Olson *et al.*, 1993; Sarma, 2002; Wyrtki, 1962). In theory, the extent of water column denitrification could be significantly altered by changing the export rate of organic matter to the subsurface (Altabet *et al.*,1995; Ganeshram *et al.*, 2000), and/or by changing the oxygen supply through a physical mechanism (Broecker and Peng, 1982; Galbraith *et al.*, 2004; Meissner *et al.*, 2005). The relative balance between surface ocean fertility and the oxidizing capacity of the subsurface can be approximated by the ratio of nutrient to oxygen concentrations of global thermocline waters; all else being equal, when this is high, denitrification is abundant. Because the concentrations of nutrients and oxygen in the thermocline are both strongly influenced by those at high latitude outcrop regions (Galbraith *et al.*, 2004; Meissner *et al.*, 2005; Sarmiento *et al.*, 2004), water column denitrification has an acute sensitivity to the surface conditions at high latitudes (see Section 4.3).

Since nearly all subsurface respiration on the global scale is supported by oxygen, there is no clear *a priori* reason that water column denitrification should have been similarly important at all times in the past; increases in the rate of oxygen supply relative to consumption could have nearly eliminated it. However, the strong sensitivities of water column denitrification, while rendering this process susceptible to climate-driven variability, probably also make it more responsive to stabilizing negative feedbacks (see Section 2.2.3).

2.2. Internal stabilizing feedbacks on the marine nitrogen inventory

The conceivable potential for nitrogen source and sink fluxes to vary independently of each other would appear to characterize the marine nitrogen cycle as highly unstable. However, modern process studies have illuminated stabilizing (negative) feedback mechanisms that would tend to limit excursions in the N cycle (see Fig. 34.2). The strengths of these feedbacks on centennial to millennial timescales should determine the responsiveness of the marine N cycle to climate change, but they remain largely unquantified.
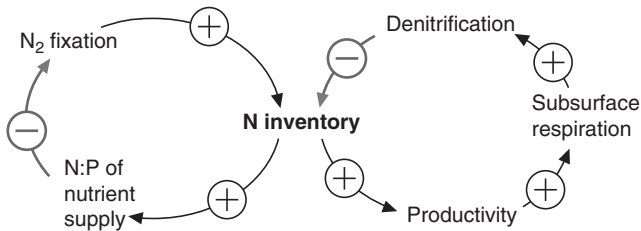
Figure 34.2 Hypothesized feedbacks within the marine N cycle on centennial-millennial time-scales. A direct proportionality, i.e., producing a change of equal sign to that of the forcing, is shown as a black arrow, while an indirect proportionality is shown as a grey arrow. Note that each complete loop contains exactly one indirect proportionality, therefore each represents a negative (stabilizing) feedback on the fixed N inventory. Modified after Deutsch *et al.* (2004).

2.2.1. N$_2$ fixation self-limiting feedback

It is widely held that N$_2$ fixers tend to be at a competitive disadvantage to non-diazotrophs under nitrate-replete conditions: only where nitrate is nearly exhausted but phosphorus remains available are N$_2$ fixers expected to provide substantial new nitrogen. If so, N$_2$ fixation would tend to be self-limiting: greater rates increase new N input to an ecosystem, reducing the degree of N limitation of non-diazotrophic competitors and, hence, the net availability of phosphate to diazotrophs (Broecker, 1982; Redfield *et al.*, 1963; Tyrrell, 1999; Deutsch *et al.*, 2007). This self-limiting feedback, illustrated on the left side of Fig. 34.2, intimately ties N$_2$ fixers to the global aggregate rate of denitrification, which sets the global deficiency of N relative to P and, consequently, the availability of nitrate-limited environments exploitable by N$_2$ fixers. The sensitivity of this feedback depends largely on the degree of disadvantage that diazotrophs suffer in the presence of nitrate, which is poorly quantified.

The residence time of marine P is 3–15 times longer than that of fixed N (Delaney, 1998; Ruttenberg, 2003) and it has been argued that its source and sink fluxes are less sensitive to glacial-interglacial climate change than those of fixed N (Ganeshram *et al.*, 2002). Given this apparent stability of the P inventory, the relatively robust relationship between N and P concentrations in bulk algal stoichiometry (Redfield *et al.*, 1963) is critical when considering potential changes in the global N$_2$ fixation rate. This ratio reflects the nutritional requirements of the surface ocean algal community, thereby determining its ability to utilize a given phosphate supply with available nitrate, and hence, the potential for excess phosphate to be available to N$_2$ fixers. Although there is some degree of localized discrepancy from the canonical "Redfield" N:P value of 16:1, including among individual species and in local environments, the ratio holds remark-ably well throughout the modern ocean (see Gruber (2004) for a review and Chapter 1, Gruber, this volume). Broecker and Henderson (1998) (Section 2.1.1) invoked a global biomass N:P of ∼24:1 during glacial periods to explain lower atmospheric pCO$_2$. This degree of flexibility is not immediately suggested by the low variability of remineralized N:P in the modern ocean, particularly with near-Redfield N:P in the modern Fe-replete subtropical North Atlantic (Hansell *et al.*, 2004). Nonetheless, nitrate to phosphate ratios in the deep Mediterranean Sea can be as high as 25:1 (Krom *et al.*, 2005), indicating that substantial divergences above Redfield N:P are

possible under some conditions. Without a better appreciation of the mechanisms underlying this master variable, the possibility of significant changes in the N:P of past oceans cannot be dismissed, and the strength of the N_2 fixation self-limiting feedback remains in question.

2.2.2. Nitrogen-limitation feedback on denitrification

Denitrification in the ocean interior only occurs under extremely low oxygen concentrations. Oxygen is depleted by the respiration of reduced carbon compounds, most of which are derived from the ecosystems above. If the ability of those ecosystems to export fixed carbon is impaired due to nitrogen limitation of export production, the resulting alleviation of the subsurface oxygen deficiency will cause a reduction in denitrification. This constitutes a general stabilizing feedback on both types of denitrification, illustrated on the right side of Fig. 34.2.

The extent of water column suboxia is sensitive to organic matter flux from the surface, and would quickly respond to changes in export production. This sensitivity is evident in the delicate balance between oxygen supply and oxidant demand in the global subsurface: the respiration history of suboxic waters (oxygen utilization plus denitrification) is almost entirely supported by oxygen, typically having consumed $\sim$250 μM O_2 versus 15 μM NO_3^- (Codispoti *et al.*, 2001). Denitrification in suboxic waters is, therefore, a relatively small residual term in the difference between total respiration and oxygen supply. As a result, small changes in the total respiration, driven by export production, could have large effects on denitrification. Because waters from denitrification zones supply the local upwellings that are, in turn, largely responsible for maintaining the suboxia below, this is a geographically tight coupling and would be expected to provide a strong feedback. The sensitivity of this feedback depends on the degree to which diazotrophs near the upwelling zone are capable of eliminating any N-limitation caused by a nitrate deficit in upwelled waters, thereby mitigating changes in export production. In this regard, a stronger expression of the N_2 fixation self-limiting feedback posed above would weaken the denitrification-driven feedback described here, and vice versa.

Sedimentary denitrification would respond similarly to a productivity change caused by nitrate limitation, as it is largely determined by the organic carbon flux to the seafloor (Middelburg *et al.*, 1996). Hence, if a decrease in denitrification rates promotes higher export production via a relaxation of nitrate limitation, the enhanced organic carbon flux to the seafloor would reinvigorate sedimentary denitrification. Conversely, an increase in denitrification encourages nitrogen limitation and limits large increases in export production. This reflexive feedback would tend to dampen any massive swings in the N inventory.

2.3. Nitrate distribution and the nutrient-rich surface regions

The macronutrient-rich surface waters of the equatorial upwellings and polar oceans are a particularly dynamic component of the global carbon cycle. In the Southern Ocean, for example, the nutrient-rich and CO_2-charged waters of the deep sea are exposed to the atmosphere and returned to the subsurface before the available nutrients are fully utilized by phytoplankton for carbon fixation. This incomplete utilization of upwelled nutrients allows for the leakage of biologically sequestered

CO_2 back into the atmosphere, raising atmospheric pCO_2 (Knox and McElroy, 1984; Sarmiento and Toggweiler, 1984; Siegenthaler and Wenk, 1984). On this basis, increased nutrient utilization in the Southern Ocean has been invoked as the cause of the lower pCO_2 concentrations of glacial times, through a reduced leakage of carbon into the atmosphere (Francois *et al.*, 1997; Martin and Fitzwater, 1988).

The potential changes in the polar ocean over glacial/interglacial cycles are too diverse to be usefully covered in this review (Sigman and Boyle, 2000; Sigman and Haug, 2003). Nevertheless, it is illustrative to consider how the nutrient status of the polar ocean would respond to a given change in ocean circulation, depending on the parameters that most strongly control algal growth in these regions (a topic of ongoing research in the modern ocean).

We consider the hypothesis that the polar oceans were more vertically stable during the last ice age than they are today (Francois *et al.*, 1997; Jaccard *et al.*, 2005; Robinson *et al.*, 2004; Sigman *et al.*, 2004; Toggweiler, 1999), focusing on the Antarctic region. According to this hypothesis, increased density stratification of the upper Antarctic water column would have reduced the rate of exchange between nutrient-rich deep water and the euphotic zone. If algae growing in the Antarctic are most critically light limited, their growth rates would be expected to have remained similar regardless of climate state, so that decreased access to the deep nutrient pool would have led to greater relative nutrient utilization (i.e. a greater ratio of nutrient assimilation to gross nutrient supply).

On the other hand, if suggestions that Fe availability provides the overriding control on algal production in the Antarctic are correct (Martin *et al.*, 1991), the predominant route of Fe supply to the surface would become a critical parameter. If the Fe supply has always been dominated by dissolved Fe upwelled from below, its supply would have decreased in step with the glacial decrease in the gross supply of the macronutrients nitrate and phosphate (assuming a constant Fe: macronutrient ratio in Antarctic subsurface water). In this case, the degree of relative nitrate utilization in the glacial surface would have been more similar to that of today. By contrast, if direct atmospheric Fe supply is important (either during the last ice age or both during the last ice age and today), the relative utilization of nitrate and phosphate in the glacial Antarctic surface would have increased with increasing stratification, as in the case of light-limitation. Sedimentary records of export production, which should be proportional to the net flux of macronutrients to the euphotic zone at steady state, can be used to help untangle competing scenarios such as these. Clearly, reconstructing the nutrient status of the polar oceans in the past and developing an understanding of the controls on algal growth in these regions go hand in hand.

3. Recorders of the Past Nitrogen Cycle

Unfortunately, there are no existing direct records of past nitrate concentration; one must rely instead on proxy evidence. We focus here on the most specific and frequently used proxy for studying the history of the ocean N cycle, the nitrogen isotopic ratio of organic matter preserved in marine sediments.

3.1. Nitrogen isotope systematics

There are two stable isotopes of N, ^{14}N (which constitutes 99.63% of the N in the environment) and the rare isotope ^{15}N (see Sigman and Casciotti (2001) for a review and Chapter 29, Montoya, this volume). Measurements of the $\delta^{15}N$ of nitrate ($\delta^{15}N_{nitrate}$) in the deep sea (below 1000 m) have shown it to be fairly homogeneous, at about 5‰ relative to atmospheric N_2 (Sigman *et al.*, 2000) ($\delta^{15}N = (^{15}N/^{14}N_{sample}/^{15}N/^{14}N_{air} - 1) \star 1000$‰). In certain regions and depth intervals of the ocean, the $\delta^{15}N_{nitrate}$ diverges significantly from the mean deep $\delta^{15}N_{nitrate}$ because of regionally specific processes, as described below. To understand the signals that might be retrieved from sedimentary N isotopes, we first look individually at each of the key fractionating processes (Fig. 34.3).

3.1.1. Inputs

Inputs of nitrogen to the marine environment appear to be isotopically similar to atmospheric N_2. N_2 fixation occurs with little effective isotope discrimination, so that marine N_2 fixers produce reduced nitrogen with a $\delta^{15}N$ only slightly lower than that
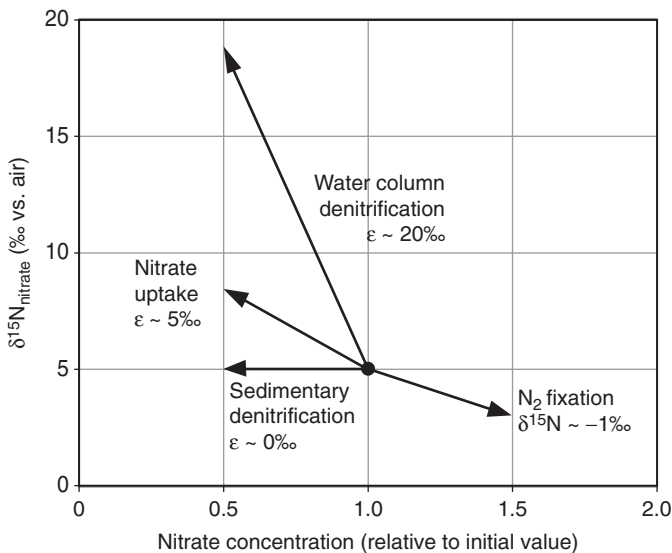
Figure 34.3 Nitrogen isotopic fractionation processes. The vectors approximate the isotopic changes associated with the indicated addition or removal of nitrogen from an initial nitrate pool with a $\delta^{15}N$ of 5‰ (black filled circle). Note that the fractionating processes (water column denitrification and nitrate uptake) are actually nonlinear, due to the progressive enrichment of $\delta^{15}N$ in the residual pool, a feature not evident in this discrete view. The return of organic N to the nitrate pool by remineralization is not shown despite a large isotope effect associated with nitrification (Casciotti *et al.*, 2003), since ammonium in typical open-ocean subsurface water is completely oxidized to nitrate. As a result, the nitrate produced should be similar to the organic matter being remineralized. However, remineralization can lead to significant ^{15}N enrichment in the small fraction of organic matter that survives extensive degradation, as in the case of deep sea sediments (see Section 3.3.1). After Sigman and Casciotti (2001).

of air (0 to $-2‰$, e.g., (Carpenter *et al.*, 1997)). In regions of the subtropical ocean, thermocline $\delta^{15}N_{nitrate}$ can be as low as 2‰, $\sim$3‰ lower than the mean deep ocean $\delta^{15}N$. This observation is most readily explained by N_2 fixation in the overlying surface waters and the subsequent remineralization of this low-$\delta^{15}N$ organic material in the waters below (Karl *et al.*, 2002; Knapp *et al.*, 2005; Liu *et al.*, 1996; Pantoja *et al.*, 2002) (Fig. 34.4, North Atlantic).

Fixed N is also transferred from terrestrial to marine ecosystems by riverine and atmospheric vectors, in roughly equal parts (Galloway *et al.*, 2004). This probably contributed no more than one quarter, and perhaps less than one eighth, of the input flux of fixed N to the pre-industrial ocean (Galloway *et al.*, 2004). Although it has been suggested that the $\delta^{15}N$ of terrestrial inputs sums to near 0‰ (Brandes and Devol, 2002), their isotopic compositions are very poorly constrained and may in fact be distinct from the N introduced by oceanic N_2 fixation. There is currently a need for a systematic study of the isotopic composition of N inputs to the ocean from rivers and groundwater that takes into account the multiple chemical forms of N involved as well as the reactions that occur as rivers deposit N into the coastal ocean. Terrestrial
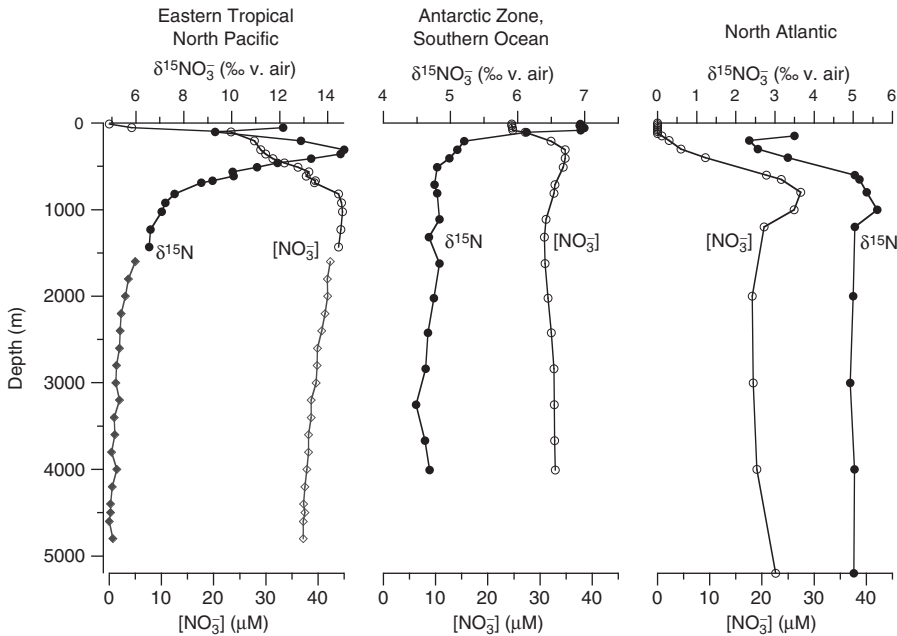
Figure 34.4 Water column profiles of nitrate concentration (open symbols) and $\delta^{15}N_{nitrate}$ (filled symbols) in the Eastern Tropical North Pacific (ETNP, coastal Baja California), Southern Ocean and North Atlantic (Sargasso Sea). The ETNP shows a large increase in $\delta^{15}N_{nitrate}$ in the thermocline owing to local water column denitrification. The Southern Ocean shows little deviation from the global deep mean $\delta^{15}N_{nitrate}$ except at the surface, where partial NO_3^- assimilation leaves residual nitrate enriched in ^{15}N. The North Atlantic profile shows low $\delta^{15}N_{nitrate}$ in the thermocline owing to the nitrification of locally fixed N. Note that the ETNP profile also includes deep measurements from near Hawaii (diamonds); the smooth transition between samples at distant locations emphasizes the homogeneity of the deep Pacific.

inputs of fixed N are also a concern in paleoceanographic work given their potential to be delivered directly to ocean margin sediments, thereby contaminating the paleoceanographic signal in sediment records (see Section 3.3.2).

3.1.2. Outputs

Unlike N_2 fixation, nitrate reduction has a strong isotopic bias, favoring the light N isotope and leaving behind a residual nitrate pool that is progressively enriched in ^{15}N with increasing degree of nitrate consumption. Culture studies collectively yield a relatively broad range in the isotope effect, ϵ (i.e. $(^{14}k/^{15}k - 1)^{\star}1000$‰, where ^{14}k and ^{15}k are the rate coefficients of the reactions for the ^{14}N- and ^{15}N-bearing forms of NO_3^-, respectively) for nitrate consumption by denitrifying bacteria, from roughly 15‰ to 30‰ (Barford et al., 1999; Mariotti et al., 1981; Granger et al., in press). By analogy with work on nitrate assimilation (Granger et al., 2004; Needoba et al., 2004), it would appear that isotopic discrimination primarily takes place upon reduction of nitrate to nitrite by the intracellular enzyme nitrate reductase which, in its assimilatory form, has an intrinsic isotope effect of 15–30‰ (Ledgard et al., 1985; Schmidt and Medina, 1991). The expression of this fractionation is registered by the $\delta^{15}N$ of unconsumed nitrate that diffuses from the cells back into the ambient waters (Granger et al., in press).

Water column studies, in which denitrification rates are calculated from nitrate deficits relative to phosphate, have led to estimates of 22–30‰ for the isotope effect of denitrification (Altabet et al., 1999b; Brandes et al., 1998; Cline and Kaplan, 1975; Liu and Kaplan, 1989; Sigman et al., 2003; Voss et al., 2001). Regardless of the exact value of the expressed isotope effect, water column denitrification clearly results in very high $\delta^{15}N_{nitrate}$ in the thermocline within, and adjacent to, actively denitrifying regions of the water column (see Eastern Tropical North Pacific (ETNP) profile, Fig. 34.4). We note that although there are no existing measurements to explicitly resolve the isotopic impact of anammox, it is implicitly included in all existing water–column (and sedimentary) denitrification studies as part of the overall fixed N loss.

Sedimentary denitrification, in contrast, shows very little expression of the enzymatic isotope effect. Benthic flux experiments have shown that, even with high rates of sedimentary denitrification, minimal increase of $\delta^{15}N_{nitrate}$ is observed (Brandes and Devol, 1997, 2002; Lehmann et al., 2004). The common explanation is that nitrate is nearly completely consumed at the locus of sedimentary denitrification. Because no nitrate escapes the denitrification zones, there is no expression of the isotope effect. This view is supported by the observation of extreme ^{15}N enrichment of nitrate in sediment porewaters (Sigman et al., 2001; Lehmann et al., 2007), which indicates that the lack of isotopic expression of denitrification in sediment cannot be explained by a lack of isotopic fractionation at the scale of the organism. In any case, in the context of budgetary calculations, sedimentary denitrification is typically assumed to have a negligible isotopic effect (Brandes and Devol, 2002; Deutsch et al., 2004; Sigman et al., 2003).

These two extremes of expression of the organism-level isotope effect of denitrification—complete expression in the water column and no expression in sediments—are unlikely to apply perfectly in the ocean. Instead, we might expect

some degree of under-expression of the isotope effect in the water column under some conditions (Deutsch *et al.*, 2004; Sigman *et al.*, 2003), particularly if alternate dissimilatory pathways, such as anammox, fractionate differently and are of variable importance depending on environmental conditions. An extreme case of this is provided by the Cariaco Basin, where water column denitrification appears to consume nitrate completely very near the oxic/anoxic interface, leading to minimal expression of the isotope effect of denitrification (Thunell *et al.*, 2004). In addition, measurements of N_2/Ar in the Arabian Sea (Devol *et al.*, 2006) imply that the loss of nitrogen through denitrification there is greater than previously thought, which would suggest that the isotope effect of water column denitrification has been significantly overestimated. Reciprocally, isotopic discrimination by denitrification is partially expressed in some sediments, albeit to a small degree relative to water column denitrification (Brandes and Devol, 2002; Lehmann *et al.*, 2004, 2007). Nevertheless, the end-member view presented above provides a useful conceptual starting point.

3.1.3. Internal cycling

Fixed nitrogen occurs in many forms in the marine environment, and most transformations among nitrogen pools involve some degree of isotopic fractionation. The most important fractionation for understanding changes in nitrate distribution (as opposed to changes in the input/output budget) occurs during nitrate assimilation, when discrimination by the enzyme nitrate reductase causes the $\delta^{15}N$ of the first organic matter produced from a given pool of supplied nitrate to be lower than $\delta^{15}N_{nitrate}$. The $\delta^{15}N$ of both the residual nitrate and the subsequently produced organic N increases as nitrate consumption progresses. Measurements of $\delta^{15}N_{nitrate}$ in the upper ocean suggest an isotope effect of 4–10‰ for nitrate assimilation, with most estimates closer to 5–8‰ (Altabet, 1988; Altabet and Francois, 2001; Altabet *et al.*, 1999b; DiFiore *et al.*, 2006; Sigman *et al.*, 1999b; Wu *et al.*, 1999). Fractionation in cultures appears to be more variable, with isotope effects as high as 20‰, although many cultures yield effects of ≤ 6‰ (Needoba *et al.*, 2003).

Developing a predictive understanding of the isotope effect of nitrate assimilation has been a goal of marine N isotope studies. The isotope effect varies among species grown under similar growth conditions (Needoba *et al.*, 2003; Wada and Hattori, 1978; Waser *et al.*, 1998) and, for at least some species, as a function of growth conditions (Granger *et al.*, 2004; Needoba and Harrison, 2004). For example, the cultured diatom *Thalassiosira weissflogii* displays a greater organism-level isotope effect under light-limitation than under iron-limitation or maximal growth rate (Needoba and Harrison, 2004; Needoba *et al.*, 2004). The potential variability of the isotope effect represents a critical uncertainty in paleoceanographic application of nitrogen isotopes to changes in nutrient utilization in the nitrate-replete regions of the surface ocean.

When nitrate is completely utilized, the accumulated organic matter has the same $\delta^{15}N$ as the original nitrate source. Hence, in regions where the consumption of nitrate is complete over the course of the year, isotope fractionation during nitrate assimilation has only a transitory (i.e. seasonal) effect that disappears when integrated over the seasons of growth. From the perspective of paleoceanography, the $\delta^{15}N$ of

organic N exported from such an environment must be identical to that of the N supply; if subsurface nitrate is the dominant supply term, then the export should have the same δ^{15}N as the sub-euphotic zone nitrate transferred to the sunlit surface ocean. In contrast, within nutrient-rich regions of the surface ocean, where nitrate consumption is persistently incomplete, the δ^{15}N of sinking N is lower than sub-euphotic δ^{15}N$_{nitrate}$, and the δ^{15}N of the residual surface nitrate is higher than both the sub-euphotic zone nitrate and the sinking N (Fig. 34.4, Southern Ocean) (DiFiore *et al.*, 2006; Lourey *et al.*, 2003; Sigman *et al.*, 1999b).

3.1.4. Deep nitrate

Despite dramatic variations of δ^{15}N$_{nitrate}$ within the nutrient-bearing surface ocean, nitrate assimilation has little effect on the global distribution of N isotopes. Complete nitrate utilization in most of the surface ocean and virtually complete remineralization in the subsurface prevents the accumulation of isotopically distinct non-nitrate pools. Dissolved organic nitrogen, the only other pool of appreciable size, has a very similar isotopic composition to that of nitrate where it has been measured (Knapp *et al.*, 2005) and therefore can have little impact on the global distribution of ^{15}N. Nitrate concentrations in the deep sea are so high that the small amount of low-δ^{15}N organic matter supplied from the nutrient-rich surface has only a minimal effect on the δ^{15}N of deep ocean nitrate (Sigman *et al.*, 2000) (compare Southern Ocean deep water *vs.* Sargasso Sea, Fig. 34.4).

Instead, the modern deep sea is remarkably homogeneous with nitrate δ^{15}N of $5 +/- 0.5‰$, so that it should represent a well-integrated measure of global input and output rates, rather than internal cycling. Mean deep ocean δ^{15}N$_{nitrate}$ therefore has the potential to provide a monitor of whole-ocean inventory change.

3.2. Ocean δ^{15}N$_{nitrate}$ through time

It seems safe to assume that N$_2$ fixation, water column denitrification and sedimentary denitrification have been the dominant input/output terms of the marine N budget throughout, at least, the past 2 Myr. With this assumption in place, one can begin to consider how potential changes would have altered the δ^{15}N of marine nitrate, both within a given region and at the scale of the whole ocean.

3.2.1. Local variations

Because the isotope fractionating processes are not evenly distributed throughout the ocean, temporal variations at fixed points will produce predictable changes in the δ^{15}N of local nitrate pools. As examples: (1) an increase in denitrification rates within the water column on the Oman margin would increase the δ^{15}N$_{nitrate}$ of the Arabian Sea thermocline (Altabet *et al.*, 1995; Schafer and Ittekkot, 1993), (2) an increase in N$_2$-fixation in surface waters of the eastern Mediterranean would cause a decrease in δ^{15}N$_{nitrate}$ in the eastern Mediterranean thermocline (Struck *et al.*, 2001), and (3) an increase in relative nitrate consumption in Antarctic surface waters would cause an increase in local near-surface δ^{15}N$_{nitrate}$ (Francois *et al.*, 1997). However, it must be kept in mind that these local signals are imprinted upon any variation in the δ^{15}N baseline of mean ocean nitrate. A similar principle operates at finer scales, with

changes in a N-cycle process in one region contaminating adjacent regions to various degrees (e.g., Kienast *et al.*, 2002; Martinez *et al.*, 2006; Galbraith *et al.*, 2008).

3.2.2. Steady state mean

At steady state, the flux-weighted mean $\delta^{15}N$ of fixed N outputs (dominated by water column and sedimentary denitrification) must equal that of the inputs (dominated by marine N_2 fixation). The $\delta^{15}N$ of nitrogen supplied by N_2 fixation is tied to the $\delta^{15}N$ of the atmosphere, which does not vary on a million year time scale. The $\delta^{15}N$ of nitrogen removed by water column and sedimentary denitrification, on the other hand, is tied to the mean $\delta^{15}N$ of oceanic nitrate, subject to the effective isotope discriminations. That the mean ocean $\delta^{15}N_{nitrate}$ is higher than the $\delta^{15}N$ of atmospheric N_2 is fundamentally due to isotope discrimination during denitrification.

At steady state, the mean ocean $\delta^{15}N_{nitrate}$ approaches the value at which total denitrification (occurring in both the water column and sediment) removes nitrate with the same $\delta^{15}N$ as N added to the ocean by N_2 fixation, i.e. ~ -1‰. Given this condition, the ratio of rates of sedimentary versus water column denitrification provides the overarching control on the mean $\delta^{15}N$ of nitrate (Brandes and Devol, 2002). Roughly speaking, since water column denitrification discriminates against ^{15}N-nitrate while sedimentary denitrification does not, a higher ratio of water column to sedimentary denitrification will tend to yield a higher mean nitrate $\delta^{15}N$ (Fig. 34.5). For example, if water column denitrification were homogeneously distributed throughout the oceans, an expressed isotope effect of 25‰ for water column denitrification would require that sedimentary denitrification be responsible for roughly 80% of the modern loss of fixed nitrogen from the ocean in order to maintain a mean ocean nitrate $\delta^{15}N$ of 5‰ (Brandes and Devol, 2002). The fact that water column denitrification takes place in <0.2% of the ocean's volume, with relatively high degrees of nitrate loss (typically exceeding 40%), means that water column denitrification causes less ^{15}N enrichment than would arise if water column denitrification were homogeneously distributed. The first effort to account for this "dilution effect" (Deutsch *et al.*, 2004) indicates that the preindustrial balance would require $\sim 30\%$ of denitrification to take place in the water column (i.e., 50% more than if the ocean were approximated as homogeneous). But such quantitative estimates remain preliminary; there is more to be learned about the net isotopic effect of both water column and sedimentary denitrification and their potential to vary over time.

3.2.3. Application to the past

As mentioned above, a given sediment core will simultaneously provide information on at least three separate quantities: changes in mean ocean nitrate $\delta^{15}N$, changes in regional ^{15}N depletion or enrichment relative to the whole ocean, and changes in the completeness of nitrate consumption in the local surface layer. Obviously, the existence of a record of past variations in the mean ocean $\delta^{15}N_{nitrate}$ would allow quantitative assessment of local and regional phenomena in other records; however, the global background signal has yet to be explicitly resolved from among the array of local changes. Sediment cores from the low and mid-latitudes contain the remains of plankton grown on N supplied from the thermocline and not directly from the
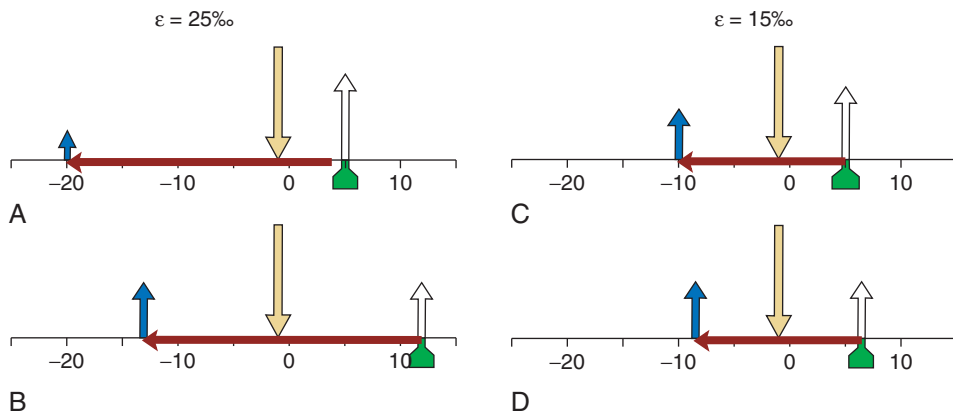
Figure 34.5 Mean ocean steady-state N isotope balance. Vertical arrows represent global input and output fluxes, in which the flux is proportional to the length of the arrow and the isotopic composition is shown by the position on the horizontal axis (showing δ^{15}N in ‰). The yellow downward-pointing arrow represents the N_2 fixation flux, the white arrow represents the sedimentary denitrification flux, and the blue arrow represents the water column denitrification flux. Sedimentary denitrification removes N of δ^{15}N close to that of mean ocean nitrate. Water column denitrification removes nitrogen depleted in ^{15}N by the expressed isotope effect, ϵ (red arrow). The green reservoir below the axis represents the δ^{15}N of mean ocean nitrate, which is determined by the ratio of sedimentary to water column denitrification. At steady state, mass and isotopic balance requires that the sum of the denitrification fluxes equal the N_2 fixation flux and that the denitrification fluxes be balanced about the N_2 fixation "fulcrum." Hence, a balanced isotope budget with an expressed isotope effect of 25‰ for water column denitrification requires a much larger flux of sedimentary denitrification in order to maintain a mean ocean δ^{15}N of 5‰, close to the fulcrum (the δ^{15}N of N_2 fixation, -1‰), as shown by (A). Keeping the total N_2 fixation flux equal and increasing the relative proportion of water column denitrification to equal that of sedimentary denitrification pushes the mean ocean reservoir to a higher value (B). If the expressed isotope effect of water column denitrification is less than 25‰, the mean ocean nitrate would be less sensitive to changes in the relative proportion of water column denitrification, but deviations from 5‰ would still be possible (C, D).

deep sea; given the regional heterogeneity of thermocline δ^{15}N$_{nitrate}$ (which exhibits a ≥ 15‰ range in the modern ocean), it is difficult to extract the global mean background variability from a foreground of changes in surface and thermocline nitrate δ^{15}N. Although polar ocean regions sample deep nitrate more directly, the possibility that the degree of nitrate consumption has varied means that records from these regions cannot be interpreted solely in terms of the deep nitrate supply. Before exploring this further, however, we must discuss the development of sedimentary records of the N isotopes.

3.3. Sedimentary nitrogen isotope records

The most common paleoceanographic proxy used to reconstruct past changes in the oceanic N cycle is the δ^{15}N of total combustible, or "bulk," sedimentary nitrogen (hereafter δ^{15}N$_{bulk}$). Such measurements provide a record, with a variable degree of accuracy, of the N sinking out of the surface ocean at times past (Altabet and Francois,

1994). Below, we review the robustness of the connection between the organic N sinking out of the surface ocean and that preserved in paleoceanographic records as bulk sedimentary N. We also briefly discuss the ongoing development of alternatives to the bulk measurement.

3.3.1. The δ^{15}N of sinking nitrogen relative to local nitrate

In low latitude regions where nitrate is nearly completely consumed, sedimentary δ^{15}N records are typically thought to reconstruct the δ^{15}N of the sub-euphotic zone nitrate that is supplied to the euphotic zone by upwelling and/or mixing. But, in some cases, the δ^{15}N of the sinking flux may not match the δ^{15}N of the nitrate supplied to the euphotic zone. For instance, in some regions, diazotrophic biomass may account for a significant fraction of the sinking N, such that the δ^{15}N of N sinking out of the surface ocean may be significantly lower than the subsurface δ^{15}N$_{nitrate}$ (Brandes *et al.*, 1998; Horikawa *et al.*, 2006; Karl *et al.*, 1997; Pantoja *et al.*, 2002). As discussed in Section 3.1.3, in nutrient-rich regions such as the Antarctic and Subarctic Pacific, the gross nitrate supply to the surface is only partially consumed before being mixed or subducted back into the ocean interior or transported laterally out of the system. In this case, the δ^{15}N of sinking organic matter will be lower than the δ^{15}N of the nitrate initially supplied to that region of the surface ocean (Altabet and Francois, 1994, 2001; Sigman *et al.*, 1999b; Wu *et al.*, 1999). It has been suggested that the progressive consumption of nitrate can also be gleaned from sedimentary transects underlying coastal upwelling cells (Holmes *et al.*, 1996), though delicate gradients such as these would be prone to obliteration by lateral sediment transport on the slope (Mollenhauer *et al.*, 2003) and should be interpreted with caution.

Beyond these upper ocean processes, poorly understood phenomena alter the δ^{15}N of sinking nitrogen during transit through the water column and burial. In a variety of oceanographic settings, sinking particulate δ^{15}N has been observed to decrease with depth in the water column (by up to 2‰ over several kilometers of depth) (Altabet and Francois, 1994; Lourey *et al.*, 2003; Nakanishi and Minagawa, 2003). Explanations for this range from the addition of low-δ^{15}N bacterial biomass, to the contamination of shallower traps by zooplankton ''swimmers''. More recently, other deviations from expectations have been observed. The sinking nitrogen δ^{15}N in the Southern Ocean is highest during the wintertime low flux period and then decreases into the higher flux, spring bloom period as [NO$_3^-$] declines (Altabet and Francois, 2001; Lourey *et al.*, 2003). This sense of change is the opposite of what is expected from simple isotopic fractionation during algal assimilation of surface nitrate. It may reflect domination of the wintertime sinking flux by very slowly sinking material that has been enriched in ^{15}N by progressive degradation, analogous to observations of high δ^{15}N in deep suspended particles (Saino and Hattori, 1987).

3.3.2. The δ^{15}N of sedimentary nitrogen

Despite these observations, excellent correlation is found between the δ^{15}N of bulk surface sedimentary N and that of the local sub-euphotic zone nitrate at sites where export production is high and/or organic matter preservation is good (many of which are from continental margins, Fig. 34.6) (Thunell *et al.*, 2004). In these
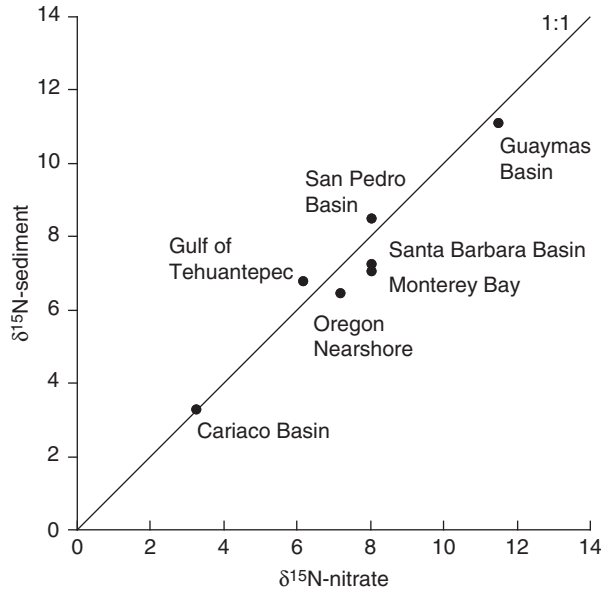
Figure 34.6 Fidelity of sedimentary $\delta^{15}N$ as a recorder of $\delta^{15}N_{nitrate}$ at coastal margins. This plot shows the $\delta^{15}N_{nitrate}$ of water at the base of the euphotic zone versus the $\delta^{15}N$ of surface sediment at or near the same locations. Despite the complexity of nitrogen cycling within the water column, the sediment records the $\delta^{15}N$ of thermocline nitrate with a high degree of accuracy at all of these diverse locations. Data from (Altabet *et al.*, 1999b; Emmer and Thunell, 2000; Kienast *et al.*, 2002; Liu, 1979; Pride *et al.*, 1999; Thunell *et al.*, 2004), figure modified after Thunell *et al.*, (2004).

settings, alteration during sinking and incorporation in the sediments does not appear to confound bulk sedimentary N as a recorder of the $\delta^{15}N$ of the N sinking out of the surface ocean. It also attests to the generally tight connection between the $\delta^{15}N$ of the nitrate supply to the surface and that of the annually integrated sinking flux under conditions of essentially complete nitrate consumption in surface waters (Altabet *et al.*, 1999b).

Measurements of $\delta^{15}N_{bulk}$ from slowly accumulating sediments at the deep sea floor have uncovered spatial patterns that qualitatively mirror expectations for the spatial variation in the $\delta^{15}N$ of sinking N (Altabet and Francois, 1994; Farrell *et al.*, 1995; Holmes *et al.*, 1996). However, in contrast to the ocean margin settings, a significant ^{15}N enrichment (on the order of 1–5‰) is observed in bulk sedimentary N at core tops relative to sinking particles (Altabet and Francois, 1994; Gaye-Haake *et al.*, 2005). This $\delta^{15}N$ increase is apparently due to preferential remineralization of ^{14}N during the oxidation of organic matter in sediments, and the isotopic effect of this process appears to be greatest in organic-poor deep-sea sediment, where accumulation rates are low (Prokopenko *et al.*, 2006). As one might expect, there is some evidence for a continuous range in the $\delta^{15}N$ difference between sinking and sedimentary N, from no difference in the high-preservation margin environments described above to ~5‰ in the most organic-poor, slowly accumulating sediments of the open ocean (Nakanishi and Minagawa, 2003; Pichevin *et al.*, 2007). As a result,

there is concern that pronounced changes over time in sedimentation rate, or in the conditions at the sediment-water interface, could produce a time-varying degree of diagenetic enrichment of sediment δ^{15}N (Sachs and Repeta, 1999).

Sedimentological processes introduce additional uncertainties that deserve mention. Two sedimentary δ^{15}N records from adjacent sites on the northern margin of the South China Sea show quite different features over the past 40 kyr despite being separated by less than 15 km (Higginson *et al.*, 2003; Kienast, 2005). This is apparently due to sediment transport that redistributes the fine fraction of sediment, including the organic matter in which the N resides, over large distances (Freudenthal *et al.*, 2001; Kienast, 2005). Other complicating factors can include the presence of terrestrial organic matter, which may have a distinct N isotopic composition (McKay *et al.*, 2004; Peters *et al.*, 1978), and inorganic N, which can be a significant component in the lattices of some clays, particularly illite (Schubert and Calvert, 2001).

Because of concerns about seafloor processes affecting the δ^{15}N of bulk sedimentary N, the isotopic composition of targeted sedimentary N fractions is being pursued. Most work of this type has focused on the N bound within the silica frustules of diatoms, which should record past surface ocean processes while being protected from the isotopic effects of bacterial degradation during sinking and incorporation in the sedimentary record (Crosta and Shemesh, 2002; Robinson *et al.*, 2004; Shemesh *et al.*, 1993; Sigman *et al.*, 1999a). The δ^{15}N of organic matter in foraminiferal tests has been explored following the same rationale (Altabet and Curry, 1989). Chlorophyll degradation products have also been isolated from sediments for N isotopic analysis (Ohkouchi *et al.*, 2006; Sachs and Repeta, 1999).

The ongoing research into such specific N fractions is critical to address the fundamental issue of isotopic alteration of exported organic N in the water column and sediments. This work also raises a host of new questions. Consider the case of diatom frustule-bound N (Fig. 34.7). The organic N of diatoms exported from the surface layer could differ from the integrated sinking N, for instance, if the isotope effect of nitrate assimilation by diatoms differs from that of the entire phytoplankton population. Moreover, the δ^{15}N of N trapped and preserved within the diatom frustule (here δ^{15}N$_{\text{frustule}}$) appears to be quite different from the bulk biomass of the diatom that produced it. Thus, δ^{15}N$_{\text{frustule}}$ need not be a direct reflection of integrated sinking flux δ^{15}N, and the relationship between the two could change over time (Galbraith, 2006). Nevertheless, the concerns regarding the interpretation of bulk δ^{15}N demand that approaches be developed for the analysis of specific N fractions, at least for open ocean records. The combination of bulk δ^{15}N with specific N fractions from the same sediment samples may yield important complements in the effort to reconstruct changes in the N cycle.

4. Observations and Interpretations

Although questions remain regarding the fidelity of sedimentary N isotopes as recorders of oceanic N dynamics, significant progress has been made by comparing multiple records from different sedimentary regimes and by using additional
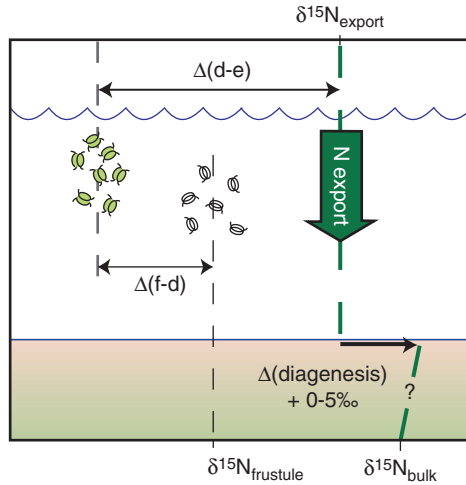
Figure 34.7 The $\delta^{15}N$ of integrated N export (represented by the downward pointing arrow, "$\delta^{15}N_{export}$") versus $\delta^{15}N_{bulk}$ and $\delta^{15}N_{frustule}$, two sedimentary measurements intended to reconstruct the $\delta^{15}N$ of N export. During sinking and burial in slowly accumulating deep-sea sediments, organic N may undergo ^{15}N enrichment or depletion owing to diagenesis. The amplitude of diagenetic alteration is environmentally variable, being virtually absent in organic-rich ocean margin environments but as high as 5‰ in the less productive open ocean. The $\delta^{15}N$ of N bound within diatom frustules ($\delta^{15}N_{frustule}$) does not appear to be affected by diagenesis. However, measurements to date indicate that it is different than "$\delta^{15}N_{export}$". This difference may be due to (1) a $\delta^{15}N$ difference between diatom biomass sinking out of the euphotic zone and the bulk sinking N ("$\Delta(d\text{-}e)$"), and/or (2) a $\delta^{15}N$ difference between the organic N incorporated in diatom frustules and the bulk biomass N of the diatom ("$\Delta(f\text{-}d)$"). The amplitude of either of these $\delta^{15}N$ differences may be sensitive to changes in surface ocean conditions (e.g., because of changes in phytoplankton assemblage or in the growth conditions of diatoms). Thus, given current information, neither $\delta^{15}N_{bulk}$ nor $\delta^{15}N_{frustule}$ can be assumed equal to $\delta^{15}N_{export}$.

proxies to better constrain other environmental parameters. Indeed, many of the records generated to date show coherent variations on different spatial and temporal scales, indicating that they are undoubtedly recording meaningful signals with strong links to global climate. Here we review some of the observations to date and their current interpretations, in relation to the conceptual mechanisms outlined in Section 2.

4.1. Glacial–interglacial inventory changes

The most robust finding to arise from sedimentary N isotope records is of climatically-linked changes in $\delta^{15}N_{nitrate}$ (Fig. 34.8) near each of the major thermocline oxygen minimum zones of the global ocean: the Arabian Sea and the eastern tropical North and South Pacific (Altabet et al., 1995; Ganeshram et al., 1995, 2000; Pride et al., 1999). Although it remains possible that these signals represent changes in the degree to which ^{15}N-enriched nitrate from denitrification zones invaded the
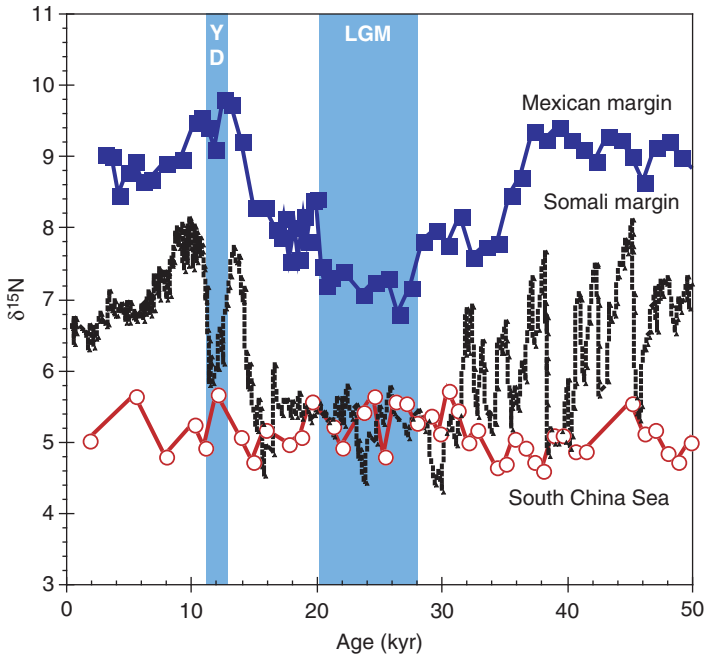
Figure 34.8 Plots of $\delta^{15}N_{bulk}$ versus sediment age over the past 50,000 years for cores from the Mexican margin (Ganeshram et al., 1995), the Somali margin of the Arabian Sea (Ivanochko et al., 2005) and the South China Sea (Kienast, 2000). The Last Glacial Maximum (LGM) and Younger Dryas (YD) cold intervals are indicated by the blue shading. The $\delta^{15}N$ offsets among the records over the Holocene (the last ~10 kyr) are consistent with the effect of modern water column denitrification, which causes high thermocline $\delta^{15}N_{nitrate}$ along the Mexican margin and in the Arabian Sea. During the last glacial maximum, this range collapsed to roughly half that of the present day (~2‰). This appears to be due to a reduction of water column denitrification, reducing the preferential loss of ^{14}N from the oceans. The remarkable stability of the South China Sea $\delta^{15}N$ record in the face of this glacial reduction in water column denitrification supports compensatory global-scale reductions in both sedimentary denitrification and N_2 fixation.

neighboring water masses (Kienast et al., 2002), we follow here the common assumption that the records are dominated by past changes in the extent and/or activity of the thermocline denitrification zones. In addition, both the west African margin and the subarctic Pacific, which have intense thermocline O_2 minima but lack denitrification today, yield similar sediment $\delta^{15}N$ records, suggestive of water column denitrification during past periods (Galbraith et al., 2004; Lavik, 2002; Pichevin et al., 2005). We suggest three factors supporting the assumption that these records document variable denitrification in the thermocline. First, the modern thermocline denitrification zones are all associated with highly productive margin environments. As described in Section 3.3.2, the sediments from these environments have been shown to accurately record the $\delta^{15}N$ of subeuphotic zone nitrate (Altabet et al., 1999b; Thunell et al., 2004), making many of them good monitors of nearby denitrification in the thermocline. Second, the isotope

effect of water column denitrification is large, so that the isotopic signal of denitrification readily overcomes the noise of other processes (see ETNP, Fig. 34.4). Third, a number of other sediment properties (e.g., presence of laminations, redox-sensitive metal contents) corroborate regional variations in water column oxygenation that parallel the inferred denitrification changes.

All of the denitrification records show a general pattern of low $\delta^{15}N$ during glacial periods and high $\delta^{15}N$ during warm interglacial periods (Fig. 34.9). These changes are accompanied by parallel evidence of water column suboxia from trace metal concentrations (Hendy and Pedersen, 2005; Ivanochko and Pedersen, 2004; Kienast et al., 2002; McKay et al., 2005; Nameroff et al., 2004), benthic foraminiferal assemblages (Ohkushi et al., 2003) and sediment laminations (Ganeshram et al., 2000; Suthhof et al., 2001; Thunell and Kepple, 2004; van Geen et al., 2003), confirming that thermocline suboxia was expanded during periods with high $\delta^{15}N$. The inverse correlation between denitrification in the ETNP and global ice volume has been shown to have reigned throughout, at least, the past 1.8 My (Liu et al., 2005). The deglacial increase in thermocline denitrification does not appear to have been contemporaneous everywhere; in particular, records from Peru (Higginson and Altabet, 2004), Chile (De Pol-Holz et al., 2006; Robinson et al., 2007; Martinez et al., 2006) and within the core of the ETNP shadow zone (Hendy and Pedersen, 2006) show $\delta^{15}N$ rises prior to 16 kybp, while most well-dated records from near the ETNP and Arabian Sea increase rapidly after 15 kybp. Nonetheless, on a glacial–interglacial timeframe, there appear to be grossly coherent changes in integrated denitrification rates in the global thermocline, with local variability modulated by regional-scale fluctuations of circulation and productivity.

The globally recognized, millennial-timescale climate oscillations that punctuate the broader glacial cycles (Dansgaard et al., 1993) also exhibit significant changes in denitrification. For example, during the Younger Dryas, a brief return to ice-age-like conditions that interrupted the last deglaciation between 12.9 and 11.5 kybp, there was less denitrification in the ETNP (Hendy et al., 2004; Ivanochko and Pedersen, 2004) and Arabian Sea (Altabet et al., 2002; Higginson et al., 2004; Ivanochko et al., 2005; Suthhof et al., 2001). Increased oxygen concentrations at intermediate depth are corroborated by decreased sediment lamination, lower trace metal concentrations, and decreased age of intermediate waters (Ahagon et al., 2003; Behl and Kennett, 1996; Ivanochko and Pedersen, 2004; Keigwin et al., 1992; Pride et al., 1999; van Geen et al., 2003). Earlier, between 25 and 60 ky ago, the Daansgaard–Oeschger climate oscillations seem to have involved similar changes in denitrification intensity within both the Arabian Sea (Altabet et al., 2002; Ivanochko et al., 2005; Suthhof et al., 2001) and the North Pacific (Emmer and Thunell, 2000; Hendy et al., 2004). Although determining precise chronologies for sediments in this time range is difficult, the oscillations appear to be in phase with climate records from the North Atlantic, with warm conditions in the North Atlantic co-occurring with enhanced denitrification. In contrast, denitrification records in the Southern Hemisphere appear to be roughly antiphased to those of the north, so that denitrification is instead in phase with Antarctic warm events (Martinez et al., 2006; De Pol-Holz et al., 2006; Robinson et al., 2007).
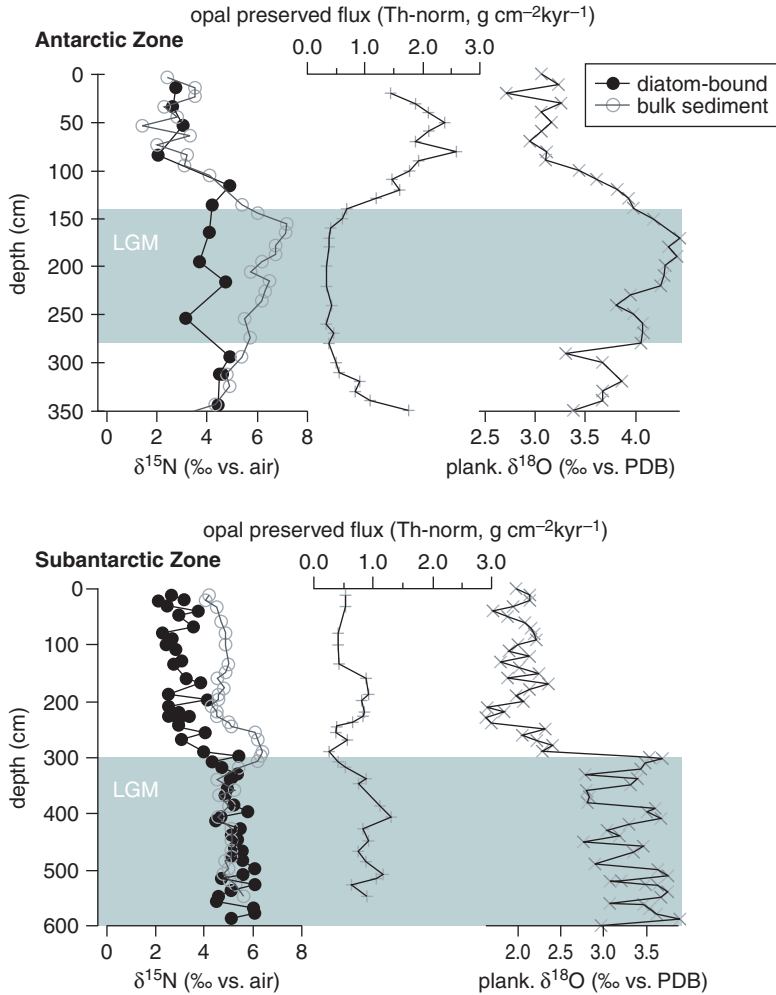
Figure 34.9 Paleoceanographic records from the Antarctic (MD84–552) and Subantarctic (MD84–527) zones of the Southern Ocean. Foraminiferal $\delta^{18}O$ is shown as a proxy for global ice volume, to indicate the Last Glacial Maximum, which is shaded gray. The ^{230}Th-corrected opal flux is shown in the centre of each panel as a proxy for diatom export production, and both $\delta^{15}N_{bulk}$ and $\delta^{15}N_{frustule}$ are shown on the left (open and filled circles, respectively). During the LGM, diatom export was lower in the Antarctic Zone, and virtually unchanged in this (Indian) sector of the Subantarctic Zone. The $\delta^{15}N_{bulk}$ records appear to be affected by diagenetic alteration in this well-oxidized, low–accumulation-rate environment. However, the $\delta^{15}N_{frustule}$ shows that the $\delta^{15}N$ of diatoms was higher in both zones during the LGM. The simplest way to reconcile these data is to invoke, during the LGM, decreased upward macronutrient supply throughout the surface of the Antarctic. After Robinson *et al.*, 2004, 2005.

The dependence of water column suboxia on sinking organic matter makes variable export productivity a clear contender for control of denitrification (Altabet et al., 1995; Ganeshram et al., 1995), and there is evidence for increased export production during warm periods in oxygen-sensitive regions (Altabet et al., 1999a; Crusius et al., 2004; Ganeshram et al., 2000; Hendy et al., 2004; Ortiz et al., 2004). Such changes have been most commonly ascribed to changes in local wind-driven upwelling, although changes in thermocline preformed nutrient concentrations (Robinson et al., 2005) and the global nutrient distribution (Boyle, 1988; Schmittner, 2005, 2007) may also play an important role, which we return to in section 4.3 below. An enhanced supply of oxygen to poorly ventilated subsurface regions, through a combination of lower water temperatures (higher O_2 solubility) and accelerated circulation of thermocline water masses, would also have contributed to reducing glacial denitrification rates (Fig. 34.9, (Galbraith et al., 2004; Kennett and Ingram, 1995; Meissner et al., 2005)). A global coupled ocean–ecosystem model has shown that both reduced oxygen consumption and increased intermediate-depth oxygen supply can arise from a shutdown of NADW, as might be induced by a massive iceberg discharge to the North Atlantic region (Schmittner et al., 2007). This has the potential to explain the Dansgaard–Oeschger and Younger Dryas observations from the Northern Hemisphere, and hints that the antiphased Southern Hemisphere denitrification records might reflect correspondingly pronounced changes in the southern oceanic circulation.

Despite the large reorganization of Earth's climate system and apparent changes in thermocline denitrification, the data in hand do not suggest a clear change in mean ocean $\delta^{15}N_{nitrate}$ between the LGM and present day (Altabet and Curry, 1989; Kienast, 2000). This constraint is based on the observation of little glacial–interglacial change ($<0.5‰$) in many cores, including in the North Atlantic (Huon et al., 2002), Southern Indian Ocean (Francois et al., 1997), Sulu Sea (Horikawa et al., 2006) and South China Sea (Kienast, 2000; Tamburini et al., 2003). This is in stark contrast to marine oxygen isotopes, for example, which are overwhelmed by a global glacial–interglacial signal (Lisiecki and Raymo, 2005). The South China Sea records appear to be compromised to some degree by sedimentary processes (Kienast, 2005), which may mask a glacial–interglacial change to some degree. More importantly, all low-latitude records are highly sensitive to the opposing, dramatic isotopic signals of denitrification and N_2 fixation that permeate the thermocline (Galbraith et al., 2004); lack of variability at any given site could be due to fortuitous location at an isotopic balance point between regions of denitrification and N_2 fixation. Furthermore, as noted in Section 3.3.2, some of these deep-sea records are likely affected by diagenesis, which casts doubt on their fidelity as recorders of the glacial–interglacial change. Nonetheless, lack of an obvious global change between the LGM and Holocene is an important constraint, given that the short residence time of marine N ($\sim$3 kyr, (Codispoti et al., 2001)) requires that steady state was approached during the LGM and has been approached again since the deglaciation. Under the reigning N isotope paradigm, the global $\delta^{15}N_{nitrate}$ during the LGM could only have been similar to that of today if the ratio of water column to sedimentary denitrification during the LGM was also similar to the present day (see Section 3.2.2, Fig. 34.5). Given the strong evidence for lower water column denitrification rates during the

glacial, this implies that the global rate of sedimentary denitrification was significantly decreased as well, in agreement with the expected effect of lower glacial sea level (see Section 2.1.2). As a result, the total glacial denitrification rate must have been substantially lower than today.

Given the constraint that the LGM N budget was near steady state, we can infer that the global rate of N_2 fixation was lower, by an amount roughly equal to the aggregate decrease in denitrification. This corresponding decrease in N_2 fixation does not preclude changes in the N inventory, however; a small sustained surplus in the N budget, possibly abetted by an increased P inventory or trace-metal fertilization of diazotrophs (see Section 2.1.1), could have allowed for a greater N inventory during the glacial period, subject to the strength of the N_2 fixation self-limiting feedback (see Section 2.2.1). All things considered, it would seem likely that the glacial N inventory was somewhat higher, though a quantitative estimate is not currently possible. A tentative upper bound, provided by a numerical analysis of observed deglacial $\delta^{15}N$ patterns, constrains the glacial inventory to no more than 30% larger than the present day inventory, despite the possibility of much larger shifts in fluxes (Deutsch *et al.*, 2004). More precise quantification of the inventory change will require a more global distribution of $\delta^{15}N$ records, better understanding of the denitrification isotope effects, and numerical modeling.

It is possible that local manifestations of the deglacial acceleration of N_2 fixation left traces in sediments, and records from the Cariaco Basin provide a tantalizing candidate for this (Haug *et al.*, 1998). However, N_2 fixation within the Cariaco Basin may respond to local, as well as global changes in denitrification (Haug *et al.*, 1998; Meckler *et al.*, 2007), and no published open ocean records show a sustained Holocene decrease in $\delta^{15}N$ below LGM values. The lack of clear N_2 fixation records may result from a close physical proximity of N_2 fixers to denitrification zones (Brandes *et al.*, 1998; Sigman *et al.*, 2005; Westberry *et al.*, 2005; Deutsch *et al.*, 2007), such that the smaller isotopic signal of N_2 fixation is overwhelmed by the amplitude of the denitrification signal. Otherwise, it may be the lack of good deep-sea records from oligotrophic gyres that is hindering our view of N_2 fixation. The resolution of this issue is bound up in the current debate over where the bulk of modern N_2 fixation occurs (Capone *et al.*, 2005). We note that although clear records of nitrogen fixation from the open ocean are elusive, dramatic reductions of $\delta^{15}N$ within the organic-rich sapropels that punctuate Quaternary sediments of the Mediterranean (Calvert *et al.*, 1992) have been shown to reflect large increases in nitrogen fixation (Struck *et al.*, 2001). Similar $\delta^{15}N$ reductions within ancient organic-rich sediments have been interpreted as reflecting analogous periods of intense N_2 fixation during the Cretaceous (Kuypers *et al.*, 2004; Rau *et al.*, 1987).

Intriguingly, despite the lack of a global LGM-Holocene change, a deglacial maximum in $\delta^{15}N$ is observed in the majority of sediment records, followed by a $\delta^{15}N$ decrease during the Holocene (Bertrand *et al.*, 2000). Given the arguments for large variations in water column denitrification, the most straightforward explanation for the maximum invokes a peak in water-column denitrification rates, followed by a decrease during the Holocene. This sequence could conceivably have been driven by either an environmental change or progressive strengthening of the negative feedback

on water column denitrification through local nitrate limitation (Section 2.2.3; Deutsch *et al.*, 2004; Galbraith *et al.*, 2004). The deglacial δ^{15}N maximum is such a pervasive signal in sediment records, however, that it may reflect a transient maximum in the mean ocean δ^{15}N$_{nitrate}$ itself, caused by a maximum in the global ratio of water column to sedimentary denitrification (Deutsch *et al.*, 2004). Indeed, this could have arisen simply due to a temporal offset between early and rapid increases in water column denitrification (17–14 kybp) vs. the more gradual rise of sea level and flooding of the continental shelves (18–10 kyr). Nevertheless, the largest δ^{15}N maxima occur in sediments close to the denitrification zones, suggesting that the absolute rate of thermocline denitrification also peaked during the deglaciation and has decreased to some degree since then.

The pronounced changes in thermocline oxygenation that have accompanied glacial–interglacial climate changes also imply large changes in the marine flux of N$_2$O to the atmosphere. The production of N$_2$O in the ocean is greatly increased under low oxygen concentrations, and oxygen–poor waters broadly associated with denitrification dominate the marine source (Suntharalingam *et al.*, 2000). Given that the modern marine N$_2$O flux is approximately one third of the global source (Hirsch *et al.*, 2006), any large change in water column denitrification related to a regional change in subsurface oxygenation should have a significant impact on atmospheric N$_2$O concentrations. Rapid changes of atmospheric N$_2$O concentrations recorded in ice cores, including a sharp rise 14.6 ky ago as well as millennial-scale fluctuations during the last ice age (Fluckiger *et al.*, 1999, 2004) may have been largely caused by the same changes of thermocline oxygenation that resulted in variable denitrification rates (Suthhof *et al.*, 2001).

In summary, the globally aggregated denitrification rate was generally lower during glacial periods, oscillated on millennial timescales in concert with climate, and accelerated rapidly during deglaciations. The emergent conclusion is that the marine nitrogen budget is highly dynamic, responding to changes in the physical environment, yet its input and output terms are coupled, leading to a nearly balanced budget on timescales of centuries to millennia. Whether this coupling allows for significant changes in the ocean N reservoir is a matter of debate. As described earlier, the apparent lack of a glacial–interglacial global δ^{15}N change, and the characteristics of certain deglacial signals in the N isotope records, suggest to us that the ice age ocean N reservoir was no more than 30% greater than that of the Holocene (Deutsch *et al.*, 2004).

4.2. Glacial–interglacial changes in nutrient-rich regions

Nutrient-rich regions of the surface ocean have undergone significant changes in export production over glacial–interglacial cycles. In the Antarctic Zone of the Southern Ocean, numerous studies have converged to show that export production was lower during the ice ages than during interglacials (Anderson *et al.*, 1998; Francois *et al.*, 1997; Kohfeld *et al.*, 2005). In contrast, studies of the Subantarctic Zone generally indicate higher export production during ice ages, the inverse of the Antarctic climate cycle in productivity (Chase *et al.*, 2003; Frank *et al.*, 2000; Kumar

et al., 1995; Mortlock *et al.*, 1991). In the Equatorial Pacific, reconstructions of glacial–interglacial export productivity are conflicting, and the picture remains equivocal (Farrell *et al.*, 1995; Loubere, 1999; Loubere *et al.*, 2004; Marcantonio *et al.*, 2001; Paytan and Kastner, 1996; Pedersen, 1983), while in the subarctic Pacific, export productivity seems to have been generally lower during glacial periods (Brunelle *et al.*, 2007; Jaccard *et al.*, 2005; Kienast *et al.*, 2004; Narita *et al.*, 2002), similar to observations from the Antarctic. The observation of variable export productivity in these nutrient–rich regions prompted sedimentary N isotope studies to explore the underlying physical mechanisms, assuming that $\delta^{15}N$ variability there would be dominated by changes in the degree of relative nitrate consumption.

In the glacial Antarctic, evidence for reduced export production is accompanied by higher sedimentary $\delta^{15}N_{bulk}$ (Fig. 34.9) (Francois *et al.*, 1997), implying a greater degree of relative nitrate consumption despite the diminished export production. Although $\delta^{15}N_{frustule}$ records suggest that the glacial/interglacial change in environmental conditions was less homogeneous across the Antarctic zone than indicated by $\delta^{15}N_{bulk}$ (Robinson *et al.*, 2004; Robinson and Sigman, 2008), they remain consistent with equal or greater relative nitrate utilization during the glacial. This requires that the gross rate of nitrate supply to the Antarctic euphotic zone was reduced during glacial times. Physically, this change could be explained by reduced exchange of water between surface and deep waters, as would result from an increase of density gradients or a change in the wind–driven circulation (see Section 2.3). Similarly, increased $\delta^{15}N_{bulk}$ and $\delta^{15}N_{frustule}$ in the subarctic Pacific co–occur with decreased export production during glacial periods, mirroring the Antarctic trend (Brunelle *et al.*, 2007; Galbraith *et al.*, 2008), consistent with theories of glacial water column stratification in the polar oceans (Haug *et al.*, 1999; Jaccard *et al.*, 2005; Sigman *et al.*, 2004). Enhanced aeolian Fe supply during the glacial may have contributed to nitrate drawdown by supplementing the Fe from deep waters (Archer and Johnson, 2000; Lefevre and Watson, 1999; Parekh *et al.*, 2004), but this cannot be resolved from the current data.

Although $\delta^{15}N_{bulk}$ measurements from the Subantarctic show few systematic glacial/interglacial patterns of change (Francois *et al.*, 1997), $\delta^{15}N_{frustule}$ records from the Subantarctic reveal a coherent pattern of elevated $\delta^{15}N$ during glacial times (Fig. 34.9) (Crosta *et al.*, 2005; Robinson *et al.*, 2005). The combination of increased opal fluxes with elevated $\delta^{15}N$ is consistent with higher nutrient consumption as expected under enhanced Fe supply. Given the greater proximity of the Subantarctic Zone to potential dust sources in Patagonia, South Africa, and Australia, and the zonality of the winds, the $\delta^{15}N$ may indicate a greater susceptibility to iron fertilization during ice ages than the higher latitudes of the Southern Ocean (Robinson *et al.*, 2005).

In the equatorial Pacific, $\delta^{15}N_{bulk}$ is low during glacial episodes and elevated during interglacials (Farrell *et al.*, 1995). While this may indicate lower nitrate consumption during the ice age, there are two important caveats to consider. First, the correlation of low $\delta^{15}N_{bulk}$ with periods of rapid sediment accumulation is reminiscent of the Subantarctic records, in which $\delta^{15}N_{frustule}$ data have suggested a diagenetic origin for the $\delta^{15}N_{bulk}$ changes (Robinson *et al.*, 2005). Second, the equatorial Pacific exchanges water with the ETNP shadow zone (Higginson *et al.*,

2003; Yoshikawa *et al.*, 2005), so the apparent glacial decrease in denitrification within the ETNP may have lowered the regional $\delta^{15}N_{nitrate}$ enough to impact the $\delta^{15}N$ of glacial-age sediment in the equatorial Pacific. Thus, the nutrient status of the ice-age equatorial Pacific remains enigmatic.

4.3. Connections

Although we have presented inventory changes and nutrient utilization as separate processes, they are undoubtedly linked. Perhaps most interesting is the potential role of high latitude circulation and nutrient utilization on the nutrient content of the thermocline, a key parameter for setting the productivity of low latitude surface waters (Sarmiento and Orr, 1991; Sarmiento *et al.*, 2004; Schmittner, 2005).

The nutrient cycling scenarios presented above show the potential for Southern Ocean surface waters to become relatively nutrient-poor under alternate climate states. If such nutrient depletion did indeed occur in the glacial ocean, the net result would likely have shifted nutrients from the surface and thermocline to the deep sea (Boyle, 1988; Keir, 1988). Glacial decreases in the formation rate of North Atlantic Deep Water (NADW), intimately linked to North Atlantic surface conditions, would also have decreased the rate at which nutrients were returned from the deep ocean to the surface (Schmittner, 2005). Both of these mechanisms fit with extensive evidence from foraminiferal $\delta^{13}C$ and Cd/Ca for thermocline nutrient depletion during the last ice age, in the Atlantic (Marchitto *et al.*, 1998), the North Indian Ocean (Boyle, 1988, 1992; Kallel *et al.*, 1988), and the Pacific (Herguera *et al.*, 1991; Keigwin, 1998; Matsumoto *et al.*, 2002). The loss of nutrients from the sunlit surface ocean would have driven a decrease in low latitude export production, for which there is considerable evidence (Ganeshram and Pedersen, 1998; Loubere *et al.*, 2004; Marcantonio *et al.*, 2001; Ortiz *et al.*, 2004; Ivanochko *et al.*, 2005). This, in turn, would have reduced oxygen consumption in the thermocline at the same time as the subsurface oxygen supply was increased by physical means (Fig. 34.10). Furthermore, global variations in the nutrient richness of the thermocline would have caused sedimentary denitrification to vary in parallel with water–column denitrification, maintaining a stable global mean $\delta^{15}N_{nitrate}$ as implied by the observations. Although this general mechanism remains speculative, it offers an intriguing paradigm that is consistent with the evidence gathered to date.

Reflexively, it seems likely that changes in the subsurface nitrate $\delta^{15}N$, driven by low latitude processes, will become more apparent in high latitude records. For instance, recent work has shown that sedimentary $\delta^{15}N$ records in the subarctic Pacific represent the sum of broad, regional changes caused by denitrification in the tropical Pacific, and local nutrient utilization within the subarctic itself (Galbraith *et al.*, 2008). The Antarctic, in contrast, is more intimately connected to the deep ocean and thus may be most sensitive to $\delta^{15}N$ changes of deep nitrate. N isotopes aside, past changes in the oceanic N reservoir could have affected relative nitrate consumption in nutrient-rich regions of the ocean, as the gross nitrate supply to the surface would have changed. Given that the low and high latitude ocean regions can affect one another in these and other ways, future research will likely reveal complex relationships between the input/output budget for oceanic fixed N and its internal cycling.
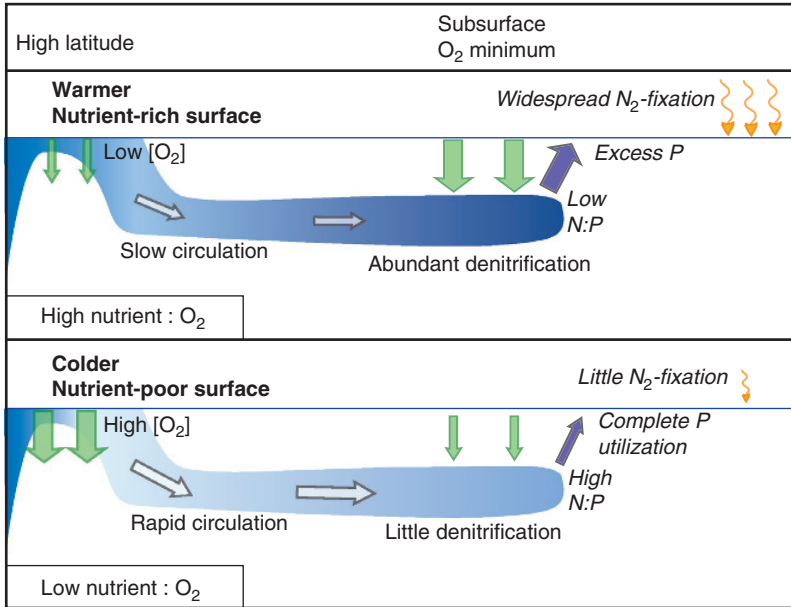
Figure 34.10 Modulation of marine N budget by high latitude climate change. The colored layer indicates the ventilated thermocline, in which darker blue represents greater phosphate concentrations. Downward-pointing green arrows represent export production, which consumes subsurface oxygen, while the upward pointing blue arrows represent the upwelling of nutrients. In the upper panel, low relative nutrient utilization in high latitude source water regions produces a nutrient-rich thermocline, fuelling abundant low-latitude export production. Meanwhile, warm conditions tend to produce low initial O_2 concentrations and the waters may circulate more slowly. The high nutrient:O_2 waters maintain abundant suboxia, with widespread denitrification and, consequently, low N:P in thermocline water (i.e. waters with excess phosphate), which is subsequently upwelled to the surface. Algal growth in this upwelled water eventually yields nitrate-free, phosphate-bearing water, which drives N_2-fixation. In contrast, the lower panel shows high relative nutrient utilization in the source regions, producing a nutrient-poor thermocline that limits export production at low-latitudes. In addition, cool conditions are likely to produce high initial O_2 and more rapid thermocline circulation. As a result, thermocline suboxia and denitrification are reduced. With low nutrient:O_2 conditions inhibiting denitrification, the N:P ratio in upwelling water will be high, encouraging complete P utilization by non-N_2 fixing algae, discouraging N_2 fixation.

5. Perspectives on Progress

5.1. Looking backward

It may seem as though the current view of the past marine N cycle is highly uncertain, but this should be viewed in the context of the last several decades. The first proposition that climate change can influence the marine N cycle was made by McElroy (1983), who pointed out that the short residence time of fixed marine N (then believed to be ∼10 ky) made it a good candidate for change over glacial–interglacial cycles. The marine nitrogen budget estimates of the day appeared to

show a large deficit, so McElroy suggested that the present–day situation was part of a grand ∼100 ky cycle: during ice age inception, advancing glaciers would have bull-dozed terrestrial N into the ocean, while the erosion of continental shelves exposed by sea level drop would have flushed N-rich sediments into the deep sea, to create a glacial ocean rich with nitrogen in which phosphate was the limiting nutrient everywhere. When shelf sediments were worn down and the ice sheets reached their maximum extents, the supply of terrestrial N would have returned to something like its low, modern rate. Denitrification would have eaten away at the accumulated surplus of N, bringing us eventually to our modern-day position of a nitrogen-limited ocean with a large N deficit. This hypothesis, which was novel and fully defensible at the time, was predicated on the view of the marine N cycle as unresponsive, in that the marine fluxes of fixation and denitrification were assumed to be ineffective at compensating for changes in the external supply of N.

Over the past twenty years, modern process studies have taught us that the input and output fluxes of the marine N cycle are dominated by marine microbes, not by terrestrial inputs or sediment burial. Meanwhile, sedimentary N isotope studies have shown that extensive changes in the $\delta^{15}N$ of thermocline nitrate have occurred, with coherent connections to climate dynamics. These observations indicate that the marine nitrogen cycle responds dramatically to changes in the physical environment on timescales shorter than envisioned by McElroy, with large changes in thermocline denitrification occurring within centuries. Moreveover, the realization that the resi-dence time of marine nitrogen is only a few thousand years, coupled with the stability of global mean nitrate $\delta^{15}N$ suggested by sediment records, requires that steady state is roughly achieved on a thousand year time scale and not over tens of thousands of years. These observations are consistent with a reactive, internally stabilized nitrogen cycle, unlike the unresponsive, terrestrially controlled cycle described by McElroy.

Although quantitative interpretation of the paleoceanographic data has just begun, the insights gained thus far suggest a tightly coupled system that reacts dramatically to external forcings but is resistant to extreme inventory variations. This, in turn, requires the existence of effective stabilizing feedbacks within the marine N cycle. Thus, the remaining questions do not concern whether or not these feedbacks exist, but rather how strong they are, how tightly they constrain the system, and by precisely what mechanisms they operate.

5.2. Looking forward

Valuable information will continue to arise from measurements of the isotopic composition of bulk sediments, especially in margin environments. Expanding the network of high-resolution $\delta^{15}N_{bulk}$ records is crucial to map out patterns of changing $\delta^{15}N_{nitrate}$ in space and in time, without which mechanistic hypotheses cannot be tested. However, it is critical that we work to address the ambiguities that afflict $\delta^{15}N_{bulk}$ in open ocean records, with the ideal goal of quantifying diagenetic isotope effects so that they can be removed from the bulk records. One promising approach is the isolation and analysis of organic matter that is of known origin and protected from diagenesis, such as microfossil-bound N. The development of compound-specific techniques, such as for chlorophyll and amino acids, also holds

promise for the future, as does the coupling of compound-specific techniques with the use of microfossil-bound organic matter (Ingalls *et al.*, 2003).

The interpretation of isotopic data will become more quantitative and mechanistic. The use of numerical models with realistic circulation, coupled with the inclusion of other paleoceanographic proxies, will lead to much better-defined pictures of, for example, how water column denitrification zones have interacted with physical circulation and respiration. The inclusion of nitrogen isotope dynamics in ocean general circulation models will allow estimates of past rates of denitrification, N_2 fixation and high latitude nitrate utilization to be drawn from the expanding array of $\delta^{15}N$ records. Also, although we have limited our discussion here to the past 2 My, the number of studies exploring the nitrogen isotopic dynamics of ancient oceans is increasing rapidly, and is sure to reveal exciting insights. Together, these advances will illuminate the connections of N cycle processes to other aspects of the climate system and to each other.

This work will necessarily go hand in hand with studies to link N isotopic patterns in the modern ocean to underlying N transformations. Some important processes such as sedimentary denitrification can only be studied indirectly with available or foreseeable paleoceanographic tools. These processes should be considered critical targets for improved understanding through study of modern environments. Finally, paleoceanographic techniques that, to date, have focused mostly on open-ocean processes, can be brought to bear on the critical environment of the coastal zone. This prospect offers a wealth of unexploited avenues of research with implications for preindustrial human impacts on the environment, fisheries, and coastal zone management.

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Feedbacks Between the Nitrogen, Carbon and Oxygen Cycles

Ilana Berman-Frank, Yi-Bu Chen, Yuan Gao, Katja Fennel,
Michael J. Follows, Allen J. Milligan, *and* Paul G. Falkowski

Contents

1. Evolutionary History of the Nitrogen, Carbon, and Oxygen Cycles

1.1. Nitrogen in the preoxygenated world/oceans

All organisms on Earth are composed primarily of the six light elements: H, C, N, O, S, and P. Hydrogen was created 13.7 Ga (billion years ago) in the ''Big Bang'' while the other five elements were created via fusion reactions in stars. Their relative

abundance scales, to a first order, with the inverse of their atomic mass. Hence, among these five elements in our solar system, H is the most abundant and P is the rarest (Williams and Frausto Da Silva, 1996). When our solar system was formed, *ca.* 4.6 Ga, a complement of elements was imported from supernovae and distributed among the planets and planetary bodies (e.g., moons and asteroids) and our sun. During planetary accretion, formation of dense phases caused siderophile elements (e.g. Fe metal) to move towards the core, while lighter elements tended to migrate toward the mantle or the atmosphere.

In early models of planetary accretion, the process was thought to be relatively slow, such that elements such as N and C had high probabilities of combining with H to form highly reduced molecules such as NH_3 and CH_4 (Urey, 1952). However, based on more detailed observations of how planets are formed, as well as on theoretical calculations about the rate of outgassing of H_2 to space, a much larger fraction of C and N probably existed in higher oxidized states such as CO_2 and N_2 (Holland, 1984), but almost certainly not NO_x. Chemically, N_2 is highly inert. The biologically accessible form of the element requires that it be used in dissimilatory metabolism (such as bacterial conversion from nitrate to nitrite) or react either at a lower reducing level, with H, to form NH_3, or at a higher oxidized level, NO_x, from which it can be biologically reduced to NH_3. Assuming the described initial conditions, the environment of the Archean oceans exerted a strong selection pressure for processes that could reduce N_2 to NH_3, thereby making N_2 a sink for electrons in an electron market where oxidized species are relatively rare. Under such conditions, biological reduction of N_2 could serve as a pathway for the oxidation of organic carbon under anaerobic conditions, or as a detoxyase for detoxification of cyanides, which could have been common in the ancient oceans (Fani *et al.*, 2000; Postgate, 1998). This redox couple, namely the oxidation of organic carbon and the reduction of N_2, is sensitive to oxygen, and hence the biologically-mediated geochemical cycles of the three elements, C, N, and O, are inextricably interconnected (Box 35.1).

In this chapter we examine the connections between these three elements and focus on biological nitrogen-fixation to illustrate the regulatory control of biological processes on the geochemistry of the oceans and Earth over the past 4 billion years.

1.2. Oxygenation of the early atmosphere: The influence of shelf area and nitrogen fixation rates

Geochemical evidence suggests that there were delays of several hundred million years between the rise of oxygenic photosynthesis, the oxygenation of the atmosphere, and the oxygenation of the deep ocean. Photosynthesis (evidenced by cyanobacterial microfossils and biomarkers) rose as early as 3.5 Ga (billion years before present; (Schopf, 1993)) and had been solidly established by 2.7–2.5 Ga (Brocks *et al.*, 1999; Knoll, 1996; Schopf, 1993; Summons *et al.*, 1999). Data from red beds, detrital mineral deposits, and sulfur isotopes indicate the rise of atmospheric oxygen around 2.4 Ga (Bekker *et al.*, 2004; Chandler, 1980; Des Marais *et al.*, 1992;

Box 35.1 Links Between Oceanic Nitrogen and Oxygen Cycles

The oceanic cycles of nitrogen and oxygen are intimately linked through the processes of nitrification and denitrification. These processes constitute the key source and sink of fixed nitrogen in the ocean and control its global budget. The coupling of the nitrogen and oxygen cycles has implications for the long-term chemical evolution of the ocean and the atmosphere, variations in the oceans' nitrogen budget, and the nitrogen limitation of marine ecosystems over centennial to very long timescales.

During photosynthesis nutrients, including nitrogen, are assimilated into organic matter and molecular oxygen is produced, depicted schematically by

$$106CO_2 + 16NO_3 + H_2PO_4 + 122H_2O \leftrightarrow C_{106}H_{263}O_{110}N_{16}P + 138O_2 \qquad (35.1)$$

Here we assume a source of fixed nitrogen in the form of nitrate. Alternatively, diazotrophs can fix their own nitrogen, reducing N_2 gas to the level of ammonium in order to be assimilated for protein synthesis. Ammonium may be oxidized by nitrifying bacteria to produce nitrite and nitrate and consuming molecular oxygen:

$$NH_4 + 2O_2 \leftrightarrow NO_2 + 2H_2O. \qquad (35.2)$$
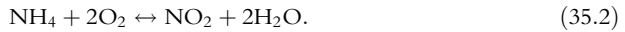
The sequence of nitrogen fixation and nitrification is a source of fixed nitrogen and a sink of dissolved oxygen in the ocean. Aerobic respiration of organic material by heterotrophic bacteria also consumes oxygen and releases nitrogen to the dissolved inorganic pool; the reverse of (35.1).

Under very low oxygen conditions, denitrification, resulting from the anaerobic reduction of nitrate by heterotrophic bacteria, consumes fixed nitrogen in the form of nitrate, ultimately regenerating N_2 gas (Froelich *et al.*, 1979).

$$C_{106}H_{263}O_{110}N_{16}P + 84.8HNO_3 \rightarrow 106CO_2 + 42.4N_2 + 16NH_3 + H_3PO_4 + 148.4H_2O$$
$$(35.3)$$
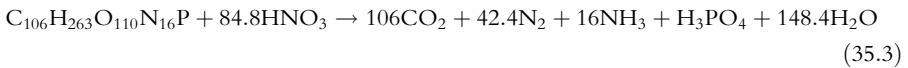
Hence, the sources and sinks of dissolved inorganic nitrogen and dissolved oxygen in the ocean are intimately linked and their global cycles coupled.

Farquhar *et al.*, 2000; Holland and Rye, 1997; Knoll, 1996; Summons *et al.*, 1999). The deep ocean appears to have remained anoxic at least until the disappearance of banded iron formations at 1.8 Ga (Holland, 1984), and possibly as late as 0.8 Ga (Arnold *et al.*, 2004; Canfield, 1998; Canfield and Teske, 1996).

It is likely that oxygen feedbacks on N and C cycles substantially delayed the rise of atmospheric oxygen. This hypothesis is supported by box model calculations, incorporating ocean circulation and ocean–atmosphere gas-exchange with the geochemical cycles of C, N, and O (Fennel *et al.*, 2005; Fig. 35.1). The model has four ocean boxes (high and low latitude surface, deep ocean and shelf seas) coupled to a single atmospheric reservoir and represents the nitrogen and oxygen cycle interactions excluding the sedimentary processes (Fig. 35.1). Application of this model shows that, when initialized with anaerobic conditions, ammonium is originally stable in the reduced
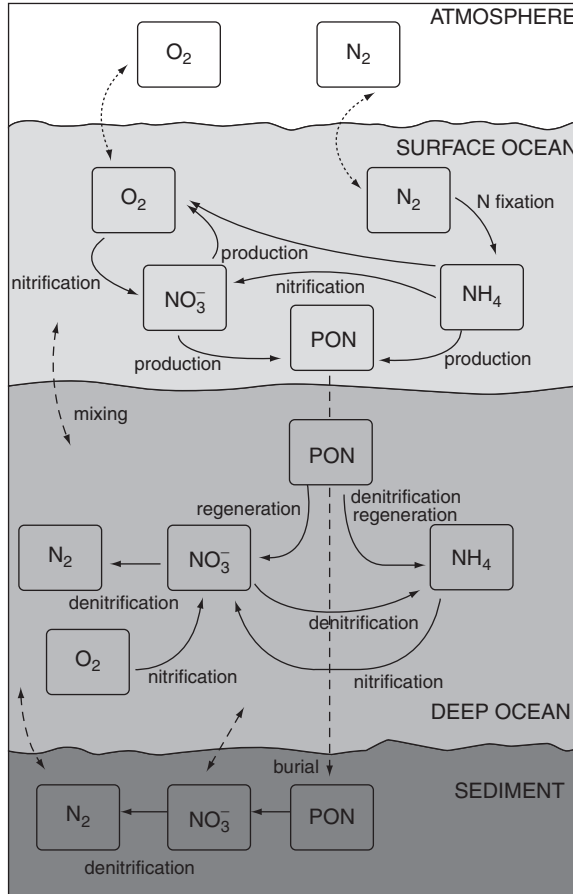
Figure 35.1 Schematic depiction of the coupled, global ocean nitrogen and oxygen cycles. Dissolved inorganic nitrogen and nitrogen fixation fuel oxygenic photosynthesis in the surface ocean, helping to sustain atmospheric and deep ocean oxygen levels. In the deep ocean, oxygen is typically consumed during the microbial respiration of organic matter, producing dissolved inorganic nitrogen. However, in low oxygen waters and sediments, denitrification fuels the oxidation of organic matter, ultimately removing dissolved inorganic nitrogen from the water column. In the early ocean, initial oxygenation of the water column may have inhibited the build up of nitrate which would have been quickly consumed as the oxidant of organic matter.

deep ocean. Yet, as free oxygen becomes available in the ocean, ammonium is converted to nitrate, which can be rapidly reduced to N_2, thus decreasing the pool of fixed inorganic nitrogen. Hence, the interaction between N, C, and O during a transition from an anaerobic to aerobic ocean leads to a strong negative feedback. During this low–nitrogen stage, which can last several 100 million years in our simulations, export production is severely attenuated. Unless some exported carbon is buried, oxygen cannot accumulate in the upper ocean or atmosphere.

The net rate of oxygen production during the low (fixed)-nitrogen stage at the anoxic/oxic transition depends on several factors, including the burial of organic matter and the rate of oxygen consumption during nitrification. In our model studies, the oxygenation of the ocean and atmosphere critically depend on the presence of shallow continental shelf seas (Fennel *et al.*, 2005). The burial of organic matter is much more efficient in shallow continental shelves than in the deep ocean (Hartnett *et al.*, 1998) hence, rates of organic matter burial and net oxygen production should increase as a function of the relative area of continental shelves and shallow seas (Berner, 1984). The relationship between the area of continental shelves, carbon burial, and oxygen production highlights the fundamental role of tectonic processes, especially seafloor spreading, in controlling eustatic sea level and the area of shallow seas (Miller *et al.*, 2005), and in determining the rate of oxygen evolution. The model also demonstrates that an increase in the oceanic phosphate inventory will enhance the net rate of oxygen production owing to higher export. In a fully oxidized ocean, it also potentially leads to an increased inventory of fixed inorganic nitrogen. Furthermore, the rate of net oxygen production is very sensitive to the N_2 fixation rate, which suggests that the initial increase of oxygen had an additional negative feedback on oxygen production by compromising nitrogenase activity both directly and through trace metal limitation (Anbar and Knoll, 2002; Falkowski, 1997) (see below and Fig. 35.2)

An extended period of severe nitrogen limitation and low productivity during the Proterozoic is consistent with the $\delta^{13}C$ record of carbonates, which indicates large variations at the beginning and end of the Proterozoic, but not in the intervening period (Des Marais *et al.*, 1992). Our model studies suggest that during the enhanced tectonic activity at the end of the Proterozoic, changes in the global shelf area or in the oceanic phosphate inventory could have triggered the oxygenation of the deep ocean.

2. NITROGEN-FIXATION, NITROGENASE, AND OXYGEN. EVOLUTIONARY CONSTRAINTS, ADAPTATIONS OF CELLULAR PATHWAYS AND ORGANISMS, FEEDBACKS TO GLOBAL NITROGEN, CARBON, OXYGEN CYCLES

2.1. Evolutionary constraints for nitrogen fixation and adaptive strategies

Over a 200 million year period, centered around 2.3 billion years ago (Bekker *et al.*, 2004), the partial pressure of oxygen (pO_2) rose from $<4 \times 10^{-6}$ atm to >0.03 atm (Pavlov and Kasting, 2002; Rye and Holland, 1998). This emerging environment especially challenged diazotrophic cyanobacteria, the only diazotrophs capable of oxygenic photosynthesis, yet having to contend with the innate sensitivity of the nitrogenase enzyme to molecular oxygen. Diazotrophic cyanobacteria provide an elegant case-study of how the interplay between the N, C and O cycles influenced the adaptation of key metabolic processes, and how these cellular processes feed back
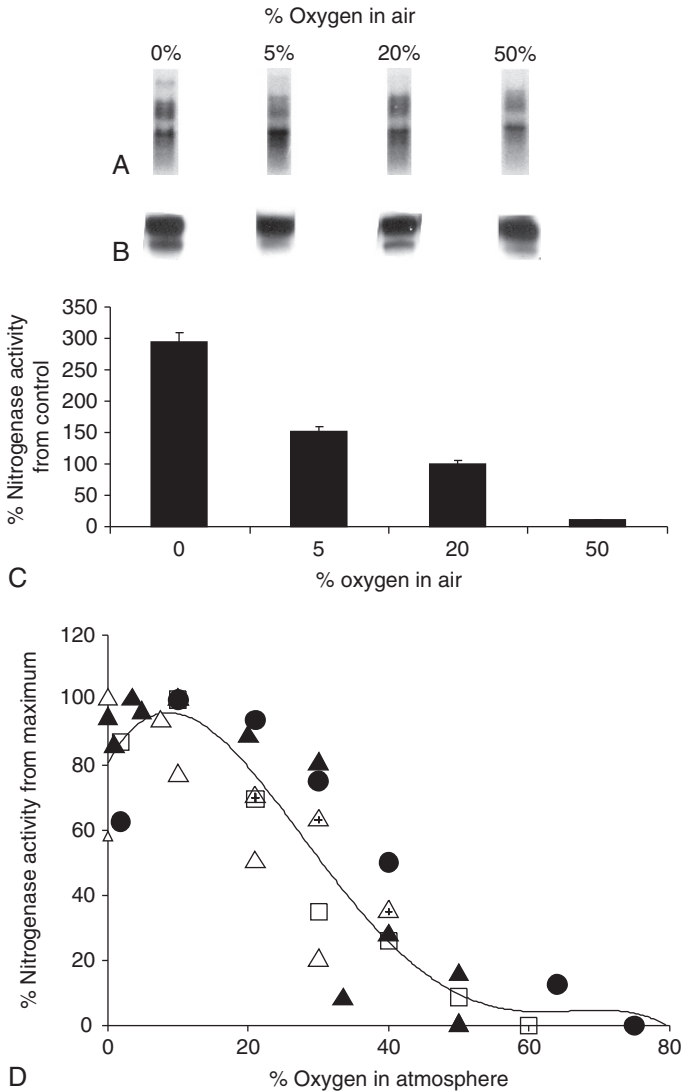
Figure 35.2 (A) Northern blots of *nif* HDK transcript abundance for *Trichodesmium* IMS101 cultures bubbled with nitrogen (0% oxygen), 5%, 20%, and 50% O_2 for 5 h. (B) Western blots of nitrogenase protein abundance (challenged with universal Fe–protein polyclonal antibodies) for the above experiment (C) Nitrogenase activity (presented as % of air-control) measured by acetylene reduction for the above experiment 1 h after induction of bubbling. (D) Relationship between O_2 concentrations and nitrogen fixation (% from maximum rates) for *Trichodesmium* IMS101 and other diazotrophs. Filled triangles: experimental data from our cultures of *Trichodesmium* IMS101; empty triangles with cross—*Trichodesmium* NIBB1067 (Ohki and Fujita, 1988), empty triangles—*Trichodesmium* spp. field populations (Mague *et al.*, 1977; Saino and Hattori, 1982), black circles—*Anabaena cylindrical* (Gallon *et al.*, 1993), black squares—*Gloeothece* (Nageli) (Gallon *et al.*, 1993). Short-term (1–2 h) anaerobic incubation yields maximum nitrogenase activity for aerobic diazotrophs (C). On longer time scales, respiratory requirements will yield maximum activity (*in vivo*) at microaerobic oxygen concentrations (D).

into the aquatic and global systems. In this section we focus on the evolutionary constraints on nitrogen-fixation in cyanobacteria. We then discuss how these biological controls feed back into the geochemical cycles.

The evolutionary roots of nitrogen fixation, inferred from molecular phylogenetic analyses of *nifH* genes, suggest that the genes encoding for nitrogenases are ancient and underwent horizontal gene transfer, gene duplication, recruitment and fusion (Raymond *et al.*, 2004). Indeed, phylogenic analyses do not rule out the possibility that nitrogenase was present in the last universal common ancestor (LUCA) and, therefore predates the divergence of the archaea and bacteria (Fani *et al.*, 2000; Raymond *et al.*, 2004; Woese, 1998), or if it evolved in methanogenic archaea and was subsequently transferred to a primitive bacterium (Raymond *et al.*, 2004). In either scenario, the catalytic subunits of the enzyme complex clearly indicate that the original enzyme existed long before the oxygenation of Earth's atmosphere (Broda and Pesheck, 1983) and that trace elements such as Fe, Mo, and V additionally modulated its evolutionary trajectory.

The influence of required trace elements on the evolution of nitrogenase has been discussed by several authors (Anbar and Knoll, 2002; Kustka *et al.*, 2003; Raven, 1988; Raymond *et al.*, 2004). The availability of iron influences N_2 fixation in diazotrophic cyanobacteria, from its direct effect on Fe-rich protein synthesis such as nitrogenase, to effects on photosynthesis, growth, and global productivity (Berman-Frank *et al.*, 2001, 2007; Falkowski, 1997; Kustka *et al.*, 2003; Mills *et al.*, 2004; Paerl *et al.*, 1994; Reuter *et al.*, 1988). In the anaerobic environments of the Archean oceans, Fe would have been found predominantly in its reduced form (FeII) rather than FeIII (Anbar and Knoll, 2002 and Table 1 in Berman-Frank *et al.*, 2003). The necessity for nitrogen selected for nitrogenase, while the availability of reduced iron selected for the metal complement of nitrogenase. Both laboratory studies (Berman-Frank *et al.*, 2001; Berman-Frank *et al.*, 2007, Shi *et al.*, 2007; Kupper *et al.*, 2008) and field simulations (Mills *et al.*, 2004) show a direct link between the availability of nitrogen fixation rates in environmentally important diazotrophs (see Section 3.1). Thus, diazotrophy and subsequent primary production were predicted and found to be limited in the iron deficient regions of the subtropical and tropical oceans, such as the contemporary South Pacific (Moutin *et al.*, 2008; Raimbault and Garcia, 2008).

Molybdenum (Mo) is another essential metal co-factor of nitrogenase. Mo, a rare trace element is much more abundant in the contemporary ocean than under the anaerobic conditions prevailing when nitrogenase first evolved, and is also required in the bacterial reduction of nitrate to nitrite (Williams and Frausto Da Silva, 2002). In the anoxic, asulfidic Archean oceans, up to 90% of the Mo would have been complexed in relatively insoluble sulfide minerals in igneous rocks (Anbar and Knoll, 2002; Falkowski, 2001; Williams and Frausto Da Silva, 1996). Limited availability of Mo may have been exacerbated in the mid-Proterozoic, where weathering, under a moderately oxidizing atmosphere, would have enhanced the delivery of sulfate (SO_4^{2-}) and molybdate to the deep ocean. Combined with primary production in the surface waters, this would have resulted in extremely high H_2S concentrations and removal of Mo via increased precipitation and formation of active thiomolybdate (MoS_4^{2-}) (Anbar and Knoll, 2002; Arnold *et al.*, 2004).

Whereas alternative, older nitrogenases exist, in which Fe or V were replaced by Mo, these forms are catalytically less efficient. Interestingly, Mo-independent nitrogenases have been found only in heterocystous diazotrophs (phylogenetically the most recently diverging group—see discussion below) but not in non-heterocystous species (Bergman et al., 1997).

Past depletions in dissolved inorganic phosphate may have also limited nitrogen-fixation and primary production due to high adsorption of phosphate by iron-rich minerals. Indeed, based on adsorption isotherms in banded iron formations, sea-water phosphate concentrations in the Archean have been suggested to have been more than ten fold lower than the present values (0.15–0.6 µM versus the modern value of 2.3 µM) (Bjerrum and Canfield, 2002). Thus, present restrictions of phosphate on N_2 fixers in some oligotrophic areas such as the western subtropical Atlantic (Mills et al., 2004) and in the tropical Pacific (Moutin et al., 2007) may have correlations in the paleoceanographic records of the late Archean eon.

Nitrogen fixation is metabolically costly, requiring relatively large inputs of energy, reducing power, ancillary antioxidant enzymes and metal co-factors such as iron and Mo (Raven, 1988; Kustka et al., 2003). A priori, the nitrogenase enzyme is notorious for its sensitivity to molecular oxygen and for the irreversible damage to the the the Fe_4S_4 clusters, in-vitro (Burgess and Lowe, 1996). Moreover, high oxygen stress causes proteolysis of nitrogenase subunits (Durner et al., 1996), suppresses nitrogenase synthesis, and leads to a shortage of respiratory substrates and reductants necessary for nitrogen fixation and assimilation (Gallon, 1992). Inhibitory effects of moderate oxygen levels, or short exposure times, in-vivo may be reversed, leading to an increase in nitrogen fixation rates (Gallon, 1992; Ludden and Roberts, 1995; Pan and Vessey, 2001; Prosperi, 1994; Yakunin et al., 2001) and, in some diazotrophs, to post-translational modification of the Fe protein from an inactive to active form (Ernst et al., 1990; Jouanneau et al., 1983; Ohki et al., 1991; Sweet and Burris, 1982; Zehr et al., 1993).

The fundamental constraint of the two conflicting processes has led to several adaptations including spatial (e.g. heterocystous cyanobacteria) and temporal separation of photosynthesis and N_2 fixation (e.g. unicellular species such as Cyanothece), or a combination of both (e.g. in the non-heterocystous colonial forms such as Trichodesmium). The oxygen protection mechanisms have been the focus of several reviews examining molecular, phylogenetic, physiological, morphological, regulatory, and evolutionary adaptations in cyanobacteria (Adams, 2000; Bergman et al., 1997; Berman-Frank et al., 2003; Bohme, 1998; Fay, 1992; Gallon, 1992, 2001; Tuli et al., 1996). The various strategies to overcome the inherent conflict between oxygenic photosynthesis and nitrogen fixation reflect the wide-ranging flexibility and niches occupied by cyanobacteria: from anaerobic sediments to pelagic waters saturated with oxygen. Molecular phylogenies, of N_2 fixers using the nifH gene and small subunit ribosomal RNA sequences, showed no correlation between the phylogenetic relationships and the type of N_2 fixation (Raymond et al., 2004; Turner et al., 2001). This lack of correlation suggests multiple gains and/or losses of N_2 fixation among the different cyanobacteria (Raymond et al., 2004; Turner et al., 2001). The loss of cyanobacterial nif genes implies that different strategies arose early in the evolution of the clade, where some organisms

were able to adapt to an oxic world, while others were not (e.g. *Oscillatoria*). Commonly, many phylogenies show the most recent phylogenetic branching (both for *nifH* and RNA trees) in filamentous species where complete segregation of N_2 fixation and photosynthesis was achieved with the evolution of heterocystous cyanobacteria (Wolk *et al.*, 1994).

Heterocystous cyanobacteria are predominantly terrestrial, fresh water and coastal species, inhabiting eutrophic or brackish environments (e.g. the Baltic Sea), with a few epiphytic and symbiotic representatives in the marine environment (Paerl and Zehr, 2000) (see Chapter 4, Carpenter and Capone, this volume). Very few heterocystous free-living species are found in the pelagic oceans, though a novel species was found, designated *Anabaena gerdii* (Carpenter and Janson, 2001). The heterocyst glycolipid envelope may confer an advantage for cyanobacteria inhabiting temperate brackish and fresh waters. These waters are characterized by an increase in O_2 flux by $\sim$25% from that in sea water, and thus the thick glycolipid layer can help decrease diffusion of oxygen into the heterocysts (Staal *et al.*, 2003a). At higher temperatures, typical of tropical waters, the glycolipid envelope of the heterocyst does not provide additional protection against oxygen, which may explain the paucity of heterocystous free-living bacteria in such environments (Staal *et al.*, 2003a).

In addition to specialized niche selection and avoidance strategies, whereby nitrogen fixation is spatially or temporally separated from photosynthesis, oxygen concentrations influence transcription, translation, and changes in nitrogenase activity of cyanobacteria. We demonstrate this with results from the bloom-forming cyanobacterium *Trichodesmium* IMS101, which contributes significantly to nitrogen fixation in the tropical and sub-tropical oceans. Sequence and structural analyses of its nitrogenase are similar to those of other diazotrophic organisms (Zehr *et al.*, 1997), and there is a post-translational modification between the active and non-active form of the protein under natural and induced conditions (Ben-Porath *et al.*, 1993; Ohki *et al.*, 1991). The abundance of *nifH* transcripts and its corresponding gene product were assayed in *Trichodesmium* cultures incubated for 5 h with 0, 5, 21 (present atmospheric level—PAL), and 50% O_2 (Fig. 35.2A). The results indicate that extracellular O_2 does not significantly influence transcription or translation of *nifH* on this time scale (Fig. 35.2A and B). In contrast, nitrogenase activity was strongly depressed by O_2; at 50% O_2 >90% of the nitrogenase activity was inhibited within 1 h (Fig. 35.2C).

Post-translational modification of activity appears relatively common in diazotrophic cyanobacteria and operates at much lower concentrations of oxygen then those required for repression of *nif* genes (Dominic *et al.*, 1998; Tuli *et al.*, 1996). A compilation of published data on the response of nitrogen fixation to varying ambient O_2 concentrations suggests a general relationship that is consistent with both the short-term response of the enzyme to O_2 and the organisms' longer evolutionary adaptations where O_2 is used as sink for electrons in respiration (Fig. 35.2D). On average, 20–30% of the enzymatic activity is inhibited at present atmospheric oxygen concentrations of 21% (Fig. 35.2D).

In *Trichodesmium* spp., maximum nitrogen fixation occurs at oxygen concentrations of 2.5–5% PAL. Under truly anaerobic conditions, the Embden–Myerhof

(glycolysis) pathway apparently cannot supply sufficient substrates through fermentative reactions to meet the demands of nitrogen fixation. Hence, *in vivo,* the activity of the enzyme declines. As oxygen concentrations increase above 5%, the enzyme activity is inhibited, with only ~70–80% activity measured at atmospheric oxygen concentrations of 21%. At oxygen concentrations greater than 30%, a sharp drop in activity reduces fixation efficiency (potential) to <10% within minutes. This pattern of an inefficient nitrogenase is corroborated in data from other aerobic cyanobacteria such as in the heterocystous *Anabaena* and in the unicellular *Gloeothece* (Fig. 35.2D). In these cyanobacteria, lowering the oxygen below atmospheric concentrations also enhances relative nitrogenase activity at short durations. However, at very low oxygen concentrations, lack of respiratory production of energy and substrates ultimately affects nitrogen fixation. Thus, in *Trichodesmium* for example, low oxygen stress caused faster mortality and biomass crashes after ~48 h (Berman-Frank, unpublished).

2.2. Adaptive strategies. Oxygen consumption—The Mehler reaction

In cyanobacteria, oxygen is potentially consumed through aerobic respiration and two light-dependent reactions, the oxygenase activity of RubisCO (photorespiration) and the photosynthetic reduction of O_2, termed pseudocyclic photophosphorylation or the Mehler reaction (Box 35.2). In contrast to terrestrial C3 plants, which have relatively high rates of photorespiration, photorespiration of oceanic phytoplankton is usually low when dissolved inorganic carbon concentrations in seawater are at equilibrium (~2 mM) with the atmosphere. Moreover, cyanobacteria operate a CO_2 concentrating mechanism (CCM) which raises the CO_2 concentration in the vicinity of RubisCO and inhibits oxygenase activity (Kaplan and Reinhold, 1999).

The Mehler reaction is a photochemical reduction of O_2 to H_2O_2 or H_2O in photosystem I (Box 35.2). Mehler activity is thought to be a mechanism for energy dissipation under high light intensities or when carbon fixation is limited by supply

Box 35.2 The Mehler reaction (pseudocyclic photophosphorylation)

In 1951, the late Alan Mehler (1951) observed that chloroplasts can use oxygen as an electron acceptor. The reaction sequence is

$$H_2O + 2O_2 \rightarrow 2O_2 + 2H^+ + \tfrac{1}{2}O_2,$$

$$2O_2 + 2 H^+ \rightarrow H_2O_2 + O_2$$
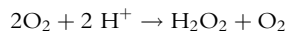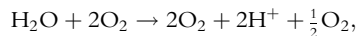
and, in the presence of catalase:

$$H_2O_2 \rightarrow H_2O + \tfrac{1}{2}O_2.$$
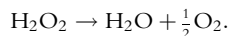
Net gas exchange is absent since the overall electron transport reaction which involves both Photosystems II and I is:

$$H_2O + 2O_2 \rightarrow H_2O + 2O_2$$
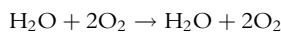
of inorganic carbon (Helman *et al.*, 2003). Since the products of O_2 reduction are often superoxide and hydrogen peroxide (with superoxide dismutase catalyzing the reduction of superoxide to peroxide), Mehler activity has been hypothesized to be a metabolic defect rather than an adaptive strategy (Patterson and Myers, 1973). However, in the cyanobacterium *Synechocystis* sp. (PCC 6803), superoxide is reduced directly to water without a hydrogen peroxide intermediate (Helman *et al.*, 2003). This single step reduction of superoxide to water is catalyzed by A-type flavoproteins; two of which (flv1, flv3) were identified as essential for this activity (Helman *et al.*, 2003). Examination of the genome of *Trichodesmium*, identifies homologous genes to flv1 and flv3 with 62% and 67% sequence identity, respectively (Milligan *et al.*, 2007).

Mehler activity is generally considered a process which can only consume photosynthetically derived O_2, and it cannot cause net consumption of O_2 because PSI activity relies on photosynthetically derived electrons (Kana, 1993). Yet, the shared-arrangement of photosynthetic and respiratory electron transport chains in cyanobacteria allows electrons from respiratory derived NAD(P)H to feed into the plastoquinone pool of the photosynthetic electron transport chain and reduce PSI (Schmetterer, 1994). Through the translocation of reductant (i.e. glucose 6-phosphate) from cells with functional PSII, Mehler activity can result in a net consumption of O_2 in cells (or heterocysts) which have no PSII activity and in which nitrogen is fixed (Fig. 35.3).

Results from *Trichodesmium* provide an example of the Mehler pathway's role in modulating oxygen and facilitating nitrogen-fixation. In this species, under nitrogen-fixation conditions, approximately 50% of gross O_2 production is consumed through Mehler activity (Fig. 35.4); this is about twice the rate reported for *Synechococcus* ($\sim$25% of gross O_2 production) exposed to photoinhibitory irradiances (Kana, 1992). Mehler activity is dependent both on the time of day and the nitrogen source (Fig. 35.4). The period of maximum N_2 fixation is coincident with a decline in the net production of O_2 and a rise in the consumption of oxygen via Mehler activity, which is consistent with the hypothesized role of this pathway as a mechanism to protect nitrogenase from O_2 damage. In nitrate-grown *Trichodesmium*
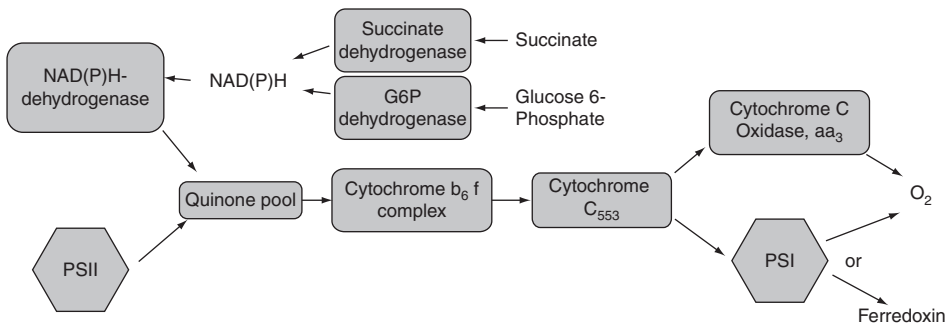
Figure 35.3 Photosynthetic and respiratory electron transport chains in cyanobacteria showing the shared electron carriers of each pathway (based on Schmetterer, 1994).
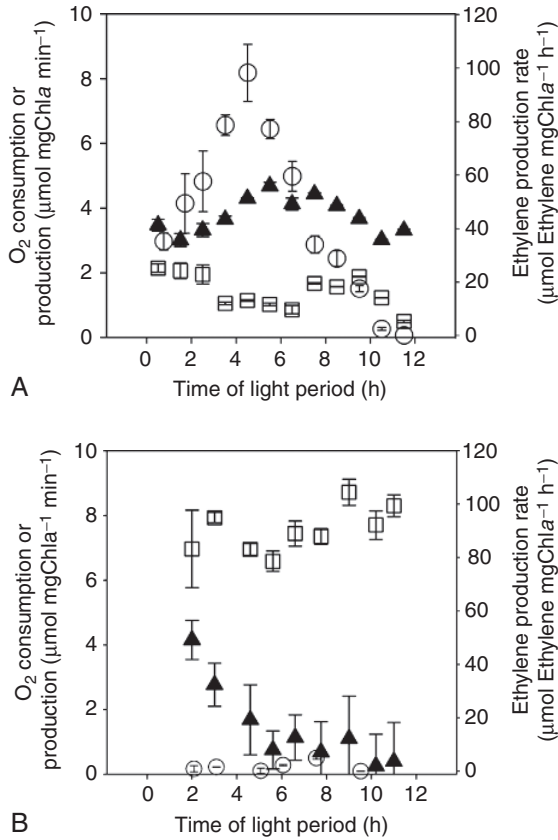
Figure 35.4 Time course of oxygen production (□), consumption (▲) and acetylene reduction (○) during the photoperiod in *Trichodesmium* sp. IMS 101 grown with N_2 (A) and nitrate (B) as nitrogen sources. Error bars are ±1 standard deviation.

cultures (with negligible nitrogen-fixation), Mehler activity increases with light induction, but quickly drops to low and constant rates (10% of gross production) through the rest of the photoperiod (Fig. 35.4).

In *Trichodesmium*, Mehler activity is essential, as *Trichodesmium* relies on short term regulation of PSII and nitrogenase activities to separate these functions within a trichome (Berman-Frank *et al.*, 2003). PSII activity is regulated on time scales of 10–15 min and appears to involve the association/disassociation of the light harvesting complex (LHC II) from PSII (Küpper *et al.*, 2004). Nitrogenase activity is also regulated on similar time scales when incubated at different oxygen concentrations, with transcriptional and translational regulation requiring longer time-scales and higher concentrations of exposure (Fig. 35.2). While PSII activity is repressed in N_2 fixing cells, the activity of PSI is responsible for net O_2 consumption relying on the translocation of reductant for the donor side of PSI and the flux of photons driving the oxidation. Cultures of *Trichodesmium* grown under low (5%) oxygen showed some nitrogenase activity during the night; this

activity was absent in controls (21%) and in high (50%) oxygen cultures (Küpper *et al.*, 2004). The lack of Mehler activity at night in the controls (21%) and in the high oxygen cultures may thus reduce the total possible oxygen consumption and prevent nitrogenase activity. At lower O_2 concentrations, N_2 fixation can proceed in darkness because the respiratory rates are sufficient to consume O_2.

2.3. Biological feedbacks to the global nitrogen, carbon, and oxygen cycles

Oxygen-consuming pathways, including the Mehler reaction, deliver a positive feed-back to nitrogenase and nitrogen fixation by reducing the concentrations of molecular and reactive oxygen that inhibit the enzyme. Yet the presence of oxygen is essential as respiratory pathways provide the required substrates for subsequent nitrogen assimilation. Thus, oxygen concentrations will exert both a positive and negative feedback towards adaptations at the organism-scale, which reflect a compromise of opposing metabolic requirements. At the molecular/biochemical scale, oxygen concentrations impose an upper limit on the nitrogenase enzyme for all types of cyanobacterial diazotrophs. Whether the regulation is at the level of transcription, translation, or metabolic activity, depends on the concentration and duration of inhibition by oxygen. Chronic inhibition of nitrogenase at PAL of O_2 imposes a metabolic inefficiency that reflects an extraordinarily slow tempo of evolution for this critical biogeochemical process. The high conservation within the primary sequence of *nif* genes suggests that the evolutionary risks associated with modifying nitrogenase outweigh the costs of its production. Nitrogenase is analogous to other core metabolic proteins, such as the reaction center protein of photosystem II, D1 and ribulose 1,5 bisphosphate carboxylase/oxygenase (Rubisco), which also operate at a fraction of their capacities under ambient atmospheric conditions or undergo extremely high rates of turnover as a result of post-translational damage. The inhibition of all of these proteins, either directly by elevated O_2, or indirectly through reactive oxygen species, potentially exerts a strong biological control on the upper bound of the concentration of the gas in Earth's atmosphere, leading to metabolic and biogeochemical inefficiency in N_2 fixation. The inefficiency imposes a major elemental taxation on diazotrophic cyanobacteria, both in the costs of protein synthesis and in the scarce trace elements, such as iron, which has, in turn, led to a global limitation of fixed nitrogen in the contemporary oceans (Falkowski, 1997).

We extended and applied the systems-analysis based approach of Beerling and Berner (Beerling and Berner, 2005) to the feedbacks between (aquatic) nitrogen fixation and the cycles of carbon and oxygen (Fig. 35.5). While the role of sustained forest fires in maintaining PAL of oxygen (Lenton and Watson, 2000; Watson *et al.*, 1978) is currently debated (Wildman *et al.*, 2004) (Fig. 35.5), nitrogen fixation (via the evolutionary sensitivity of nitrogenase) acts as an additional control, limiting the upper concentration of oxygen in the atmosphere. The reduction of nitrogen-fixation due to high oxygen concentrations could lead to a decline in both diazotrophic and total aquatic primary productivity (with parallel declines in the terrestrial biomass due to higher photorespiration under high oxygen), and to reduced rates of organic burial (Fig. 35.5 path 1–2–3). Lower burial rates would lead to a decrease in oxygen and an
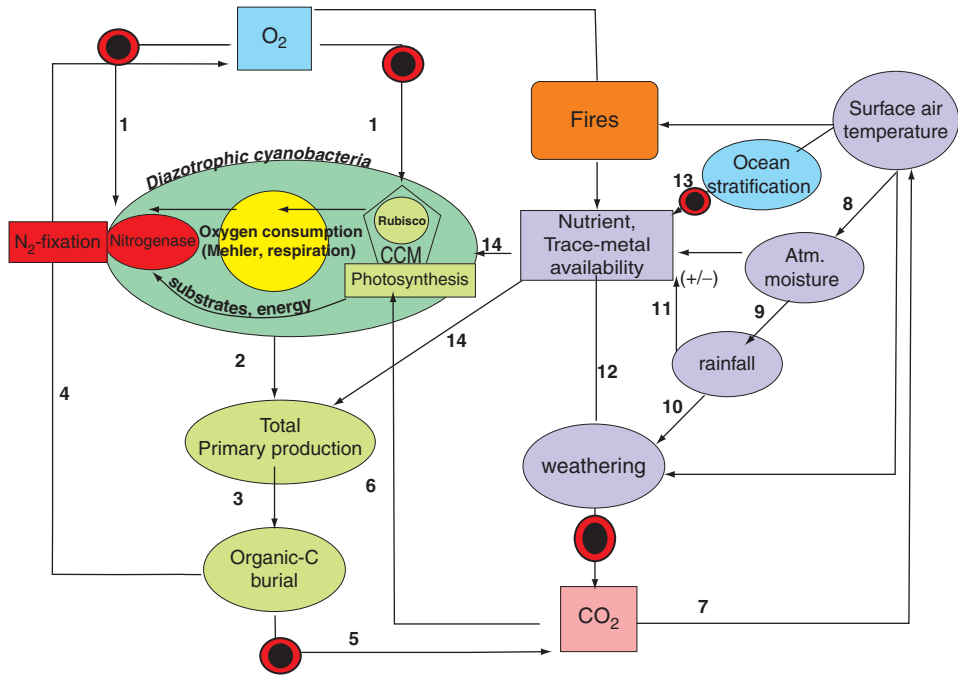
Figure 35.5 Simplified systems analysis diagram of the feedbacks between nitrogen fixation (mainly by aquatic cyanobacterial diazotrophs) and the oxygen and carbon cycles. Plain arrows show direct responses while arrows with bull's-eyes illustrate inverse effects. The $+/-$ indicates feedbacks could go in either direction depending on conditions. The feedback loop is designated by numbers adjacent to path. Note that the processes and feedbacks operate at different time-scales (from hours (e.g. N–fixation and photosynthesis) – to millenia (e.g. global O_2, CO_2 concentrations). Diagram modified from Beerling and Berner (2005).

increase in atmospheric CO_2 (Fig. 35.5 paths 1-2-3-4, 1-2-3-5). If oxygen concentrations are maintained at PAL or slightly lower (see Fig. 35.2D), nitrogen fixation may be sufficient to drive high total primary production and higher burial rates, and a negative feedback to both C and O concentrations (Fig. 35.5).

3. SENSITIVITY OF THE NITROGEN, CARBON, AND OXYGEN CYCLES TO CLIMATE CHANGE

Biological feedbacks into the global nitrogen and oxygen cycles are intricately dependant on climate patterns and oceanic circulation which also influence trace metal availability in the upper ocean (Fig. 35.5). In this section we explore future scenarios in the oceanic nitrogen, carbon and oxygen cycles due to shifts in aeolian iron availability, variations in climate, and ocean circulation.

3.1. Aeolian iron and stratification of the surface mixed layer in the future

The major source of Fe in the surface waters of several large ocean regions is the aeolian dust transported from the continents (Duce and Tindale, 1991; Fung *et al.*, 2000; Jickells *et al.*, 2005). Yet the evidence for the relative importance of aeolian sources versus *in situ* iron supply by upwelling waters from depths is unclear (Coale *et al.*, 1996). The distribution of aeolian Fe varies strongly with the seasons and from one ocean region to another. The predominant fraction of the Fe inputs enters the oceans in the Northern Hemisphere, where the high Fe fluxes are primarily concentrated in low and mid-latitudes (Gao *et al.*, 2001). These are characterized by disproportionate seasonal flux rates, with summer Fe fluxes approximately twice those of winter fluxes (Gao *et al.*, 2001). This non-uniform distribution feature is largely reflected by the distribution of dust sources (Ginoux *et al.*, 2001; Zender *et al.*, 2003). The current estimate of the total Fe deposition to the ocean is $\sim 14 \times 10^{12}$ g year^{-1}, which includes substantial uncertainties. The major processes that control the delivery of aeolian Fe to the ocean are dry deposition by gravitational settling of particles, turbulence in the surface layer of the atmosphere, and wet deposition through precipitation scavenging. Any variation in the strength and distribution of dust sources, and removal processes caused by climate change and land use, could affect dust emissions (Tegen *et al.*, 2004) and consequent delivery of aeolian iron to the ocean (Fig. 35.5 path 8-13).

One example is the increasing concentrations of atmospheric CO_2 and enhanced global temperatures which have altered the global hydrological cycle. This is reflected by changes in precipitation patterns that may have profound impacts on dust emissions and subsequent deposition to the oceans. Over the past century there has been a global increase in rainfall over land (Dai *et al.*, 1997). Enhanced precipitation over desert regions, in particular, has been considered as a negative feedback to desertification (Miller *et al.*, 2004). The increased rainfall could directly impact the emissions of dust through altering soil moisture (aridity) in the source regions and dust burden in the atmosphere and, consequently, affect the concentrations of dust over the oceans. Observations made at Barbados in the tropical North Atlantic indicate that variations in dust concentrations from 1965 to 1998 were inversely correlated with rainfall in the Soudano-Sahel region in Africa (Prospero and Lamb, 2003). This finding suggests that atmospheric dust loading over the ocean is sensitive to the strength of precipitation in the dust source regions; therefore, any increases in precipitation and rising concentrations of atmospheric CO_2 may contribute to decreased aridity and a possibly less dusty atmosphere in the future (Mahowald and Luo, 2003) (Fig. 35.5 path 7-8-9-11 inverse effect).

Increased precipitation over the oceans may increase the supply of a more "bioavailable," dissolved aeolian Fe via wet deposition (Fig. 35.5 path 8-9-11 direct effect). During long range transport, dust particles undergo heterogeneous reactions at gas-solid-liquid interfaces involving pollution-derived substances that may lead to the increased solubility of aeolian Fe (Dentener *et al.*, 1996; Meshidzke *et al.*, 2003; Underwood *et al.*, 2001). Photochemical reduction in more acidic cloud waters and precipitation promote dissolution of Fe in dust, leading to the production

of soluble Fe (II) (Siefert et al., 1999; Zhu et al., 1997; Zhuang et al., 1992). These natural processes potentially increase the amount of dissolved Fe in precipitation beyond the Fe solubility of a few percent directly measured from leaching of dry dust particles. Gao et al. (2003) suggest that the annual input of dissolved Fe by wet deposition accounts for 4–30% of the total aeolian Fe fluxes to the ocean. Hand et al, (Hand et al., 2004) reported that soluble iron associated with aerosols over the Atlantic and Pacific ranged from 0–45% in the $PM_{2.5}$ mode (particulate mass less than 2.5 µm diameter) and 0–87% in the coarse mode. These findings reveal that soluble iron could be a significant fraction of the total aeolian Fe entering the surface seawaters. Thus, increasing the supply of soluble aeolian Fe in the oceans through wet deposition may cause natural iron fertilization and induction of blooms by diazotrophic and other Fe-limited photosynthetic populations (Fig. 35.5 path 8-14). As such, variations in precipitation distributions, or changes in hydrological cycles caused by global warming, could alter carbon cycles in the ocean and contribute to the future climate change. The non-linear response of critical environmental factors over the land and oceans has restricted the characterization and predictive power of how precipitation and land–use patterns influence dust generation and subsequent aeolian Fe deposition to the oceans.

Changes in ocean stratification may further affect the degree of aeolian Fe demand by marine biota and the subsequent CO_2 fluxes (Le Quere et al., 2003). Intensified stratification in the upper ocean due to global warming could become more resistant to mixing by surface wind and prevent the flux of nutrients from deep waters to the surface layer (Sarmiento et al., 1998) (Fig. 35.5 path 13-14). This weakened mixing could also reduce the amount of recycled Fe brought from the deep ocean to the surface layer and, consequently, the supply of aeolian Fe to production in the surface ocean could be more critical in the future. This situation may occur both in the high-nutrient low-chlorophyll (HNLC) waters and also in the oligotrophic gyres, where there may be co-limitation of production by iron and phosphorous (Mills et al., 2004). In the eastern subtropical North Atlantic, where the impact of Saharan dust input is strong, utilization of nitrate supplied from dust deposition could be enhanced in the strongly stratified mixed layer during the late summer-fall period, thereby promoting phytoplankton growth (Neuer et al., 2004). Although primary productivity in the Sargasso Sea is often suggested to be phosphorus-limited, as the concentrations of dissolved inorganic phosphate in the surface waters are low (Wu et al., 2000), results from nutrient-addition bioassays in the tropical North Atlantic indicate that nitrogen fixation in this region is co-limited by iron and phosphorus (Mills et al., 2004). This finding suggests that even in a region where dust deposition is high and the amount of aeolian Fe should be sufficient, the rates of nitrogen fixation by high-Fe demanding organisms such as Trichodesmium spp. are still controlled by the supply of iron.

The combined effects of warming air temperatures and of enhanced stratification in the upper ocean may thus promote the dissolution of iron in dust and its subsequent bio-availability in the surface seawaters, possibly enhancing nitrogen fixation and export production. Future aeolian Fe input to the ocean may increase due to desertification enhanced by human activities or decrease by altered hydrological cycles (Fig. 35.5). These interactions and feedbacks could certainly play important roles in future nutrient and carbon cycles in the ocean.

3.2. Interactions of the oxygen and nitrogen cycles: Nitrification and denitrification

Changes in the ocean nitrogen budget linked to climate change have been discussed by several authors (Altabet *et al.*, 1995; Broecker and Henderson, 1998; Falkowski, 1997; Ganeshram *et al.*, 1995; Galbraith *et al.*, Chapter 34 this volume). The coupling of the nitrogen and oxygen cycles via nitrification and denitrification has implications for the long-term chemical evolution of the ocean and atmosphere, the variations in the oceans' nitrogen inventory, and the nitrogen limitation of marine ecosystems over centennial and longer timescales. Yet, the interactions and feedbacks of the coupled nitrogen and oxygen cycles are not clearly understood. Consider a reduction of nitrogen fixation leading to a global decline in the fixed nitrogen budget of the ocean, increasing nitrogen limitation and reducing total export production. In response, deep ocean respiration decreases and the low oxygen zones shrink along with the integrated rate of denitrification. While there is the possibility for a stabilizing feedback (Codispoti, 1989; Deutsch *et al.*, 2007), the processes of nitrogen fixation and denitrification are spatially (and temporally) disconnected; the former occurs predominantly in the surface, subtropical oceans while the latter occurs in the low oxygen zones of the water column and sediments (Fig. 35.1). Thus the rate of accumulation or the loss of nitrogen in the ocean must also be regulated by the processes of physical transport by which these regions are connected. In the modern ocean, the transport timescale between the subtropical Atlantic, a region of significant nitrogen fixation, and the low oxygen waters of the mid-depth Pacific basin, is on the order of hundreds of years leading to the potential for delayed responses in the feedback loop (e.g. Gruber, 2004; see Chapter 1, Gruber, this volume) and even to oscillatory behavior. A highly idealized box model of the coupled nitrogen and oxygen cycles (Fennel *et al.*, 2005) suggests complex interactions (see following section) and illustrates how these interactions were likely critical in the development of global biogeochemical cycles on the early Earth. Factoring in possible links to the carbon pumps, climate and the global iron cycle suggests even richer, and more complex, possibilities for interactions of climate and biogeochemical cycles (e.g. (Falkowski, 1997).

3.3. Ocean circulation and the global ocean cycles of nitrogen, oxygen and carbon

Ocean models forced by warmer atmospheric climates and coupled climate models in global warming scenarios suggest that weakened atmospheric temperature gradients and increased hydrological cycle might weaken the ventilation of the deep ocean (e.g. Zhang *et al.*, 2001) and expand the regions of well-stratified, subtropical surface waters (e.g. (Sarmiento *et al.*, 2004). Consider the oceanic nitrogen cycle as represented simply in Fig. 35.6. Nitrogen is supplied to the surface ocean by ocean circulation, from depth, or by fixation in the surface. Depending upon the nature of the ecosystem and its efficiency at recycling, some fraction, N_{bio}, of the deep ocean nitrogen, N_d, is brought to depth through biological export of organic matter and remineralization. The remaining fraction, or the pre-formed nitrogen N_{pre}, is brought to depth by physical advection or mixing from the surface layer. In regions
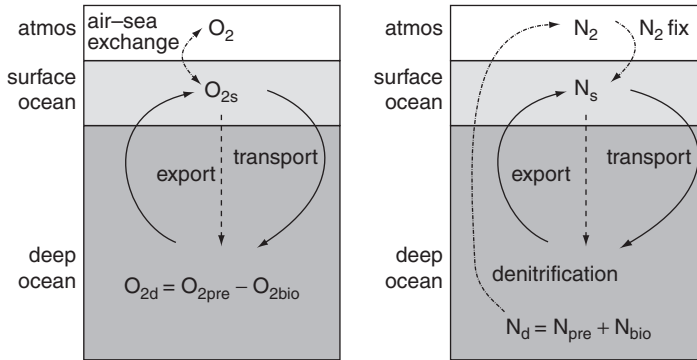
Figure 35.6 Simplified schematic views of the oceanic nitrogen and oxygen cycles. N_d and O_{2d} are the deep ocean dissolved inorganic nitrogen and dissolved oxygen concentrations respectively, N_{pre} and O_{2pre}, are the preformed nitrogen and oxygen concentrations, and N_{bio} and O_{2bio} are the contributions to deep ocean concentrations due to the remineralization of exported organic matter. N_s and O_{2s} are the concentrations of dissolved inorganic nitrogen and dissolved oxygen in the surface mixed-layer of the ocean. Exported organic matter (really export of "oxygen demand"—indicated by the minus sign) is respired in the deep ocean, providing a source of dissolved inorganic nitrogen and a sink of dissolved oxygen.

of low oxygen (including pore waters), denitrification and subsequent processes ultimately lead to a return to dinitrogen gas, and to nitrogen loss from the oceans.

The warming of the surface ocean will weaken the overturning circulation leading to shallower mixed-layer. Slower overturning suggests a longer residence time for waters in the surface, perhaps increasing the role of biological export relative to physical transport, favoring N_{bio} over N_{pre}. In addition, a coincident warming of the surface waters and reduction of turbulent mixing in the surface mixed-layer might favor diazotrophs such as *Trichodesmium* (Capone, 2001; Karl *et al.*, Chapter 16 this volume), enhance nitrogen fixation and work to increase the oceanic nitrogen budget, perhaps relieving nitrogen limitation. Consider, however, the coupled oxygen cycle. Shallower mixing and warming of the surface waters will reduce the physical supply of oxygen to the deep ocean and decrease the solubility of oxygen in the ventilated waters (O_{2pre} decreases) (which may further enhance nitrogenase activity and nitrogen-fixing species but will reduce productivity of other phytoplankton). Consequently, deep ocean oxygen concentrations, O_{2d}, will likely decrease under such conditions, increasing the extent of regions of very low oxygen concentration in the water column (Zhang *et al.*, 2001) and pore waters. In consequence, the global flux of denitrification is also likely to increase in response. The net effect of such a climate change on the oceanic nitrogen budget and productivity is not yet clear.

We examined the steady state response of the fully dynamic ocean nitrogen and oxygen cycles in our box model (see section Ib, Fennel *et al.*, 2005) and an atmospheric mixing ratio of oxygen at 20% to prescribed variations in the ocean overturning, efficiency of nitrogen fixation and mean ocean phosphate loading. Starting from modern ocean conditions under which the oceanic N:P ratio is close to 16, varying the rate of overturning between 10 and 30 Sverdrups does little to the ocean nitrogen

inventory, or N:P, though the timescale for adjustment increases as overturning decreases. Changing the efficiency of nitrogen fixation, perhaps interpreted as reflecting changes in upper ocean stratification, has some interesting and counter-intuitive consequences; since the modern ocean has an N:P ratio close to 16, increasing the efficiency of nitrogen fixation does not significantly enhance the nitrogen budget, as the model assumes diazotrophs are not competitive unless nitrogen is limiting. However, for conditions where oceanic N:P is somewhat lower than 16, *increasing* nitrogen fixation can *decrease* the fixed nitrogen inventory of the deep ocean by enhancing export production, reducing deep ocean oxygen and increasing the rate of denitrification. Increasing the oceanic phosphorus inventory above today's values also reduces N:P through a similar mechanism. These model explorations suggest there are critical non-linear interactions of between the C, N, O and P cycles which remain to be elucidated.

3.4. Implications for the global carbon cycle

What are the implications of such changes in the nitrogen cycle for the global carbon cycling and atmospheric pCO_2? A biologically mediated *net* exchange of CO_2 between the atmosphere and ocean can be brought about by changing the oceanic inventory of one or more limiting nutrients (Falkowski *et al.*, 2003). If fixed inorganic nitrogen is limiting, then how sensitive is the atmospheric exchange of CO_2 with the ocean to a change in the inventory of fixed inorganic nitrogen. To do this, we examined the effect of changing the nitrogen inventory (through biological fixation) in a coupled biogeo-chemical model. Following the treatment of the nitrogen and oxygen concentrations in the deep ocean, we may describe the ocean reservoir of dissolved inorganic carbon (DIC_d) as the sum of preformed (DIC_{pre}) and biological components (DIC_{bio}):

$$DIC_d = DIC_{pre} + DIC_{bio} \qquad (35.4)$$

Here, the preformed pool includes contributions from the saturation concentration and the degree of disequilibrium at the point of subduction. The biological fraction has contributions from both soft-tissue and carbonate pumps (Volk and Hoffert, 1985). Following Brewer (Brewer, 1978) we may, as a first approximation, interpret the soft-tissue contribution to be related to the biological fraction of nitrogen by a fixed, Redfieldian stoichiometry:

$$DIC_{bio} = R_{CN} N_{bio}.$$

If the ocean-atmosphere loading of carbon is fixed then, given these simplistic assumptions, enhancing the oceanic nitrogen budget and export production would lead to an enhancement of the oceans biological carbon pool at the expense of the atmosphere, reducing atmospheric pCO_2 in proportion to the change in DIC according to the buffer factor, B:

$$dpCO_2/pCO_2 \sim B\, dDIC/DIC \qquad (35.5)$$

(e.g. Bolin and Erickson, 1959) where $B \sim O(10)$.

Accordingly, let us consider some climate change process which leads to an increase in fixed inorganic N by 1 μmol L^{-1} in the ocean interior, N$_d$. Studies of the contemporary ocean suggest that the biological contribution N$_{bio} \sim 0.3$ N$_d$. Assuming this fraction stays constant and $R_{CN} \sim 6$, this implies a corresponding 2 M increase in DIC$_d$. The buffering relationship (2.5) suggests a modest associated decrease in atmospheric pCO$_2$ (relative to 280 ppmv) of about 3 ppmv in the steady state (see Ito and Follows, 2004) for a more detailed analysis). If all of the extra nitrogen is in the biological fraction, the sensitivity of atmospheric CO$_2$ to the mean ocean nitrogen concentration could be as much as 10 ppmv per micromole, relative to the pre-industrial state. It should be noted that this argument assumes no impact on the preformed component, DIC$_{pre}$. It also assumes a fixed ocean–atmosphere carbon budget. However, modifications of the deep ocean carbonate system will ultimately lead to interactions with the sedimentary calcium carbonate reservoir, dampening the impact on atmospheric pCO2 (e.g. Bolin and Eriksson, 1959).

4. SUMMARY AND CONCLUSIONS

The history of Earth can be broadly divided into two "super eons." The first 2.2 billion years were marked by biological innovation and experimentation, during which time all the major metabolic pathways evolved in prokaryotes. One of these pathways, oxygenic photosynthesis, would subsequently, over the second half of Earth's history, give rise to aerobic metabolism, and ultimately permit the evolution of multicellular eukaryotes. The shift from mildly reducing to strongly oxidizing conditions was, beyond doubt, the most profound transition since the origin of life itself. Our analysis suggests that feedbacks between C, N, and O cycles helped prevent the oxidation of Earth in the Paleoproterozoic. This stabilizing feedback, which was ultimately over-ridden, led to the contemporary nitrogen cycle where nitrate, rather than ammonium, was the stable form of fixed inorganic nitrogen in the oceans. Barring some minor changes in the trace element composition in nitrogenases, the core proteins remained essentially unchanged following the transition to an oxidized atmosphere. In the contemporary ocean, approximately 20—30% of nitrogenase activity is inhibited at any moment in time by O$_2$. This inhibition results in a negative feedback which constrains the upper level of O$_2$ on Earth (Figs. 35.2 and 35.5).

Three central aspects of cyanobacterial nitrogen fixation remain curious. First, although some trace elements have been altered in the evolution of nitrogenases, the core proteins have remained virtually unchanged. In effect, nitrogenase, like RuBisCO, is a "frozen metabolic accident" (Shi *et al.*, 2005). Either there is no substitute for the basic iron–sulfur cluster motif, or protein–protein interactions have severely constrained the evolutionary trajectory of this enzyme complex, or both. Whatever the reason, all nitrogenases are irreversibly inhibited by oxygen—and cyanobacteria are the only oxygenic photoautotrophs that contain the gene. Second, while there is abundant evidence of lateral transfer of nitrogenase genes between prokaryotes, in the endosymbiotic appropriation of cyanobacteria into heterotrophic hosts to photosynthetic eukaryotes, nitrogenases were lost. Indeed, there is no known eukaryote that contains an endogenous functional nitrogenase. It is not apparent whether this deficiency is

genetically insurmountable, but it is clear that (under present conditions) it made most aquatic eukaryotes dependent on cyanobacteria for fixed inorganic nitrogen. In oligo-trophic subtropical and tropical oceans large chain–forming diatoms are found in association with the endosymbiotic heterocystous cyanobacterium, *Richelia intracellularis* that contribute significant amounts of fixed N to some systems (Capone *et al.*, 1997; Carpenter *et al.*, 1999; Foster and O'Mullan, Chapter 27, this volume; Janson *et al.*, 1999; Venrick, 1974)(see Carpenter and Capone, Chapter 4, this volume). Yet, given the above dependency, the low diversity and limited biomass of endosymbiotic nitrogen fixing cyanobacteria harbored in eukaryotic host cells seems curious. Third, although free-living heterocystous cyanobacteria are abundant in lakes and brackish water eco-systems, they appear to be rare in the open ocean (Carpenter and Janson, 2001). Heterocysts are advantageous at lower temperature and salinities compared to *Tricho-desmium* types, which dominate in the tropical oceans (Staal *et al.*, 2003b). Yet, it is not clear why this, or some other biological innovation which facilitates nitrogen fixation under relatively high oxygen concentrations, is rare in temperate and polar oceanic ecosystems.

Clearly these issues will be the focus of research efforts over the next few decades because they lie at the heart of a fundamentally applied problem: why have we not been able to genetically engineer plants that feed and serve humans with the capability of fixing their own nitrogen? Should we overcome that major hurdle, we will have made a major transition in the impact of humans on the chemistry of Earth and its oceans.

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Nitrogen Fluxes from Rivers to the Coastal Oceans

Elizabeth W. Boyer *and* Robert W. Howarth

Contents

1. Introduction

Human activities, largely associated with food production and fossil-fuel combustion, have greatly modified biogeochemical cycles of nitrogen in terrestrial and aquatic ecosystems throughout much of the world (Galloway *et al.*, 2004; Smil, 2001; Vitousek *et al.*, 1997). Fifty years ago, the vast majority of reactive nitrogen on the land surfaces of the Earth was created naturally through biological nitrogen fixation, but the rate of formation of reactive nitrogen stemming from human activities now roughly matches this (Table 36.1, Galloway *et al.*, 2004). A significant quantity of this nitrogen is transported in rivers from the continental landscape to the coastal ocean zones (Boyer *et al.*, 2006). The major change in the global nitrogen cycle has been the creation of synthetic nitrogen fertilizers to fuel the green revolution, although the release of reactive nitrogen during fossil-fuel combustion has also been a factor (Fig. 36.1).

Notwithstanding unequivocal benefits associated with the acceleration of the global N cycle in terms of satisfying societal demands for food and fuel, the negative environmental consequences to estuaries and coastal seas caused by over-enrichment of nitrogen are significant (Howarth and Marino, 2006; Rabalais, 2002). For example, two-thirds of the coastal rivers and bays in the United States are moderately to severely

Table 36.1 The rate of formation of reactive nitrogen on the land surfaces of Earth in 1950 and in 2000 from natural and human-controlled processes (Tg N year^{-1})

	1950	2000
Natural processes		
Biological nitrogen fixation	100–200	100–200
Lightning	5	5
All natural processes	105–205	105–205
Human-controlled processes		
Synthetic fertilizer production	5	110
Nitrogen release during fossil-fuel combustion	5	25
Biological nitrogen fixation in agroecosystems	24	33
All human-controlled processes	34	168
Total rate of creation of reactive nitrogen	139–239	273–373

Rates of nitrogen fixation in natural ecosystems are from Cleveland *et al.* (1999). Data on notrogen creation from human-controlled processes are from Galloway et al. (2004), assuming no major loss of natural ecosystem fixation since 1950.
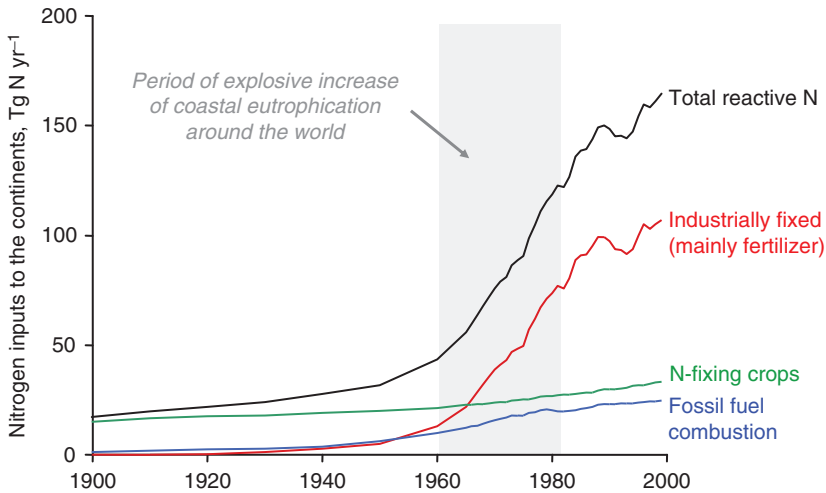
Figure 36.1 Human activities have greatly altered the global nitrogen cycle, increasing reactive nitrogen inputs to the terrestrial landscape, shown below. Major sources of reactive nitrogen to the environment include industrial processes that produce nitrogen fertilizers, cultivation of leguminous crops that host nitrogen-fixing bacteria, and combustion of fossil fuels. Coastal eutrophication problems worldwide have been largely attributed to this increase of reactive nitrogen in the environment (as indicated by the gray bar). Figure modified from Boesch (2002), Galloway *et al.* (2004).

degraded from nutrient pollution, and nutrients are now considered the largest pollution problem in the coastal zone of the country (NRC, 2000). Environmental problems in the coastal oceans associated with nitrogen pollution are global in scope (Diaz, 2001; Rabalais, 2002) and are likely to increase as population increases and as use of

inorganic fertilizers and fossil fuels continues to intensify (Galloway *et al.*, 2004; Glibert *et al.*, 2006). As we present below, the geographic variation in the problem is large.

We estimate current inputs from rivers to the coastal zone as approximately 48 Tg N year^{-1} (Boyer *et al.*, 2006; Galloway *et al.*, 2004). In comparison, net inputs from atmospheric deposition in excess of volatile losses from the oceans are estimated as approximately 30 Tg N year^{-1} and biological nitrogen fixation in the oceans is estimated as approximately 120 Tg N year^{-1} (Galloway *et al.*, 2004). Although there is considerable uncertainty in all of these terms, riverine inputs of nitrogen to the coastal zone as of the 1990s represented roughly one quarter of all the nitrogen inputs to landscape. Much of the nitrogen delivered from rivers to coastal waters is subsequently consumed by denitrification on the continental shelves. Shelf sediments are a very large sink for nitrogen, thus only a small fraction of nitrogen inputs reach the open ocean (Nixon *et al.*, 1996; Seitzinger and Kroeze, 1998; Seitzinger *et al.*, 2006). However, nitrogen input to offshore oceanic waters may be significant from very large rivers that can transport water and nitrogen across the continental shelves, or from rivers that discharge into coastal waters where the continental shelves are narrow. The major influence of riverine nitrogen on ecological functioning in the oceans is in estuaries and waters on the continental shelves themselves. In many estuaries, inputs from rivers are by far the largest source of nitrogen (Nixon *et al.*, 1996; Seitzinger *et al.*, 2006). Nonetheless, the importance of nitrogen from rivers in the nitrogen budgets of continental shelf regions varies greatly, with much of this variation related to differences in the extent of advection of nitrate-rich bottom waters onto the continental shelves (Table 36.2; Howarth, 1998).

In this chapter, we review information from previous studies regarding nitrogen inputs to landscapes and the associated delivery of nitrogen to coastal waters around the world. We focus on fluxes of total nitrogen rather than dissolved inorganic nitrogen (DIN, typically including nitrate, nitrite, and ammonium), since most of the organic nitrogen added to the oceans in rivers is likely converted to DIN on ecologically meaningful time scales; that is, terrestrially derived organic nitrogen in marine sediments is only a very small proportion of the inputs (probably $< 6\%$, assuming that all nitrogen in deltaic sediments represents terrestrially derived organic nitrogen; Galloway *et al.*, 2004). Several models have been developed to estimate nitrate fluxes to the global ocean (see for example Dumont *et al.*, 2005; Seitzinger and Kroeze, 1998, Seitzinger *et al.*, 2002; Smith *et al.*, 2003); these generally yield estimates of nitrate fluxes that are one-third to one-half of global estimates for total nitrogen fluxes in rivers. However, in considering the role of human activities, it is important to note that the nitrate fluxes represent a much higher proportion of the anthropogenically-increased nitrogen fluxes (Boyer *et al.*, 2002; Galloway *et al.*, 2004; Howarth *et al.*, 1996). Though ammonium fluxes are generally much lower than nitrate fluxes in streams and rivers, they also may represent a substantial proportion of the increased fluxes resulting from anthropogenic activities, such as in some urban areas with inputs of wastewater (Paul and Meyer, 2001; Shuiwang *et al.*, 2000).

There are many complimentary approaches presented in the recent literature for considering fluxes of total nitrogen in rivers at regional and global scales, all of which

Table 36.2 Sources of nitrogen to the continental shelves in major temperate-zone water regions of the North Atlantic Ocean

Major water region of the North Atlantic Ocean	N delivered from rivers and estuaries Tg N year^{-1}	N from direct atmospheric deposition Tg N year^{-1}	N delivered from deep ocean Tg N year^{-1}	N flux due to human activities (%)
North Canada rivers	0.16 (0.16)	0.10 (0.03)	0.77	7
St. Lawrence basin	0.34 (0.11)	0.13 (0.01)	1.26	25
NE coast of US	0.27 (0.03)	0.21 (0.01)	1.54	28
SE coast of US	0.13 (0.03)	0.06 (0.01)	1.36	11
Gulf of Mexico	2.10 (0.50)	0.28 (0.03)	0.14	275
Nort Sea and NW Europe	0.97 (0.14)	0.64 (0.02)	1.32	98
SW European Coast	0.11 (0.04)	0.03 (0.001)	0.20	40

Nitrogen fluxes (in Tg N year^{-1}) are reported for contemporary conditions, with estimates under pristine conditions indicated in parentheses. Flux of nitrogen delivered from rivers and estuaries is the direct input of rivers that discharge onto the continental shelf plus the riverine input into estuaries, minus nitrogen consumed in estuaries. Atmospheric nitrogen deposition estimates in this table are those directly onto the waters of the continental shelf and do not include deposition onto the landscape (which is part of the flux from rivers and estuaries). The flux from the deep ocean represents the advection of nitrate-rich deep Atlantic water onto the continental shelf. Data in this table are from Howarth (1998).

rely on relatively simple descriptors of nitrogen sources (such as fertilizers, deposition, fixation, manure, sewage) and landscape characteristics (such as data on climate, soil properties, land use, and river routing) to estimate fractions of nitrogen inputs to landscapes that are ultimately discharged to rivers; calibrating or evaluating the models with information on nitrogen fluxes observed in world rivers (such as the GEMS/GLORI compendium of riverine nitrogen fluxes synthesized by Meybeck and Ragu, 1995). For example, Seitzinger *et al.* (2005) use a spatially-explicit empirical approach to predict riverine nitrogen fluxes as a function of nitrogen inputs, land use, hydrology, and other factors. Green *et al.* (2004) use aggregate measures of basin temperature and hydraulic residence times of water in soils and river reach networks to characterize transport and transformations of nitrogen inputs from landscapes to river basins with a statistical approach. Van Drecht *et al.* (2003) and Bouwman *et al.* (2005) take a more mechanistic approach, describing the fate and transport of nitrogen inputs explicitly (e.g., ammonia emissions, denitrification in soils and waters, leaching and groundwater transport) as they are transported along various hydrologic pathways.

These approaches all provide admirable first approximations at how to characterize disproportionate impacts of nitrogen inputs to a heterogeneous landscape, though they also point out a vast number of assumptions and challenges associated

with characterizing the complex factors controlling nitrogen retention and release at this scale (Bouwman *et al.*, 2005; Green *et al.*, 2004; Van Drecht *et al.*, 2003, 2005). For example, limitations are imposed by: (1) the coarse resolution of spatial databases describing point- and non-point sources of nitrogen (e.g., deposition, fertilizers, human waste, animal manure, crop uptake and fixation); (2) land characteristics and associated assumptions about their hydraulic characteristics (e.g., soils, land cover, geology); (3) characterization of nitrogen transformation processes (e.g., assumptions about in-stream retention based on physical flow regime or time-of-travel that do not aim to consider the complexity of chemical and biological conditions that influence microbial activity); and (4) estimates of riverine nitrogen loads that are based on sparse observations that are not always representative of mean annual flow conditions, and are synthesized from large numbers of literature sources with differences in measurement methods, sampling frequencies, and length of period covered by the measurements (Bouwman *et al.*, 2005).

In the face of these uncertainties, we assert that simple empirical methods remain useful for exploring broad-scale linkages among nitrogen inputs and exports at regional scales. In their original application providing a comprehensive analysis of nitrogen export from large water regions to the North Atlantic Ocean, Howarth *et al.* (1996) presented a nitrogen budgeting approach for regional watersheds, and found a very strong positive correlation between net anthropogenic nitrogen inputs per unit land area and nitrogen delivery per area to coastal waters (Fig. 36.2). This method is similar to the approaches outlined above in that it uses information on the spatial and temporal variability of nitrogen sources on the landscape to predict riverine nitrogen exports, and suggests that fates of residual nitrogen inputs (e.g., retention and gaseous losses) are controlled by a variety of factors related to functioning of terrestrial and aquatic ecosystems. Applications of nitrogen
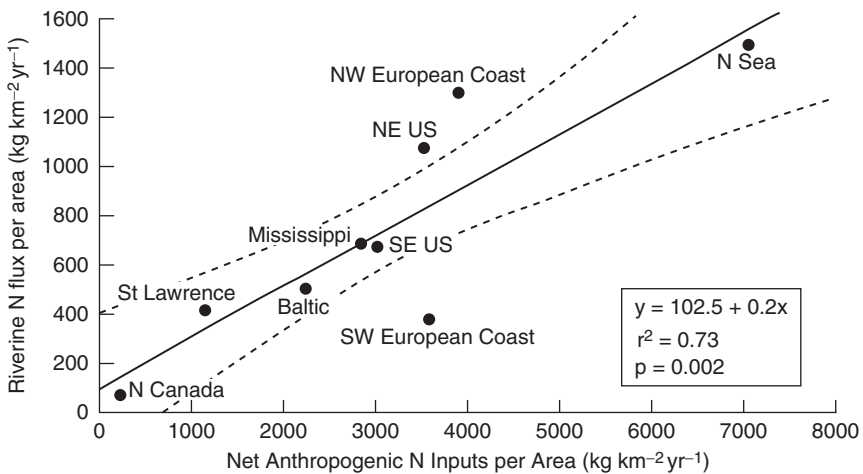
Figure 36.2 Nitrogen fluxes per area of watershed to the coastal ocean as a function of net anthropogenic nitrogen inputs to the landscape per unit area. Reprinted from Howarth *et al.* (1996).

budget models have set forth the conceptual notion that nitrogen fluxes in surface waters are controlled to a large degree by inputs of nitrogen to their watersheds, a basic fact which underlies most of the more complex modeling approaches and underpins management strategies to mitigate nitrogen pollution problems in surface waters.

Though the nitrogen budget method is subject to the same suite of limitations described for the approaches outlined above, it has been widely adapted given its simple structure. It is particularly useful because it provides a method to quantify and assess the relative importance of the various sources of nitrogen within and among regions and their associated riverine nitrogen responses; and allows systematic comparisons of regions over space and time. In a comparison of 6 large watershed models for estimating either total nitrogen or DIN fluxes, Alexander *et al.* (2002) found that the nitrogen budgeting approach of Howarth *et al.* (1996) compared very favorably, with one of the lowest factor-related biases of any of the models and the greatest precision of all, although it tended to under-predict fluxes (by ∼15% on average). Further, nitrogen budget models were found to be very robust when extended to watersheds of many different sizes and to world regions in both temperate and tropical settings (Alexander *et al.*, 2002; Bashkin *et al.*, 2002; Borbor *et al.*, 2006; Boyer *et al.*, 2002, 2006; Filoso *et al.*, 2006; Howarth *et al.*, 1996; Xing *et al.* and Xhu, 2002) For these reasons, a modified version of the nitrogen budgeting method was chosen to estimate global riverine nitrogen transfers to the coastal zone for the International Nitrogen Initiative's current global assessment of nitrogen cycles: past, present, and future (Galloway *et al.*, 2004). Regardless of the method used, quantifying the changing nitrogen inputs to world regions is critical for mitigating problems associated with nitrogen pollution.

As a basis for contrasting the contemporary distribution of nitrogen fluxes around the world, we present results from various applications of the nitrogen budget method in this chapter, and compare predictions of nitrogen fluxes to coastal waters from this method to other methods reported in published studies. In addition to consideration of global nitrogen fluxes, we emphasize regional differences, given that most reactive nitrogen cycles at spatial scales far smaller than global. Since the distribution of newly created reactive nitrogen is not uniform, some regions have seen massive increases in reactive nitrogen, while other regions have seen little change (Fig. 36.3). Associated with fossil-fuel combustion, atmospheric nitrogen deposition (Fig. 36.3A) yields nitrogen inputs to landscapes, with hot spots in the heavily developed regions (Dentener *et al.*, 2006). Associated with food production, cultivated land areas (Fig. 36.3B, showing areas in which at least 30% by area of the landscape is cultivated in a given year) result in large inputs of nitrogen to landscapes from the use of fertilizers and manure, and from biological fixation in cultivated leguminous crop lands. In 2000, heavily cultivated systems used for crop production, livestock production, aquaculture and agroforestry covered nearly 25% of the land surface (MEA, 2005).

Note that the greatest intensity of use of nitrogen by humans is often in regions where the natural rates of biological nitrogen fixation are low (Fig. 36.3C). Where natural biological nitrogen fixation tends to be highest—in the tropics—human inputs of nitrogen still tend to be relatively low in many regions, although rapid
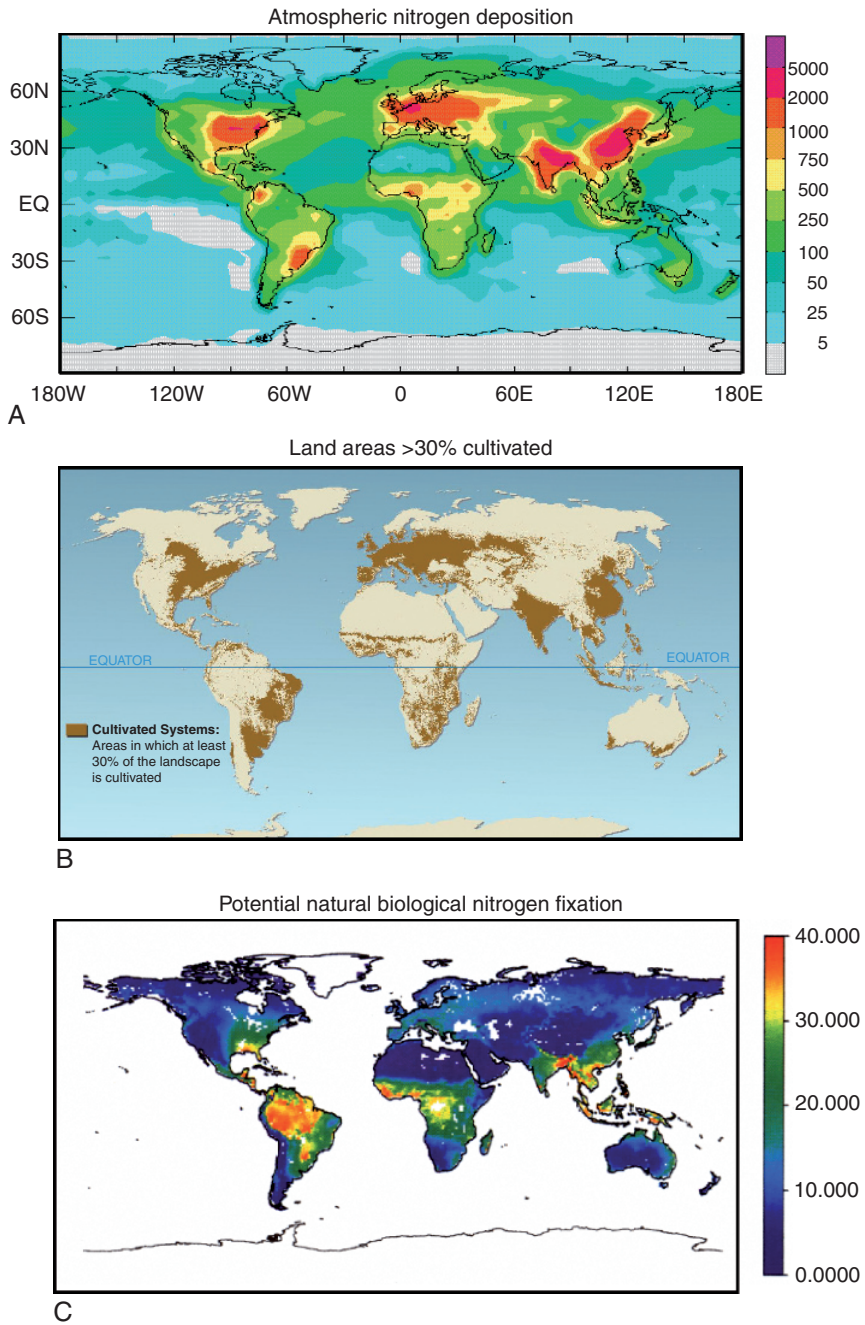
Figure 36.3 Contemporary nitrogen inputs to the landscape are unevenly distributed across the world. (A) Inputs of nitrogen to landscapes from atmospheric deposition (kg/km^2/year). Data from Frank Dentener, Reprinted from Galloway *et al.* (2004). (B) Significant inputs of nitrogen from use of fertilizers and manure and from biological nitrogen fixation occur in cultivated regions of the landscape. This map showing the extent of cultivated systems, which cover 24% of the earth's surface in 2000, is reprinted from the Millennium Ecosystem Assessment (2005). (C) Potential biological nitrogen fixation by natural ecosystems (kg/ha/year), if lands were not cultivated or otherwise converted. Reprinted from Cleveland *et al.* (1999).

future growth in nitrogen use is anticipated (Downing *et al.*, 1999; Matson *et al.*, 1999).

2. Nitrogen Budget Method

Our approach to modeling nitrogen fluxes with budgeting methods has been described extensively in our earlier publications (Boyer *et al.*, 2002, 2004; Galloway *et al.*, 2004; Howarth *et al.*, 1996, 2002A, 2006). In brief, the method involves assembling spatial and temporal data on nitrogen sources from available synoptic databases, tallying nitrogen inputs to each watershed or region from multiple sources, and developing relationships between these inputs and riverine nitrogen export. Nitrogen flux terms in the budgets are typically calculated using multiple years of data to avoid short-term climatic influences, and are typically expressed in units of mass per time (Tg N year^{-1}) or mass per unit area of watershed per time (kg N km^{-2} year^{-1}). For readers who are more accustomed with other units, 1 Tg N = 1 million metric tons N = 10^{12} gN, and 100 kg N km^{-2} year^{-1} = 1 kg N ha^{-1} year^{-1}. The goal is not to quantify the entire distribution of nitrogen in the landscape, but rather to quantify and sum the *new* inputs of reactive nitrogen to each watershed or water region from anthropogenic and natural sources. "New" inputs refer to inputs that are either fixed within a region or transported into a region. Anthropogenic nitrogen sources include fertilizer, biological nitrogen fixation in cultivated cropland, net imports of nitrogen in human food and animal feedstuffs (where a negative net import term indicates a region that is a net exporter of food and feed), and atmospheric NO$_y$-N deposition from fossil-fuel combustion. Natural nitrogen sources include biological nitrogen fixation in natural (non–cultivated) land and nitrogen fixation by lightning.

An important distinction among various implementations of the nitrogen budgeting method is how specifically the inputs are quantified. The net anthropogenic nitrogen inputs (NANI) approach considers nitrogen inputs from just the anthropogenic sources, while the net total nitrogen inputs (NTNI) approach includes natural in addition to the anthropogenic sources. Regardless of whether nitrogen inputs are tallied as NANI or NTNI, animal waste (manure) and human waste (sewage) are not considered *new* nitrogen inputs because they are recycled within a region; the nitrogen in these wastes originated either from synthetic nitrogen fertilizer, nitrogen fixation in agricultural lands, or nitrogen imported in food or feedstocks. It is important to note that nitrogen from human and animal waste sources is represented in the NANI and NTNI budget approaches, as they are included implicitly as part of the calculation of nitrogen inputs in food and feed.

Similarly, deposition of ammonium is not considered a new input, as this is largely recycled nitrogen volatilized from animal wastes within the same region or large watershed (Howarth *et al.*, 1996; Boyer *et al.*, 2002). Approximately 90% of ammonia in the global atmosphere comes from agricultural sources (Dentener & Crutzen, 1994), with major emissions coming from animal wastes (manure) and lesser contributions from volatilization of fertilizers. Because ammonia is short-lived

in the atmosphere, with residence times ranging from hours to a few weeks, ammonia may re-deposit within the same region from which it was emitted (Prospero *et al.*, 1996). The volatilization and deposition cycle is likely to be complete over the scale of a large region, such as the large water regions used in this analysis. Therefore, most nitrogen budgets computed over large spatial scales simply assume that ammonia deposition reflects local recycling and ignore it as a new input (Howarth *et al.*, 1996; Jordan and Weller, 1996). However, it is unlikely that this recycling is complete over shorter distances, and nitrogen budgets applied at smaller catchment scales have taken this into account (Boyer *et al.*, 2002).

Further, the budgeting approach assumes that the nitrogen status of soils is in steady state, and that the rate of soil nitrogen mineralization equals the rate of nitrogen immobilization on an annual basis, at least on average over a multiple year period (Howarth *et al.*, 2002B). Soil mineralization at the scale of a large watershed or region is a very big term (McIsaac *et al.*, 2001), and if mineralization were to be significantly greater than or less than immobilization, this would have a profound influence on the nitrogen budget for the region. When virgin lands are converted to agricultural use, there is a large net release of nitrogen due to mineralization in excess of immobilization. However, over a period of decades, the soil nitrogen is likely to come to a quasi- steady state (David *et al.*, 1997; McIssac *et al.*, 2001). Much of the inorganic nitrogen fertilizer added to a field is immobilized by bacterial action and only becomes available to crops in a subsequent year through mineralization (David *et al.*, 1997). That this mineralization is dependent upon the fertilizer input does not violate assumptions of steady state conditions in nitrogen budgeting approaches (Howarth *et al.*, 2002B).

The relationship between nitrogen inputs to watersheds (expressed either as NANI or NTNI) and the associated nitrogen issuing from these regions in rivers has been shown to be an excellent integrator of the human effects on nitrogen cycling at large scales (Bashkin *et al.*, 2002; Boyer *et al.*, 2002; Galloway *et al.*, 2004; Howarth *et al.*, 1996, 2002B, 2006; Jordan and Weller, 1996; McIsaac *et al.*, 2001; NRC, 2000; Quynh *et al.*, 2005; Sferratore *et al.*, 2005; Xing and Xhu *et al.*, 2002).

3. Relationship between Nitrogen Inputs to Watersheds and Riverine Outputs

We assembled data from published nitrogen budget studies that were conducted around the world, including countries draining into the North Atlantic Ocean (Howarth *et al.*, 1996), the northeastern United States (Boyer *et al.*, 2002; Howarth *et al.*, 2006), Ecuador (Borbor *et al.*, 2006), Brazil (Filoso *et al.*, 2006), Korea (Bashkin *et al.*, 2002), and China (Xing and Xhu *et al.*, 2002). All of these studies quantified nitrogen inputs to large regions or watersheds from multiple important nitrogen sources. Then, we compared total net nitrogen inputs to the watershed landscape with nitrogen fluxes leaving the watersheds in riverine fluxes of total nitrogen (in dissolved, organic, and particulate forms). The input and output data from these studies are based on monitoring data that represent conditions in
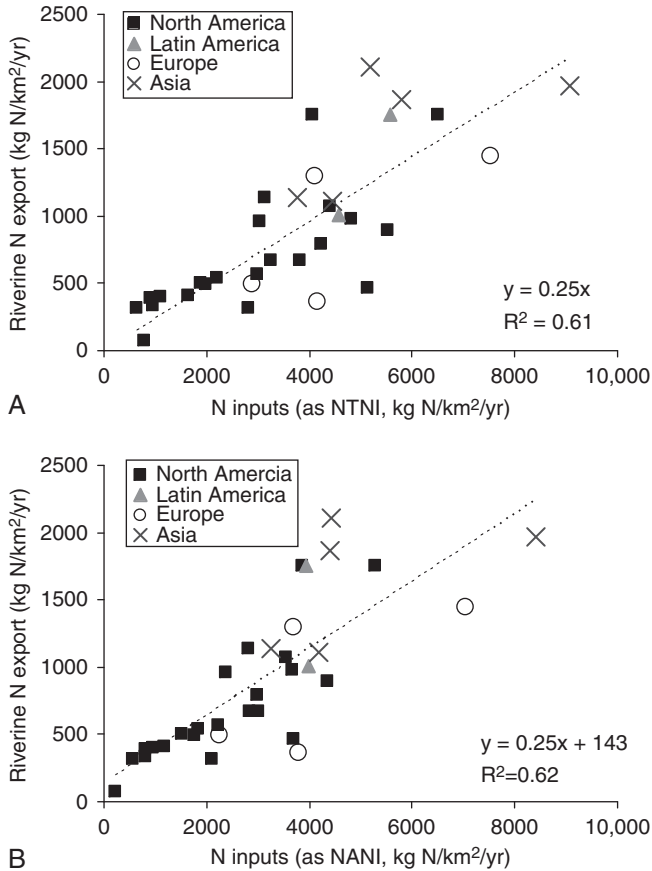
Figure 36.4 Relationship between nitrogen inputs and riverine N fluxes from watersheds spanning multiple scales and biomes of the world. Nitrogen inputs are expressed as: (A) net total nitrogen inputs; and (B) net anthropogenic nitrogen inputs. Modified from Boyer *et al.* (2006).

the mid–1990s, consistent with the timeframe of our nitrogen input data at the continental scale shown in Fig. 36.4. We aggregated data from these studies and augmented them with additional data on nitrogen sources when necessary to make all of the studies directly comparable, in order to represent nitrogen inputs as defined by our NTNI and NANI methods (Boyer *et al.*, 2006). In both cases, there is a direct, linear relationship between nitrogen inputs and the total nitrogen fluxes in these watersheds which suggests that, on average, 75% of the nitrogen inputs are retained in the landscape (that is, stored in soils and vegetation, or lost via volatilization and denitrification), while 25% of the nitrogen inputs are exported to rivers (Fig. 36.4). Note that the intercepts vary between the two plots, with the NTNI one having an intercept near zero and the NANI plot having an intercept of 143 kg N km^{-2} year^{-1}. The intercept for the NANI plot indicates that some nitrogen will be exported from watersheds even when there are no nitrogen inputs from human activities, with this nitrogen reflecting losses from natural inputs. It is

encouraging that for the NTNI plot — where natural nitrogen inputs are also included — that the intercept is approximately zero. That is, no nitrogen can be exported from a watershed that has absolutely no nitrogen inputs, considering both natural and anthropogenic sources. The positive intercept in NANI plots of this sort (see also Fig. 36.2) have been used before to estimate a baseline for nitrogen export from a "pristine," non–human impacted landscape under current climate conditions (Howarth *et al.*, 2002A, 2005; NRC, 2000).

While nitrogen inputs to a region are a good predictor of nitrogen export from the region, there is scatter in the relationships, with R^2 values ranging between 0.61 and 0.73 (Figs. 36.2 and 36.4). Regardless of the percentage of inputs transported to rivers, the strong relationship between watershed-scale nitrogen inputs and the associated losses of nitrogen in riverine export suggests that decreasing nitrogen inputs (e.g., via policy or management approaches) will improve nitrogen pollution problems in surface waters. One might expect the scatter to be even greater, given both the quality of the data and the large number of other factors that might be expected to affect nitrogen fluxes in rivers, such as topography, soils, geology, climate, and land use. In fact, a surprisingly large amount of the variation appears to be related to climate. In a detailed study of 16 watersheds in the northeastern United States, we observed that percentage of the human-controlled inputs of nitrogen (NANI) that was exported in rivers varied from approximately 10% to over 40%. This percent export was highly correlated with both average precipitation and average discharge in the watersheds (Howarth *et al.*, 2006). The combined influences of NANI and precipitation could explain 87% of the variation in riverine nitrogen flux from these watersheds. The percentage of the nitrogen inputs that was exported was greater in the watersheds where precipitation and riverine discharge were higher, perhaps suggesting less time for denitrification in riparian wetlands and low-order streams (Howarth *et al.*, 2006). Similarly, in their studies of minimally disturbed watersheds in Lewis *et al.* (1999) and Lewis (2002) found that nitrogen yields are not strongly related to watershed area but are very strongly related to runoff, when both discharge and nitrogen flux are expressed per area of watershed. This relationship, illustrating the importance of climatic controls on nitrogen exports, held true in both temperate areas (Lewis *et al.*, 2002) and tropical areas (Lewis *et al.*, 1999).

4. Comparison of Global Estimates of Contemporary Riverine Nitrogen Fluxes to the Oceans

The NTNI modeling approach yields a global estimate for the input of nitrogen to the oceans from rivers of 48 Tg N year^{-1} as of the early to mid 1990s (Boyer *et al.*, 2006; Galloway *et al.*, 2004). In addition to the flux of nitrogen in rivers to the oceans, some nitrogen transported from rivers flows to landlocked inland waters and never reaches the coast, or to inland regions that don't actively transmit significant fluxes of water to the coasts because they are too cold or arid. The NTNI model indicates endoreic fluxes of approximately 11 Tg N year^{-1}, so the total global flux
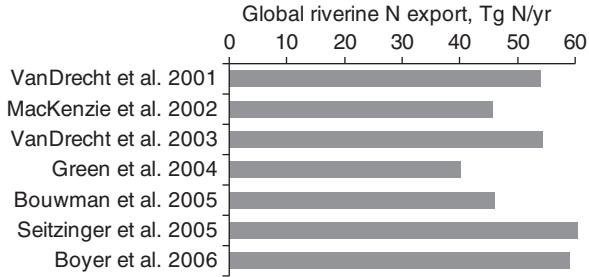
Figure 36.5 Comparison of global estimates of total riverine N export (Tg N year^{-1}) during the early to mid 1990's from studies reported in the literature. Note that these estimates include loadings to inland regions that don't reach the oceans in addition to the coastal loadings. For the Boyer *et al.* study, riverine nitrogen flows to the coasts represent 80% of the total global riverine nitrogen fluxes. Modified from Boyer *et al.* (2006).

of nitrogen in rivers is estimated as 59 Tg N year^{-1} (Boyer *et al.*, 2006). Several other recent studies present global riverine loading estimates based on different methodologies that are comparable (Fig. 36.5). A total nitrogen flux of 40 Tg N year^{-1} was predicted by Green *et al.* (2004) with an empirical model relating watershed characteristics to nitrogen export, which uses export coefficients based on basin temperature and hydraulic residence times in soils, rivers, lakes and reservoirs to transport nitrogen loads to river mouths. Van Drecht *et al.* (2001, 2003) and Bouwman *et al.* (2005) use various versions of a conceptual model based on point- and non-point sources of nitrogen coupled with mechanistic hydro-ecological models describing their fate and transport in the landscape, producing global estimates of total nitrogen fluxes of 54 Tg N year^{-1} (Van Drecht *et al.*, 2001, 2003) and 46 Tg N year^{-1} (Bouwman *et al.*, 2005). MacKenzie *et al.* (2002) use the Terrestrial Ocean Atmosphere Ecosystem Model, a biogeochemical model representing couplings among the major element cycles, to estimate a total riverine N flux of 46 Tg N year^{-1} in dissolved, particulate, and organic forms (MacKenzie *et al.*, 2002). Seitzinger *et al.* (2005) use an empirical regression approach to quantify nitrogen exports as a function of anthropogenic inputs (including sewage, fertilizers, deposition, manure, and biological nitrogen fixation), and variables influencing nitrogen retention and loss, yielding total riverine N fluxes of 66 Tg N year^{-1}, with 25 Tg N year^{-1} in dissolved inorganic forms (DIN), 12 Tg N year^{-1} in dissolved organic forms (DON), and 30 Tg N year^{-1} in particulate forms (PN). Several other global studies present estimates nitrogen fluxes to surface waters at regional scales, considering only fractions of total nitrogen export. For example, in their regression approaches put forth earlier in the literature, Seitzinger and colleagues estimated riverine N fluxes of 21 Tg N year^{-1} as DIN (Seitzinger and Kroeze, 1998) and 23 Tg N year^{-1} as PN (Seitzinger *et al.*, 2002). Similarly, Smith *et al.* (2003) used an empirical regression approach to quantify inorganic nitrogen fluxes as a function of population density and runoff, and use a statistical clustering procedure yielding estimates of global riverine DIN fluxes ranging between 19 and 23 Tg N year^{-1}, but do not estimate fluxes as DON or PN.

All of these approaches are useful for thinking about past, current, and future scenarios, because in all cases changes in riverine nitrogen flux are driven by changes in the magnitude and distribution of the nitrogen source inputs. The differences in the estimated riverine nitrogen fluxes at regional and global scales highlight large uncertainties stemming from data quality and resolution, scaling issues, and model approaches (Van Drecht et al., 2005).

5. DISTRIBUTION OF NITROGEN FLUXES IN RIVERS BY CONTINENT AND OCEAN BASIN

The use and release to the environment of nitrogen by humans varies greatly among continents (Figs. 36.6 and 36.7). Note that we used boundaries as defined by the Food and Agriculture Organization of the United Nations (FAO, 2003), so Europe is combined with the portions of Asia that were in the former Soviet Union ("FSU"), including all of Siberia. Biological nitrogen fixation in natural ecosystems dominates the nitrogen budgets in Africa (74%), Oceania (77%), and Latin America (68%) (Fig. 36.6; Boyer et al., 2004; Galloway et al., 2004). In contrast, anthropogenic nitrogen sources dominate the nitrogen budgets in Asia (76%), North America (63%), and Europe/FSU (61%) as of the 1990s. In each of the continents where human activity dominates the nitrogen cycle, the single largest source of new reactive nitrogen is synthetic fertilizer (Fig. 36.6). As is true with the pattern globally (Table 36.1, Fig. 36.1), this domination by human activity generally and by synthetic fertilizer in particular is a very recent phenomenon, particularly in Asia. For example, between the early 1960s and the late 1990s, the use of synthetic nitrogen fertilizer increased 3.6-fold in the United States and more than 20-fold in Asia (Howarth et al., 2002a; Zheng et al., 2002).

For all of the continents except Oceania, most of the nitrogen flux in rivers is delivered to the oceans rather than to landlocked inland waters (Fig. 36.7). However,
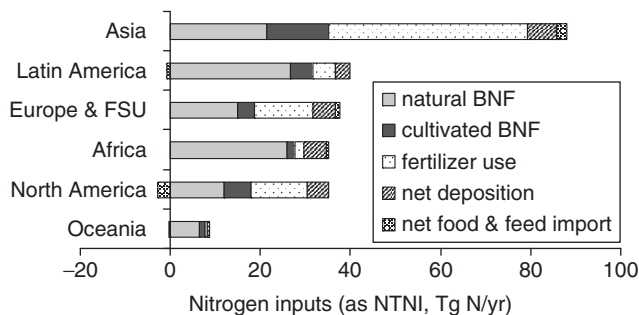
Figure 36.6 Net reactive N inputs (Tg N year^{-1}) to continental world regions during the mid-1990s, from anthropogenic and natural sources. Anthropogenic sources include N fertilizer use, N fixation in cultivated lands, net N imports in food and feed, and atmospheric N deposition from fossil–fuel combustion. The natural sources include biological N fixation in non–cultivated vegetated lands and N fixation by lightning. Modified from Boyer et al. (2004).
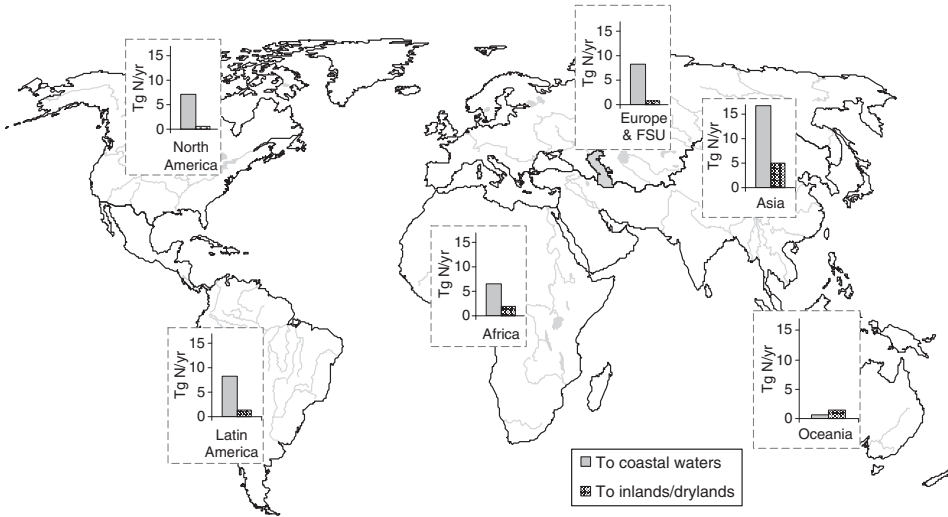
Figure 36.7 Riverine fluxes of total N from continental land masses to receiving waters during the mid-1990's. Significant N loads are transported in fluvial systems to coastal receiving waters. However, some N ends its journey inland as it is transported to landlocked endoreic watersheds, or originates in cold or arid regions that do not have enough throughput of water to transmit significant fluxes to the coastal zone. Data from Boyer *et al.* (2006).

in Oceania, more nitrogen is transported to inland receiving waters/drylands (1.5 Tg N year^{-1}) than is transported to the coastal zone (0.7 Tg N year^{-1}). This is because apart from Antarctica, Oceania is the driest continent globally, with deserts or semi-arid landscapes covering most of mainland Australia. Asia also has a significant percentage of its riverine nitrogen flux delivered to inland receiving waters or drylands, although the riverine flux to the oceans dominates (Fig. 36.7).

The global total riverine nitrogen flux parallels the total nitrogen inputs to the continents from both natural and anthropogenic sources. Thus, the total fluxes are comparable in North America, Latin America, Europe & FSU, and Africa at approximately 8 −10 Tg N year^{-1} each (Fig. 36.7). The flux from Asia is substantially higher and that from Oceania substantially lower, as is true for the nitrogen inputs to the landscape (Fig. 36.6). These fluxes are driven primarily by natural processes in Africa, Oceania, and Latin America and by anthropogenic nitrogen inputs in North America, Europe & FSU, and Asia.

Understandably, nitrogen fluxes from the continental land masses have the potential to impact the world's coastal zones. We quantified total nitrogen fluxes from the portions of each continent flowing to the major oceans based on data presented in Boyer *et al.*, 2006, and compare them to other published estimates (Fig. 36.8). The comparison among the ocean basins is fairly robust regardless of the modeling approach used, though it provides a glimpse into uncertainties associated with regional estimates of fluvial fluxes. All of the approaches are in consensus that the fluvial loadings from the continents to the coasts are tightly linked to human activities associated with food and fuel consumption. Data from Boyer *et al.* (2006)

	Van Drecht et al. 2003	Green et al. 2004	Bouwman et al. 2005	Boyer et al. 2006
■ Atlantic Ocean	25	15	23	20
▨ Mediterranean & Black Seas	3	2	3	4
☐ Indian Ocean	7	7	6	8
▨ Pacific Ocean	14	11	10	13
▨ Arctic Ocean	2	3	2	4
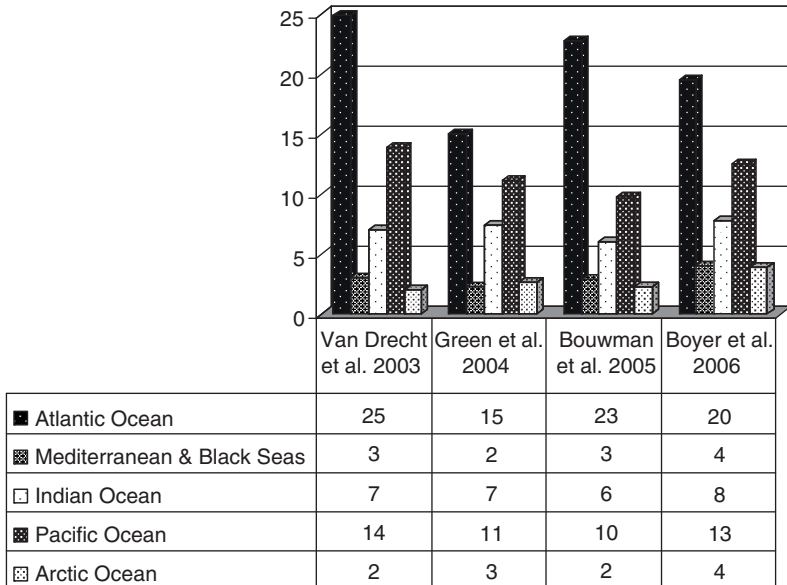
Figure 36.8 Comparison of estimates of total riverine N export (Tg N year^{-1}) from the continental land masses to coastal ocean basins during the mid-1990's, from modeling studies reported in recent literature.

indicate that the coastal Atlantic Ocean is one of the most significant receptors of nitrogen loadings, with approximately two-thirds of its fluxes contributed from the north Atlantic region, primarily from the highly developed land masses of eastern North America and western Europe. However, nitrogen fluxes to the north Pacific Ocean are also highly significant and are changing rapidly, largely influenced by the acceleration of the nitrogen cycle in Asia (Boyer *et al.*, 2004). As the region with the highest population and the most intensive and extensive agricultural practices, it also receives the highest N deposition rates globally (Galloway *et al.*, 2004). Unlike the United States and Europe which have seen rather stable rates of population growth in recent decades, East Asia continues to see very rapid increases in population, agriculture, and industrial activity, and will continue to play a major role in the global nitrogen budget in the future (Bashkin *et al.*, 2002; Bouwman *et al.*, 2005; Boyer *et al.*, 2006; Galloway *et al.*, 2004; Howarth *et al.*, 1996, Xing and Xhu *et al.*, 2002).

There is a great deal of variation in nitrogen fluxes off the landscape in rivers at scales smaller than the continents (Fig. 36.9). For example, within North America contemporary fluxes of nitrogen in rivers per area of watershed are estimated as 76 kg km^{-2} year^{-1} in Labrador and the Hudson's Bay area, as 566 kg km^{-2} year^{-1} in the Mississippi River basin, and as 1070 kg km^{-2} year^{-1} in the northeastern United States from Maine southward through Virginia (Howarth *et al.*, 1996; Fig. 36.10). At finer scales yet, more variation is observed: within the major watersheds in the northeastern United States, the riverine nitrogen flux per area varies from a low of 317 kg km^{-2} year^{-1} in the Penobscot River basin to 1755 kg km^{-2} year^{-1} in the
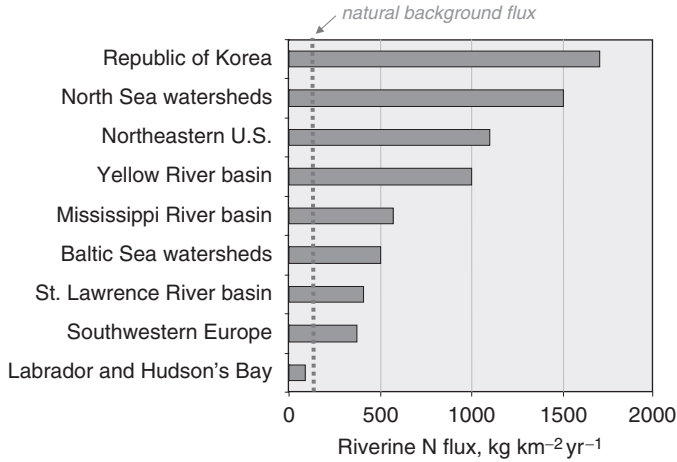
Figure 36.9 Fluxes of nitrogen (kg per km^2 of watershed area per year) from the landscape to coastal oceans in rivers for contrasting regions of the world in the temperate zone. The "natural background flux" is based on the intercept of plots of nitrogen export vs. NANI (such as shown in Figs. 36.2 and 36.4B). Data from Howarth *et al.* (1996, 2002); Bashkin *et al.* (2002).

both the Charles and Schuylkill River basins (Boyer *et al.*, 2002). Again, these variations in fluxes largely reflect the variation in nitrogen inputs to the landscape per unit area, although there is some influence of climate (Howarth *et al.*, 2006). For most of the regions shown in Fig. 36.9, the riverine flux of nitrogen is far greater—up to 15-fold greater—than is the expected natural background flux that would occur in the absence of human activity. Thus, while human activity may have roughly doubled the rate of creation of reactive nitrogen on the landscape of the planet, the distribution of this new nitrogen is far from uniform, and some regions have seen more than others an increase in the delivery of nitrogen to their coastal zones. Much of this increase has probably occurred during the last half century, since this is the time of global increase in the formation of reactive nitrogen (see Fig. 36.1).

6. DEVELOPMENT OF POLICY AND MANAGEMENT STRATEGIES FOR REDUCING COASTAL NITROGEN POLLUTION

The marine science community has long recognized that eutrophication in coastal systems is usually driven by nitrogen inputs (Boynton *et al.*, 1982; Graneli *et al.*, 1990; Howarth, 1988; Nixon *et al.*, 1986, 1995; NRC, 2000). Nonetheless, while nitrogen fluxes to coastal systems have increased dramatically over the past 30 to 40 years, management actions over much of this time were disappointing. In both the United States and Europe, national strategies for dealing with eutrophication did not distinguish between lakes and coastal marine ecosystems, and therefore focused primarily on phosphorus control, throughout the 1970s and 1980s and in fact well into the 1990s (Howarth, 2005; Howarth *et al.*, 2005; Howarth and
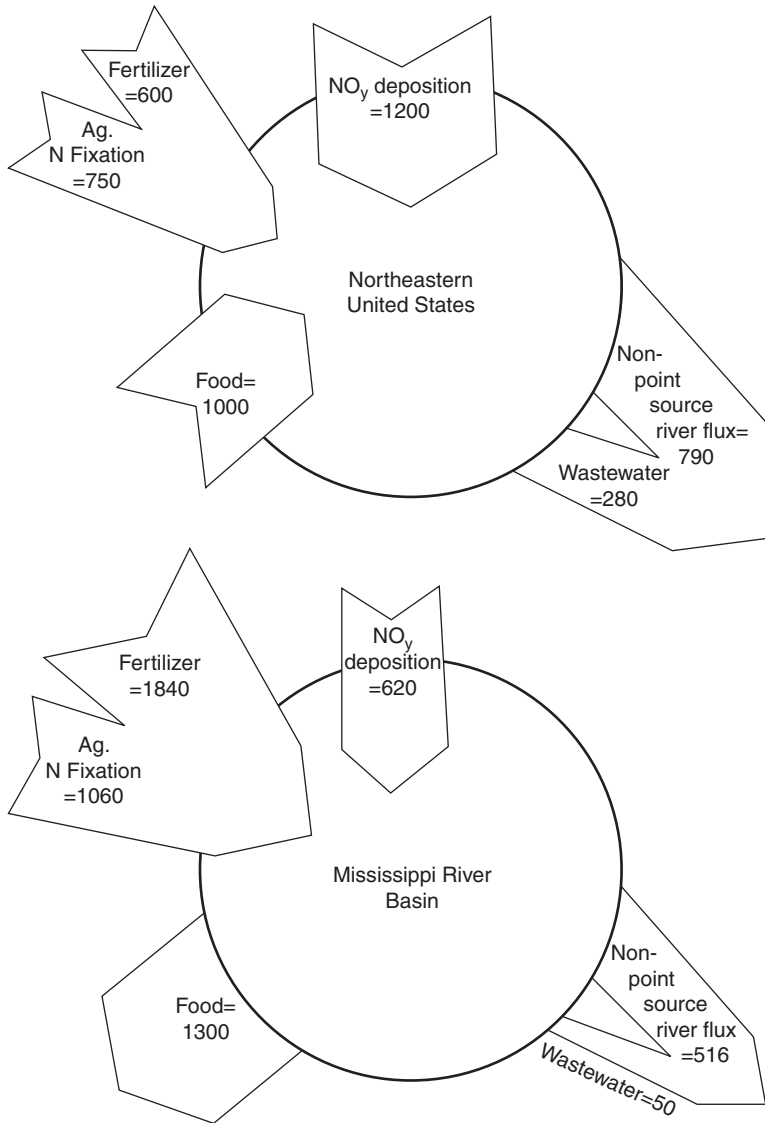
Figure 36.10 Comparison of anthropogenic nitrogen inputs and associated riverine N exports (kg per km^2 of watershed area per year) in two contrasting water regions of the USA: The northeast USA and the Mississippi River basin. Modified from NRC (2000) based on data from Howarth *et al.* (1996).

Marino, 2006; NRC, 1993, 2000). At the national scale, policy makers and water-quality managers often did not consider or accept the evidence for nitrogen control of eutrophication in coastal marine ecosystems, in part because of the political legacy left from heated debates in the 1960s and 1970s over whether phosphorus or carbon caused eutrophication in lakes (Howarth and Marino, 2006; NRC, 2000). Some

progress was made during the 1990s at removing nitrogen from wastewater treatment plants, notably in Tampa Bay (Florida, USA), Himmerfjorden (Sweden), and to a lesser extent, Chesapeake Bay (USA) (Elmgren and Larsson, 2001; NRC, 2000). However, the majority of the nitrogen inputs to most coastal marine ecosystems come from non-point sources rather than from wastewater, and to date there has been little success in significantly reducing nitrogen pollution from non-point sources in either the United States or in most of Europe (Howarth *et al.*, 2005; NRC, 2000). The fact that progress can be made was illustrated in Denmark, where strong regulatory programs and deliberate, policy-based nutrient control strategies have led to substantial reductions in non-point source nitrogen pollution (Carstensen *et al.*, 2006; Grant *et al.*, 2006). Another notable reversal of nitrogen pollution from both point and non-point sources was unintentional: nitrogen loading to the Black Sea decreased greatly as the use of synthetic nitrogen fertilizer plummeted and point sources inputs were greatly reduced after the collapse of the former Soviet Union (Boesch, 2002; Garnier *et al.*, 2002).

National-scale efforts at controlling non-point source nitrogen inputs to coastal waters in most countries only began in the 1990s and are still largely not implemented (Howarth *et al.*, 2005). In the United States the Congress in 1990 for the first time addressed the problem of non-point source nitrogen pollution in coastal marine ecosystems when the Coastal Zone Act was reauthorized (Melillo and Cowling, 2002). This was largely a planning exercise, and states were required by 1995 to submit plans for nitrogen reduction to the federal government. Actual implementation of the first phase of nitrogen reduction was not scheduled until 2004 (Melillo and Cowling, 2002), and this has not yet in fact started as of 2006. The first efforts to control non-point sources nitrogen pollution by the European Union also began in the 1990s with the Nitrate Directive, a plan for better management of nitrogen from agricultural sources that was driven largely by concerns over drinking water quality (Chave, 2001). The Water Framework Directive replaced the Nitrate Directive and other water pollution directives in 2001, substituting a watershed based approach for managing phosphorus and nitrogen in all surface and ground waters (Chave, 2001).

The good news is that many technical solutions for reducing nitrogen pollution exist, and generally at reasonable cost, at least as viewed from the perspective of developed nations (NRC, 2000; Howarth, 2005; Howarth *et al.*, 2005). This provides hope that, as national strategies for nitrogen control become fully implemented, they may be effective. Unfortunately, though past experience constrains our optimism. Regional-scale efforts to reduce nitrogen pollution in Chesapeake Bay (target of 40% reduction set in 1987), in the North Sea (target of 50% reduction set in 1987), and in the Baltic Sea (target of 50% reduction set in 1990) had come nowhere near meeting their targets by 2000 despite 10 years or more effort (Boesch *et al.*, 2001; Elmgren and Larsson, 2001; OSPAR Commission, 2000). One possible reason is delays between management action and the resulting decrease in riverine nitrogen fluxes, due to storage in aquifers and soil organic matter (Grimvall *et al.*, 2000). However, the analysis of McIsaac *et al.* (2001) for the Mississippi River basin suggests that the time lag between nitrogen use in the landscape and nitrogen flux down the river is relatively short, with most nitrogen transported downstream within a few years of its introduction to the landscape. Also, the recovery of the Black Sea following the collapse of the

former Soviet Union occurred within a few years, and a large decrease in nitrogen flows to the system was observed in less than 1 year (Boesch, 2002).

The failure to adequately control non-point source nitrogen pollution to date – in the few cases where such controls have been attempted — probably lies in structural problems with the application of the control strategies (Boesch *et al.*, 2001; Boesch, 2002; Howarth, 2005; Howarth *et al.*, 2005; NRC, 2000). An analysis of some problems with the Chesapeake Bay case is illustrative:

1. The major sources of nitrogen inputs to Chesapeake Bay remain uncertain (NRC, 2000; Howarth *et al.*, 2002b). Management decisions have been based on the source estimates of the Chesapeake Bay model, which is a highly complex and largely unverified model (NRC, 2000). This model suggests that agriculture is the largest source of nitrogen to the Bay, but other lines of evidence suggest that atmospheric deposition may equal or even exceed the agricultural sources (Howarth, 2006). Management efforts have focused largely on the agricultural sources.
2. There has been little or no monitoring of the effectiveness of management practices that have been implemented (Boesch *et al.*, 2001; NRC, 2000). For example, the watersheds of Chesapeake Bay have had some of the most extensive efforts at repairing streams and riparian wetlands of any area in the United States, but these efforts have been among the least monitored of all projects in terms of their effectiveness (Bernhardt *et al.*, 2005; Palmer *et al.*, 2005). Similarly, the effectiveness of best management practices as applied in the watersheds of the Chesapeake has received little or no assessment.
3. The best management practices that have been applied to agriculture may be less effective than assumed (Howarth, 2005). For example, while the Chesapeake Bay model suggests that no-till agriculture is an effective approach for reducing nitrogen losses from fields, this has not been demonstrated in most field studies (Randall and Mulla, 2001). On the other hand, winter cover crops and switching to perennial crops are an extremely effective approach to reducing nitrogen pollution (Staver and Brinsfield, 1998; Randall and Mulla, 2001). Water quality managers in the past often have failed to distinguish between the effectiveness of practices for phosphorus pollution and for nitrogen pollution (Howarth, 2005).
4. Climate variation and change may be aggravating efforts to reduce nitrogen pollution. Watersheds in the northeastern United States that have more precipitation and higher freshwater discharge export a higher percentage of their nitrogen inputs (Howarth *et al.*, 2006). A generally wetter climate with greater freshwater discharge to Chesapeake Bay during many years in the 1990s compared to the 1980s may have partially counteracted management efforts at nitrogen control (Boesch *et al.*, 2001).

Clearly, management strategies to decrease the flow of nitrogen pollution to coastal marine ecosystems must be grounded in policies that adequately consider the sources of nitrogen pollution, that allow for uncertainty in analysis, that monitor the effective of control strategies, that consider the greater mobility of nitrogen than phosphorus in the environment, and that understand the potential consequences of climate change and variability.

ACKNOWLEDGEMENTS

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Silicon:Nitrogen Interactions in the Marine Environment

Raphael M. Kudela

Contents

1. Introduction

A central challenge of environmental research is to understand the feedbacks that connect elemental cycling with ecosystem structure and function. This challenge has become of increasing importance as human activities continue to change the elemental ratios (Turner *et al.*, 2003b) and eutrophy the coastal ocean (Galloway and Cowling, 2002; Chapter 11 by Paerl and Piehler, this volume) and elevate atmospheric carbon dioxide concentrations, with subsequent changes to the terrestrial and marine environments (Houghton *et al.*, 2001). Phytoplankton are the link between physical, chemical, and atmospheric changes in the oceanic environment. The carbon cycle is driven by phytoplankton photosynthesis and nitrogen, phosphorous, silica, and iron are all potentially limiting nutrients for phytoplankton.

The efficiency of the "biological pump" has also been proposed as the mechanism by which atmospheric CO_2 levels are regulated on glacial/interglacial time scales (e.g., Martin, 1990). Ice core records show that for the last 420,000 years atmospheric CO_2 has oscillated between low levels (~180 ppm) during glacial periods and higher levels (~280 ppm) during interglacials (Petit et al., 1996). Thus long-term changes in the efficiency of nutrient use by marine phytoplankton, especially in those areas that have a high nutrient, low chlorophyll (HNLC; Minas et al., 1986) character in the present-day ocean, may be a key link connecting surface water geochemistry, oceanic biology, atmospheric CO_2, and global climate.

Diatoms are ubiquitous in the world's aquatic systems, including both benthic and pelagic marine ecosystems, freshwater lakes and streams, and in sea ice (Garrison et al., 1986), and are the most species-rich of all algae (Mann, 1999). Because of this ubiquity, diatoms account for a large fraction of the total carbon fixation in the modern oceans (Tréguer and Pondaven, 2000). Diatom productivity has been estimated to account for as much as 40% of the annual ocean carbon fixation, or approximately 15–20 Pg C year^{-1} (Field et al., 1998). The importance of this productivity to the global carbon cycle has been equated to the contribution of grasses to terrestrial productivity (Raven and Waite, 2004), or the total impact of the terrestrial rain forests (Field et al., 1998). This productivity forms the basis for the short, efficient transfer of energy supporting the economically and ecologically important fisheries that dominate in coastal upwelling systems (Ryther, 1969).

These unicellular, photosynthetic eukaryotes evolved relatively recently, appearing ca. 180–250 million years ago as evidenced by both the fossil record and molecular techniques, with a major expansion occurring in the Cretaceous (Falkowski et al., 2004; Raven and Waite, 2004). Diatoms have an absolute requirement for silicon (Lewin, 1962). Although there are other marine organisms which utilize silica (and nitrogen) in the marine environment, including silicoflagellates, radiolaria, and many sponges (Siever, 1991), the rise of the diatoms fundamentally altered the marine silica cycle (Harper and Knoll, 1975; Maliva et al., 1989; Racki and Cordey, 2000), and diatoms are the dominant organisms in the modern ocean which utilize both silicon and nitrogen (but see Maldonado et al., 2005). This dominance of the biogeochemical cycling of Si by diatoms in the modern ocean is so complete that each atom of Si derived from the weathering of terrestrial rocks is recycled an average of 39 times by diatoms before burial in the ocean, with an estimated residence time of 400 years in surface waters (Tréguer et al., 1995).

Although diatoms and Si cycling have long been of interest to oceanographers (e.g., Simpson and Volcani, 1981), this interest was rekindled when sediment-trap data from a variety of ocean habitats showed strong temporal correlation between the downward fluxes of particulate organic carbon (POC) and diatom opal, implying that diatoms are the autotrophic source of much of the sinking POC. Diatoms are known to play a major role not only in carbon fixation, but also in the export flux of biogenic material to depth (Dugdale and Wilkerson, 1998; Goldman, 1988; Goldman, 1993; Goldman et al., 1992; Nelson et al., 1995; Ragueneau et al., 2000; Tréguer et al., 1995; Villareal et al., 1993), including the vast regions of Fe-limited, HNLC oceans (Boyd, et al., 2007, Boyd, 2004; Boyd et al., 2000; Coale et al., 1996,

2004; Tsuda *et al.*, 2003). Fast rates of NO_3 uptake and high *f*-ratios are generally caused by a dominance of larger phytoplankton, which on death sink faster and are considered to be the major contributors of export (or new) production (Buesseler, 1998), thus providing diatoms with another key role in controlling climate (Tréguer and Pondaven, 2000). Because of their key role in the biogeochemistry of the oceans, diatoms are important components for the cycling of carbon (C), nitrogen (N), silicon (Si), phosphorous (P), and iron (Fe).

Because of the key role that diatoms play in global productivity, their dominance in regulating export flux of biogenic material, the ubiquity and diversity of diatom species, and their role in food web dynamics, diatoms as a group have been well studied, and there are many excellent review articles focusing on diatoms and their physiology, biogeochemistry, and evolution (e.g., Falkowski *et al.*, 2004; Kristiansen and Hoell, 2002; Lewin, 1962; Martin-Jézéquel *et al.*, 2000; Ragueneau *et al.*, 2000; Raven and Waite 2004; Round *et al.*, 1990; Siever, 1991; Tozzi *et al.*, 2004; Yool and Tyrrel 2003). The focus of this review is on the less well-documented interactions between silicon and nitrogen in the marine environment across a variety of scales. Towards that end, this review of Si:N interactions focuses primarily on diatoms, starting with newly available genomic information (Armbrust *et al.*, 2004), and continuing on to a review of cellular Si-metabolism, emphasizing interactions with other cellular constituents. This leads naturally to a discussion of physiological and ecophysiological Si:N interactions, with an emphasis on the (un)coupling of Si-metabolism from photosynthesis and C:N assimilation, as well as the modulating effects of trace-metal interactions such as Fe and Zn. The role of Si:N interactions in controlling export production and biogeochemical cycling, both in the modern and paleo ocean is then discussed, followed lastly by anthropogenic changes in Si:N ratios and their consequences.

2. Cellular Processes

2.1. Molecular ecology of diatoms

Although molecular techniques have been used in oceanographic studies for many years, it is only recently that the ready availability of genomic information for algae has flourished (Grossman, 2005). Of relevance to this review of Si:N interactions, nitrogen metabolism by diatoms has been reasonably well described at the molecular level, particularly with the full genome sequencing of the diatom *Thalassiosira pseudonana* (Armbrust *et al.*, 2004) and *Phaedactylum tricornutum* (*http://genome.jgipsf.org/Phatr2/Phatr2.home.html*), and the availability of many partial sequences and expressed sequence tags (ESTs) for other diatoms (Grossman, 2005), particularly *P. tricornutum* (Montsant *et al.*, 2005). This molecular information has provided the characterization of several potentially rate-limiting steps such as nitrate and nitrite transport (Armbrust *et al.*, 2004; Hildebrand and Dahlin, 2000), silicic acid transport (Armbrust *et al.*, 2004; Hildebrand and Wetherbee, 2003; Hildebrand *et al.*, 1997, 1998; Thamatrakoln and Hildebrand, 2005), glutamine synthetase (e.g., Robertson *et al.*, 1999, 2001; Takabayashi *et al.*, 2005), and nitrate reductase (Allen *et al.*, 2005),

while many other functional genes not listed here have also been characterized (e.g., review by Ward, 2005).

Molecular approaches have been especially useful in the characterization of nutrient transporter genes. Transporters are of particular interest since they are typically highly regulated, interact with specific nutrients, and are key to the environmental response of diatoms in the natural environment. Besides the silica class of transporters (SITs; Hildebrand *et al.*, 1997, 1998; Thamatrakoln *et al.*, 2006), Hildebrand and Dahlin (2000) have isolated a nitrate transporter (NAT), and more recently, Hildebrand (2005) cloned and isolated a class of ammonium transporters (AMT), while the whole–genome sequencing of *T. pseudonana* has identified multiple NAT and AMT genes, a single nitrite transporter, and transporters for other inorganic nutrients and metals (Armbrust *et al.*, 2004).

Once these transporter genes have been identified, it is possible to combine more traditional physiological experiments with molecular methods to elucidate the response, regulation, and biochemical pathways for various nutrients in diatoms. As an example, recent work has focused on the development of a "molecular *f*-ratio" by examining the genes encoding for nitrite, nitrite, and ammonium assimilation directly. Hildebrand and Dahlin (2000) showed that NAT genes are strongly induced by N starvation, but repressed in the presence of NH_4 (even NH_4NO_3); in contrast, the AMT genes are less regulated by the presence or absence of N, but are always active (Hildebrand, 2005). Further along in the biochemical pathway, Takabayashi *et al.* (2005) have shown that the gene *glnII* isolated from the diatom *Skeletonema costatum*, which encodes glutamine synthetase (GS), the isoenzyme linking C and N metabolism in the cell, is up regulated in response to external NO_3, but not NH_4. These results provide intriguing evidence for both the molecular basis for the apparent preference of NH_4 versus NO_3, as well as functional assays for "new" versus "regenerated" production (sensu Dugdale and Goering, 1967).

Despite the rapid development of molecular approaches for algal ecophysiology, much work remains to be done. For example, until the completion of the *T. pseudonana* genome, it was not known that this diatom, at least, contains a complete urea cycle, similar to animals, while about half the genes sequenced from *T. pseudonana* have no known function (Armbrust *et al.*, 2004). It is also not clear how representative a genome from a single species is; using an example relevant to this review, recent evidence shows disparate copy numbers for AMT in the diatoms *T. pseudonana, Thalassiosira weissflogii*, and *Cylindrotheca fusiformis*, which may be related to physiological function (Hildebrand, 2005), while structural differences (suggesting differences in transporter mechanisms and/or regulation) have been found in the SIT class of genes from centric and pennate diatoms (Thamatrakoln *et al.*, 2006). Using the genome for *T. pseudonana* and the EST libraries for *P. tricornutum* (the two best characterized diatoms at a molecular level), Montsant *et al.* (2005) also reported differences between pennate and centric diatoms for several other metabolic pathways, including carbon-concentrating mechanisms, cytosolic acetyl-Coenzyme A production, and fructose-1,6-biphosphate metabolism.
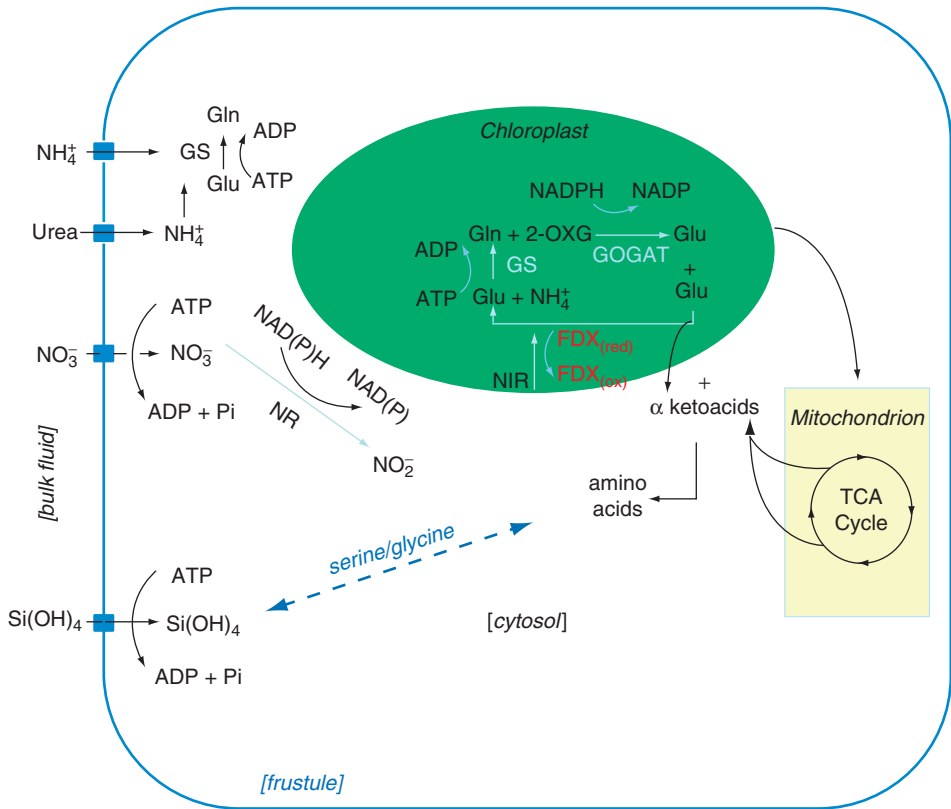
Figure 37.1 A simplified schematic of the major biochemical pathways involved in Si and N metabolism by diatoms. Metabolic steps are represented by arrows. Dashed arrow indicates a proposed, but undocumented, interaction between serine and glycine amino acid pools, Si–transport and deposition, and Rubisco. Adapted from figures and content in Armbrust *et al.*, 2004; Falkowski and Raven, 1997.

Although genomic methods have only begun to characterize the apparently wide diversity of diatoms, a general understanding of how Si and N interact at the molecular and biochemical level has emerged (Fig. 37.1). Both Si and N are actively transported across the cell membrane, although N is much more closely linked to the light reactions of photosynthesis, while Si-transport is driven by respiration (see below). Once taken up, both Si and N can efflux from the cell (Azam *et al.*, 1974; Syrett, 1981), and there is a direct coupling of Si and N metabolism due to the requirement for amino acids (particularly serine and glycine) in the formation of the cytoskeleton (see review by Martin-Jézéquel *et al.*, 2000). The evolutionary role for Si is still debated (Raven and Waite, 2004), but it has been suggested that Si–metabolism may originally have been involved in DNA synthesis (e.g., Medlin *et al.*, 2000; Okita and Volcani, 1978). Additional genome sequencing will undoubtedly refine our understanding of Si:N interactions at the molecular level in the near future.

2.2. Nutrient transport kinetics

Laboratory studies have suggested that there are three modes of transport for silicic acid (reviewed by Martin-Jézéquel *et al.*, 2000): first, silicic acid may be rapidly transported across the cell membrane, following ''surge uptake'' kinetics. This occurs primarily in Si-starved cells with cell quotas (Droop, 1968, 1973) near minimal values. Second, silicic acid uptake can be controlled internally, presumably due to regulation of silica precipitation and deposition (e.g., Hildebrand *et al.*, 1997). Third, silicic acid uptake may be controlled externally due to substrate limitation.

Under substrate limitation, silicic acid uptake is expected to follow Michaelis–Menten kinetics, and numerous laboratory and field experiments have demonstrated this to be true for both silicate and the various nitrogen species in diatoms (Azam *et al.*, 1974; Eppley *et al.*, 1969; Goering *et al.*, 1973; McCarthy, 1972; MacIsaac and Dugdale, 1969; Paasche, 1973a,b):

$$V = V_{max}[S]/(K_s + S) \tag{37.1}$$

where V is the specific uptake rate (t^{-1}), S is the substrate (N or Si in this case; μM), V_{max} is the substrate-saturated uptake rate, and K_s is the half-saturation constant, or the concentration of S at which uptake is half of V_{max}.

Although the biochemistry of silicon metabolism within the diatom is still not completely understood, multiple silicic acid transporter (SIT) genes have been characterized for various diatom species (Armbrust *et al.*, 2004; Hildebrand *et al.*, 1997, 1998), with multiple SIT genes expressed in the same organism at different times and under different environmental conditions (Hildebrand *et al.*, 1998). It is likely that these multiple SIT genes encode transporters with varying affinities for silicic acid. This strongly suggests that multi-phase kinetics should be observed for a given species, and is consistent with experimental observations. Previous investigators (e.g., Brzezinski *et al.*, 2005; Conway and Harrison, 1977; De La Rocha *et al.*, 2000; Jacques, 1983; Leynaert *et al.*, 2004) have demonstrated that the half-saturation constant (K_s) for silicic acid is highly variable ($K_s = 0.2$–55 μM), and may increase with increasing ambient concentrations, while Sullivan (Sullivan, 1977) showed that K_s and V_{max} varied as a function of the stage of cell wall biosynthesis. Similar to silicic acid, nitrogen transport also exhibits both surge uptake (Conway and Harrison, 1977) and multiphasic kinetics (see review of Collos *et al.*, 2005).

Although phytoplankton (including diatoms) commonly grow at non-Redfield proportions for C:N when either N or light is limiting (e.g., Geider and LaRoche, 2002; Goldman *et al.*, 1979; Falkowski, 2000), with few exceptions (Azam and Chisholm, 1976; Dortch and Whitledge, 1992; Dugdale, 1972; Dugdale and Wilkerson, 1998; Dugdale *et al.*, 1995; Nelson and Tréguer, 1992; Sommer, 1986, 1994a,b; Turner *et al.*, 2003a; White and Dugdale, 1997) nitrogen (*cf.*, Carpenter and Capone, 1983) and iron (Martin, 2002) have generally been assumed to be more broadly limiting in the modern ocean than Si.

Under optimal conditions, diatoms assimilate Si and N in approximately equimolar ratios (Brzezinski, 1985), essentially extending the Redfield ratio (Redfield *et al.*, 1963) to include Si. However, unlike N, many diatom species exhibit

markedly different half-saturation constants for uptake (Michaelis–Menten kinetics) versus growth (Monod, 1942), where Monod kinetics are described as in Eq. (37.1), but with the substitution of μ (specific growth rate; t^{-1}), $\mu_{\max}$, and K_μ:

$$\mu = \mu_{\max}[S]/[K_\mu + S] \qquad (37.2)$$

Although uptake (Michaelis–Menten) kinetics for silicic acid typically range from 0.2 to 55 μM, with most values <10 μM, half-saturation kinetics for Si-limited *growth* (K_μ) are substantially lower, ranging from *ca.* 0.04 to 1.5 μM (Martin-Jézéquel *et al.*, 2000; Nelson and Dortch, 1996; Ragueneau *et al.*, 2000). As a result, many, if not most, diatoms can maintain near maximal growth rates despite external limitation of Si in the media or environment. This is accomplished by allowing the frustule to become thinner, effectively reducing the per-cell concentration (quota) of Si and allowing the C:Si and N:Si ratios to increase (Brzezinski *et al.*, 1990; Claquin *et al.*, 2002; Nelson and Dortch, 1996; Paasche, 1973a).

2.3. Silica to nitrogen ratios energetics

Once Si or N substrates have been transported across the cell membrane, the substrates must be incorporated into the cell via a variety of biochemical processes. Much of this is beyond the scope of this review (see Chapter 7 by Mulholland and Lomas and Chapter 32 by Berges and Mulholland, this volume); here I will focus on the similarities and differences in the assimilation of N versus Si, and the role of photosynthetic energy.

Ultimately, photosynthetic organisms derive their energy from the conversion of sunlight. After carbon, nitrogen uptake and assimilation is the primary sink for photosynthetic reducing power, requiring a minimum of 288 kJ mol^{-1} of NH$_4^+$ (the form of nitrogen required with carbon skeletons to form amino acids; (Syrett, 1981) if the initial substrate is NO$_3^-$ (Falkowski and Raven, 1997). It is not surprising, then, that C and N metabolism, and therefore photosynthesis, are closely linked in algae (*cf.* Falkowski and Raven, 1997).

N-limitation frequently manifests as a decoupling of C and N responses to irradiance, resulting in dark-uptake of N compounds (e.g., Clark *et al.*, 2002), although dark-uptake is also influenced by diel cycles (e.g., Cochlan *et al.*, 1991a; Fisher *et al.*, 1988; Miyazaki *et al.*, 1987; Shiomoto and Matsumura, 1993). In many cases, however, particularly for coastal waters, there is a direct correspondence between irradiance and both C and N uptake rates, with a positive uptake-irradiance response for both elements (e.g., Cochlan *et al.*, 1991b; MacIsaac and Dugdale, 1972; Kanda *et al.*, 1989; Kudela and Cochlan, 2000; Kudela *et al.*, 1997). This can be mathematically described as either a Michaelis–Menten type curve with the addition of a dark-uptake value (V_D, h^{-1}), or using a photosynthesis versus irradiance (P vs E) model such as proposed by Platt and Gallegos (Platt and Gallegos, 1980), modified to account for dark uptake (Cochlan *et al.*, 1991b):

$$V = V_D + V_{\max}(E/E_K + E) \qquad (37.3)$$

$$V = V_s(1 - e(-\alpha \bullet E/V_s))(e - \beta \bullet E/V_s) + V_D \qquad (37.4)$$

where E is irradiance (μmol photons m^{-2} s^{-1}), E_K is the half-saturation value for irradiance, alpha and beta are the light-limited and light-inhibited slopes of the uptake–irradiance curve, and V_s is the uptake rate in the absence of inhibition. Thus, it is possible to describe both C and N responses to irradiance using the same mathematical models, and to extend this concept to (for example) calculation of quantum efficiency and yield for N independent of the quantum yield for carbon (Kudela and Chavez, 1997).

At the biochemical level, N-limitation manifests as a loss of chlorophyll a, and an increase in nonphotochemical carotenoid compounds (Herzig and Falkowski, 1989; Geider *et al.*, 1993). It also results in a direct reduction in the efficiency of photosystem II (PSII), primarily due to decreasing thermal efficiency (Berges *et al.*, 1996; Geider *et al.*, 1993; Kolber *et al.*, 1988). This is readily assayed by measuring the ratio of variable to maximal fluorescence (F_v/F_m), which decreases with increasing N-stress (Beardall *et al.*, 2001; Berges *et al.*, 1996; Kolber and Falkowski, 1993; Kolber *et al.*, 1998). C and N uptake and assimilation, then, are directly linked at the level of photosynthesis because of the direct coupling of C and N metabolism and the requirement for photo-reductant.

In contrast to C and N, Si-metabolism is metabolically inexpensive; for a given cell size, a silicon frustule requires *ca.* 1/10 the total metabolic energy of an equivalent cell wall composed of carbon compounds (Raven, 1983). Si-metabolism is also not directly coupled to photosynthesis. Si-transport and deposition are driven by oxidative phosphorylation (Blank and Sullivan, 1979; Blank *et al.*, 1986; Sullivan, 1976), while serine and glycine, the amino acids which provide the main fraction of the protein matrix for Si-deposition (Werner, 1977), are terminal substances produced during photorespiration (Burris, 1977). Si-metabolism is instead linked to the regulation of cell division (Fig. 37.2) and growth rates (Brzezinski *et al.*, 1990; Martin-Jézéquel *et al.*, 2000; Volcani, 1981).

The cell cycle is typically divided into four phases, G1, S, G2, and M, corresponding to DNA replication (S), mitosis and cell division (M), and two "gaps" (G1, G2) when growth occurs (Mitchison, 1971). Si-uptake and assimilation occurs predominantly just before G2 and during M (Brzezinski, 1992; Brzezinski *et al.*, 1990; Martin-Jézéquel *et al.*, 2000; Schmid, 1994). Because cell division can occur in both the light and dark, Si-metabolism remains largely independent of irradiance, with the difference between light/dark uptake most often attributed to the synthesis or activation of a transient compound in the light required for Si-transport. Blank and Sullivan (1979) demonstrated that silicic acid transport requires ATP, a byproduct of photosynthesis. They also reported, however, that it takes >6 h for transport to degrade, and that it does so fairly slowly. The implication is that silicic acid utilization is only weakly controlled by the availability of photosynthate and is essentially uncoupled from ambient irradiance, unlike C and N assimilation, which are strongly dependent on light.

Despite the potentially complex interactions between C, N, Si, irradiance, and growth stage in diatoms, progress has been made towards developing generalized mechanistic models of diatom physiology (e.g., Claquin *et al.*, 2002; Flynn and Martin-Jézéquel, 2000). Although cell division and growth rate have the most direct impact on Si-metabolism (*cf.*, Martin-Jézéquel *et al.*, 2000), light, temperature,
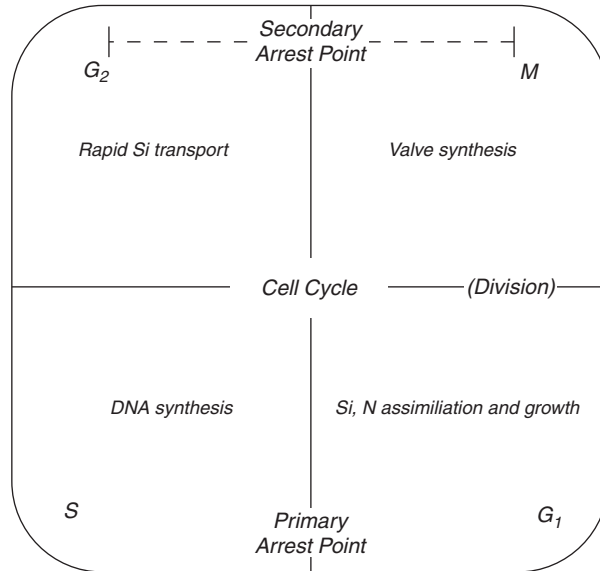
Figure 37.2 A simplified schematic view of cell cycle progression in diatoms, with critical arrest points and dominant processes indicated. Most diatoms arrest at the G1/S boundary, but a secondary arrest point can occur during the G2/M phase. M: mitosis; S: DNA replication (synthesis); G1 and G2: gap phases.

and both macronutrient (N, P) and micronutrient (Zn, Fe) concentrations also affect growth rate, and therefore indirectly, Si-metabolism. Recent genomic analysis of T. pseudonana suggests that the link between Fe and Si metabolism may also be more direct, with a set of 84 genes showing an inducible response to both Si and Fe limitation (Mock et al., 2008). Conversely, Si is strongly regulated by the cell cycle, as are some components of nitrogen metabolism (Claquin et al., 2004; Hildebrand and Dahlin 2000; Olson et al., 1996; Vaulot et al., 1987). Under nutrient limitation, the cell cycle can be prolonged, or even halted. For example, N stress prolongs G1 as well as G2 (Olson et al., 1996), and previous authors have identified the G2+M phase in diatoms as an ''arrest point'' for cell division under various conditions (Brzezinski et al., 1990; Claquin et al., 2002; Martin-Jézéquel et al., 2000). When this occurs, Si-uptake continues so long as Si-substrate limitation does not occur, but cell division is halted, resulting in increased per-cell Si content and frustule thickness. Conversely, under Si-limitation, cell division is also halted but there can be increased cell quotas of C, N, P, and chlorophyll (Harrison et al., 1977).

Although any stressor that slows growth in diatoms can result in a shift in the elemental composition of diatoms (e.g., Claquin et al., 2002; Harrison et al., 1977), the shift towards higher values in the Si:C and Si:N ratio has frequently been used as a diagnostic indicator for impaired growth due to Fe-limitation (e.g., Firme et al., 2003; Hutchins and Bruland, 1998; Takeda, 1998). When diatoms are stressed for Si rather than some other environmental factor, cell growth is also halted at the end of G1 and G2 (Brzezinski et al., 1990), although obviously there is no accumulation of Si in the cell. Intriguingly, a recent study by De La Rocha and Passow (2004)

suggests that diatoms, by arresting growth in the G2+M stage under Si-limitation, may obtain a competitive advantage relative to cells limited by N (but see discussion in Martin-Jézéquel *et al.*, 2000; Raven and Waite, 2004). This is because Si-starved cells can accumulate excess C and N, giving these cells a head start when rate-limiting nutrients are resupplied. Diatoms that are N-starved often exhibit lower cell quotas for C, chlorophyll, proteins, and amino acids (Dortch, 1982; Harrison *et al.*, 1977), thus making rapid recovery upon nutrient resupply more difficult. De La Rocha and Passow (2004) further demonstrate via the use of a model simulation that descendents of N-starved cells would never catch up to descendents of Si-starved cells under a pulsed nutrient scenario such as occurs in upwelling regions (see Chapter 17 by Wilkerson and Dugdale, this volume).

Despite this decoupling of C, N, Si, and irradiance (or the occurrence of a much more complicated interaction precluding a direct relationship), there is evidence in the field and laboratory for a direct relationship between Si-metabolism and irradiance under some conditions. As an example, Fig. 37.3 presents the results of simultaneous tracer measurements for carbon (^{14}C), nitrogen (^{15}N), and silica (^{32}Si) from a diatom-dominated natural assemblage during a typical spring upwelling event (described in more detail by Brzezinski *et al.*, 1997; Kudela and Chavez, 1997). All of the substrates demonstrate a positive uptake versus irradiance response, which is not in Redfield proportions over the short (*ca.* 4 h) duration of the experiment. These results are similar to a field study in the Hudson River estuary by Malone *et al.* (1980), who found carbon assimilation and Si-metabolism to be coupled, with strong diel periodicity evident. Despite the overall pattern of uncoupling between C (light) and Si-metabolism, some field studies have also shown a decrease in Si-uptake near the bottom of the photic zone (e.g., Brzezinski *et al.*, 1997, 2001; Leynaert *et al.*, 2001; Smith *et al.*, 1999), suggestive of, at minimum, Redfield-ratio coupling of metabolism.

There is also evidence for a feedback-mechanism between Si and photosynthetic performance, contrary to expectations. Lippemeier *et al.* (1999) found that in batch culture, the diatom *T. weissflogii* decreased photochemical efficiency in response to Si-limitation as measured by a pulsed amplitude modulation (PAM) fluorometer. This response looks functionally like Fe-limitation, with a decrease in photosynthetic performance under increasing Si stress; as with Fe-limitation, this inhibition is readily and rapidly (20 min) reversible with the addition of $Si(OH)_4$, followed by a prolonged (18 h) recovery to full health, as determined by changes in variable fluorescence. More recently, Claquin *et al.* (2004) demonstrated that photosynthetic capacity in *C. fusiformis* is highest during G1, directly linking Si-metabolism and photosynthetic competency to changes in the cell cycle. We have extended the results of Lippemeier *et al.* (1999) to Si-limited chemostats and batch cultures using the toxic diatom *Pseudo-nitzschia multiseries* (Fig. 37.4), grown under either Si or N-limitation, at a range (16–100% of maximal) of growth rates and constant light (Kudela *et al.*, 2002). The quantum efficiency of PSII (variable fluorescence: F_v/F_m) declined from the theoretical maximum (*ca.* 0.7) at 100% growth rate to *ca.* 50% of maximum at 16% growth rate. The decrease in F_v/F_m indicates that decreases in photochemical efficiency are regulated under both quasi-steady-state (chemostat) as well as nonsteady-state (batch) conditions, and have been confirmed for a second species (*P. australis*). As with *T. weissflogii*, the initial recovery upon re-addition of Si occurred nearly instantaneously, but the duration to full health (as indicated by
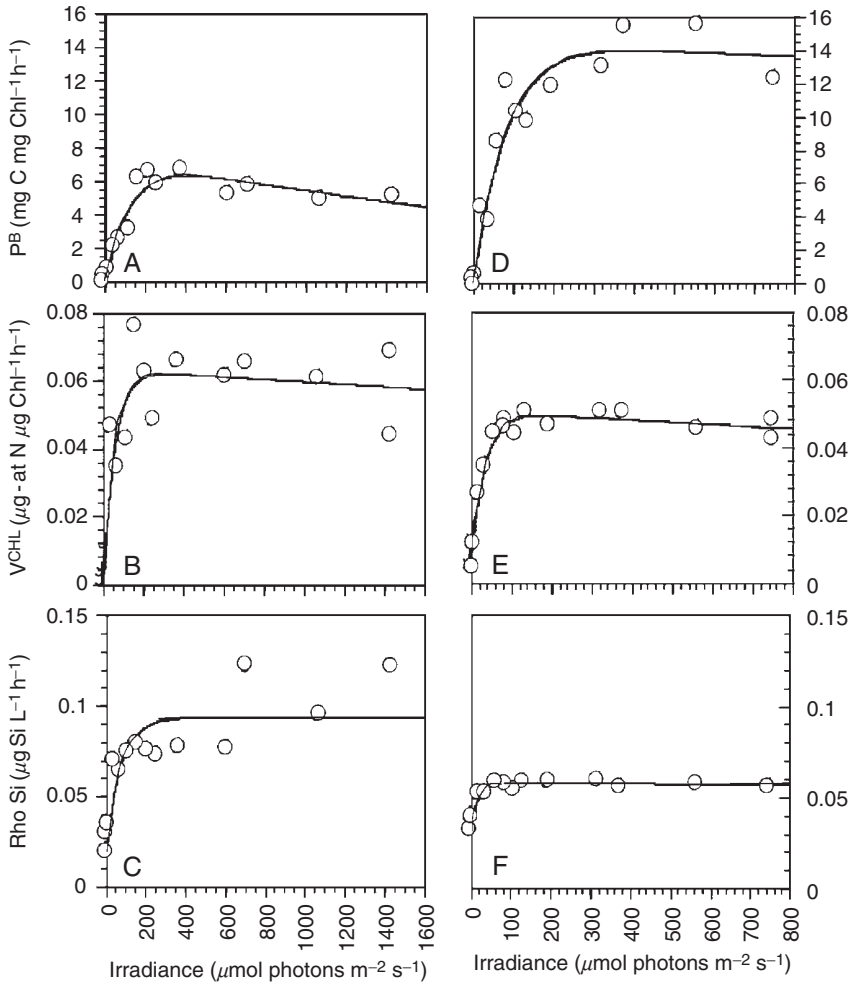
Figure 37.3 Uptake versus irradiance plots for two stations from Monterey Bay, California showing carbon (A, D) nitrate (B, E) and silicate (C, E) uptake estimated using stable (^{15}N) and radiotracers (^{14}C, ^{32}Si). The first station (A–C) was collected from freshly upwelled surface waters; the second station (D–F) was collected downstream, *ca.* 4 days from the source of upwelling. Experiments were conducted at ambient temperature for ca., 4 h at midday, as detailed in (Brzezinski *et al.*, 1997; Kudela and Chavez, 1997). Data were fitted to a PE relationship using Eq. (37.3).

F_v/F_m) depended on the amount of time the cells were left in Si-starved media. Recovery in fluorescence parameters was similar to recovery times seen for other nutrients such as N, P, and Fe (Berges *et al.*, 1996; Geider *et al.*, 1993). The rapid recovery (minutes) rules out the recovery of photochemical efficiency by a coupling to protein synthesis, which occurs on the order of hours (Werner, 1977), while the decoupling of F_v/F_m and optical density (division rates) suggest that the changes are not directly attributable to a reinitiating of the cell cycle.
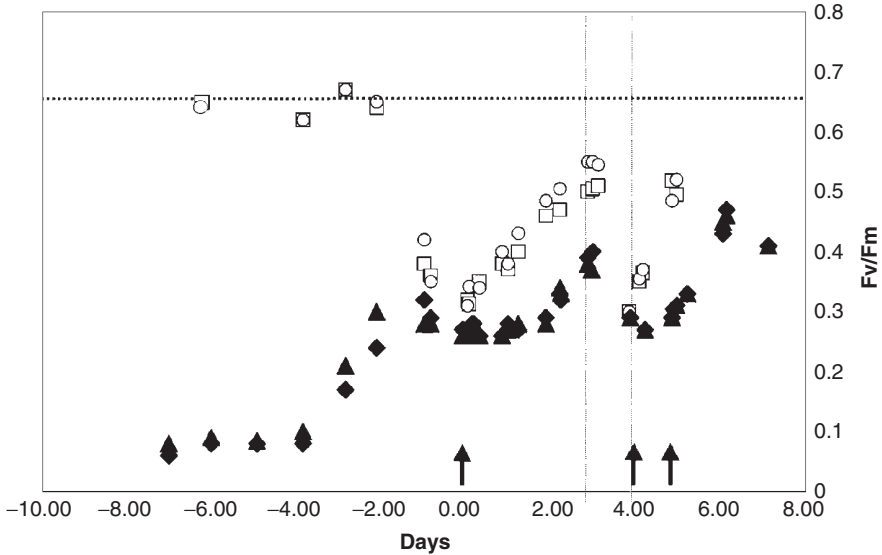
Figure 37.4 Replicate *Pseudo-nitzschia multiseries* cultures were grown under 24 h light (100 μmol photons m^{-2} s^{-1}) at 15 °C for 4 days as an auxostat (chemostat with growth limited by pump rate) with Si-limited f/2 media. On day −4, the pumps were turned off, forcing the cultures into Si-limitation. On days 0, 4 Si-spikes (10 μM) were added. On day 5, trace-metals (f/2 stock) was added, with no apparent response. Optical density (cell abundance) and variable fluorescence were determined from a PAM fluorometer and are plotted versus time (dashed vertical lines indicate additions of unenriched seawater; dashed horizontal line indicates maximal F_v/F_m values in healthy cells). A rapid decline and recovery of variable fluorescence indicates impaired photosynthetic performance, and functionally mimics the response of Fe-limitation, with recovery times dependent on the length of time spent in Si-deprived conditions.

The results of these field and laboratory experiments imply that Si-metabolism is more tightly linked to photosynthesis than previously thought. It is not clear what mechanism is responsible for this interaction; Lippemeier *et al.* (1999), posited that there was an inhibitory feedback on the assimilatory enzyme ribulose bisphosphate carboxylase/oxygenase (RubisCO). Under Si-limitation, a buildup of the internal pools of the amino acids serine and glycine, which are used as part of the protein matrix during Si-deposition, results in a down-regulation of RubisCO, and to a decrease in photosynthetic efficiency caused by a change in the electrochemical gradient of the chloroplast (details are provided in Lippemeier *et al.*, 1999). In our study we saw no evidence for a change in the ratios of serine and glycine relative to other amino acids, nor a pronounced increase in the free amino acid pools necessary for this mechanism (Smith, unpublished data).

Another potential connection between Si-metabolism and photosynthetic competency has been proposed by Milligan and Morel (2002). Those authors demonstrated that biogenic silica provides good buffering capacity in seawater, and could potentially be used as a proton donor. Specifically, Milligan and Morel suggest that to enhance the activity of RubisCO, which is undersaturated for CO_2 at ambient

seawater conditions, diatoms employ external carbonic anhydrase (CA) as a carbon concentrating mechanism. CA activity requires a proton buffer, the diatom frustule. Those authors further speculate that other biochemical processes may also benefit from a proton buffer, giving diatoms a competitive advantage relative to other microalgae. Regardless of the underlying mechanism, it is clear that there is the potential for direct coupling of photosynthesis and Si-metabolism, and hence a more direct relationship between C, N, and Si via photosynthesis in diatoms.

3. Ecological Interactions

3.1. Diatoms versus coccolithophores

Diatoms tend to dominate the phytoplankton community whenever conditions are optimal for phytoplankton growth, and provide a disproportionate fraction of the total and export primary production in the world's oceans, including the oligotrophic tropical oceans (Leynaert *et al.*, 2001). Sediment-trap data from a variety of ocean habitats showed strong temporal correlation between the downward fluxes of POC and diatom opal, implying that diatoms are the autotrophic source of much of the sinking POC (Armstrong *et al.*, 2002; Klass and Archer, 2002). Fast rates of NO_3 uptake and high *f*-ratios are generally caused by a dominance of larger phytoplankton, which on death sink faster and are considered to be the major contributors of export (or new) production. Small plankton sink relatively slowly (or not at all) and are recycled primarily within the euphotic zone by microzooplankton predation, even in systems where they are the dominant primary producers (Michaels and Silver, 1988; Probyn, 1992). Studies of natural assemblages using size-fractionation and ^{15}N tracers have demonstrated that small phytoplankton use relatively higher proportions of NH_4 and urea for growth, resulting in lower *f*-ratios (e.g., Kudela and Dugdale, 2000; Probyn, 1985; Sahlsten, 1987). Thus, diatoms also have a disproportionate influence on marine biogeochemical cycles and global climate (e.g., Field *et al.*, 1998; Raven and Waite, 2004).

Diatom dominance in the modern ocean tends to occur whenever there is more than *ca.* 2 µM Si, regardless of time of year, and over a range of environmental conditions (e.g., Egge and Aksnes, 1992). Diatom dominance includes spring blooms, coastal upwelling, equatorial upwelling, river plumes, decay of ocean eddies and transient open ocean wind events, and Fe-perturbations (see review by Ragueneau *et al.*, 2000). Diatoms are particularly well adapted to conditions favoring large cells (Chisholm, 1992). Diatoms have low surface to volume ratios, and are dominant in high-nutrient, turbulent conditions (e.g., Margalef, 1978). In addition to size-specific effects, diatoms are also well adapted to growth under low light conditions (Goldman, 1993), and there is evidence that the large capacity for dark-uptake of nitrogen by diatoms may provide a competitive advantage over other organisms such as flagellates (Clark *et al.*, 2002), while diatoms may also utilize N assimilation as an electron sink when rapidly transferred from low to high light conditions (Lomas and Glibert, 2000; Lomas *et al.*, 2000). As a result, so long as

silicic acid and other nutrients are nonlimiting, diatoms tend to exhibit higher maximum growth rates and dominate the algal assemblages (e.g., Egge and Aksnes, 1992; Furnas, 1990; Wilkerson *et al.*, 2000).

The influence of marine phytoplankton on global climate depends not only upon total primary productivity, but also on the taxonomic composition of algal assemblages. Planktonic photosynthesis contributes significantly to the oceanic uptake of anthropogenic CO_2, (Sarmiento and Le Quéré, 1996; Sarmiento and Toggweiler, 1984), but certain phytoplankton play a disproportionate role, by modifying the elemental fluxes to the deep ocean. The net effect of phytoplankton on the oceanic carbon cycle and climate is thus determined by the relative rates of photosynthesis and calcification in surface waters, and by the efficiency of organic C and calcite transport (the ''biological'' and ''carbonate'' pumps) to the deep ocean.

Ocean organic carbon storage by the biological pump is disproportionately driven by a few algal groups such as large diatoms relative to other ecologically dominant but smaller-celled taxa (Boyd and Newton, 1999; Chisholm, 1992; Michaels and Silver 1988; Wilkerson *et al.*, 2000), and by the incorporation of dense coccolithophorid calcite into sinking particles (François *et al.*, 2002; Klaas and Archer, 2002). Although diatoms tend to dominate the large phytoplankton size classes in the modern ocean, they are not the only phytoplankton cells that produce mineral ballast with correspondingly fast sinking rates and the potential for enhanced carbon export to the deep ocean. For instance, diatom nitrate reductase activity declines rapidly at elevated temperatures, potentially favoring the growth of competing species (Lomas and Glibert, 1999). Indeed, shipboard experiments in the subarctic Pacific demonstrate a shift from diatoms towards coccolithophorids at increased temperatures, and unprecedented recent *Emiliania huxleyi* blooms in the Bering Sea have been linked to changing climate regimes, including increased temperature (Napp and Hunt, 2001; Stockwell *et al.*, 2001; Sukhanova and Flint, 1998) as well as to enhanced ammonium concentrations, while silicic acid and Si:N ratios appeared not to be controlling community structure (Shiomoto, 1999). Estimates of global export production are also closely coupled to sea surface temperature via changes in community structure, and a warmer ocean may be expected to result in dramatically lower export of carbon with the Laws, 2000.

Coccolithophores produce external platelets (coccoliths) of calcium carbonate ($CaCO_3$) whose density is similar to that of SiO_2 and thus enhances the sinking of cells and their remains. In the North Atlantic Bloom (NAB), a typical succession pattern includes an initial dominance by diatoms giving way to later communities dominated by nanoplankton, especially coccolithophorids (Lochte *et al.*, 1993). This pattern has been attributed to silicate depletion during the early diatom-dominated bloom phase (Sieracki *et al.*, 1993), and *E. huxleyi* also appears to be adapted to low phosphate and high light (Tyrrell and Taylor, 1996). *E. huxleyi* has been reported to resist Fe-limitation when grown on ammonium, rather than nitrate (Muggli *et al.*, 1996); although iron is unlikely to be limiting in the North Atlantic, laboratory data on *E. huxleyi* (Sunda and Huntsman, 1995) have demonstrated that they can grow at near optimal rates with $10–100\times$ less iron than coastal diatoms, which have a high iron requirement and are easily stressed by low Fe conditions (Bruland *et al.*, 2001). Sunda and Huntsman (1995) have shown that *E. huxleyi* has a significant cobalt

requirement and concluded that variations in the Co:Zn ratios in the ocean "could influence the relative growth of diatoms and coccolithophores." They suggested that high $[Zn^{2+}]:[Co^{2+}]$ ratios may initially inhibit *E. huxleyi* growth in freshly upwelled water through an antagonism of Co uptake and nutrition. All of these factors may be part of a more general response to stratification-induced nutrient depletion favoring coccolithophores over diatoms (e.g., Tozzi *et al.*, 2004).

From a modeling perspective, understanding the processes that control the proportion of primary production carried out by diatoms versus coccolithophores can influence the marine C cycle very strongly. For example, Harrison (2000) proposed that changes in $Si(OH)_4$ supply may have influenced the relative success of diatoms and coccolithophores on glacial/interglacial time scales, and that those floristic changes may have been a key to the well known glacial/interglacial transitions in atmospheric CO_2. Experimentally, studies in mesocosms with semi-continuous nutrient additions have shown that the coccolithophore *E. huxleyi*, and other flagellates, are out competed by diatoms when silicic acid is $>\sim 2$ μM and P, N, and Fe are present in nonlimiting concentrations (Egge and Aksnes, 1992). More recently, a comparison of Si-limited, but warmer, versus Si-enriched, but colder, waters north and south of the Antarctic Polar Frontal Zone during the Southern Ocean Iron Fertilization Experiment (SOFeX) demonstrated that total productivity may be enhanced in warmer waters, despite a net decrease in diatom growth (but with a net increase in nanoflagellate growth); the resulting export of carbon was greater than Redfield predictions, resulting from differential remineralization of C and N and the effects of ballasting (Coale *et al.*, 2004). However, Arrigo *et al.* (1999) and DiTullio *et al.* (2000) demonstrated that diatoms have less potential for export of carbon and nitrogen relative to *Phaeocystis* in the Southern Ocean, due to enhanced N recycling by diatoms (relative to *Phaeocystis*).

Superimposed on these physiological and taxonomic shifts during blooms are corresponding changes in nutrient assimilation patterns. Although it is often convenient to invoke Redfield ratios, natural assemblages are rarely assimilating nutrients at Redfield proportions, such that time-integrated assimilation ratios may not be in Redfield proportions, even when averaged over reasonably long time periods (Klausmeier and Litchman, 2004). As a result, export flux is also not necessarily in Redfield proportions, which can in turn lead to variable (or unpredictable) carbon sequestration (e.g., Arrigo *et al.*, 1999; Coale *et al.*, 2004). Recent experimental (Brzezinski *et al.*, 2003a) and modeling (Tagliabue and Arrigo, 2005) evidence highlights the importance of accounting for both species composition and stoichiometry in estimates of elemental flux.

3.2. Silica, nitrogen, iron ratios interactions

Of particular interest in relation to Si:N interactions and diatoms is the relatively recent finding that iron provides a strong regulating influence on the species composition of phytoplankton assemblages, as well as on the elemental ratios of diatoms. Fe and Si supply appear to be particularly important in several large areas where high concentrations of other macronutrients (N, P) are present in surface waters. These HNLC areas (Minas *et al.*, 1986) are characterized by low phytoplankton biomass

and elevated (i.e., nonlimiting) concentrations of macronutrients (specifically nitrate), a condition shared by *ca.* 40% of the world's ocean (Fig. 37.5). Fe-enriched shipboard incubation experiments (also called grow-out experiments) have been conducted in the subarctic North Pacific (e.g., Boyd *et al.*, 1996; Coale, 1991; Martin *et al.*, 1989), equatorial Pacific (e.g., Martin *et al.*, 1991; Price *et al.*, 1991, 1994); the Southern Ocean (e.g., Cochlan *et al.*, 2002; de Baar *et al.*, 1990; Helbling *et al.*, 1991; Martin, 1990; Martin *et al.*, 1991), and the coastal waters of the north and south Pacific (e.g., Firme *et al.*, 2003; Franck *et al.*, 2005; Hare *et al.*, 2005; Hutchins *et al.*, 1998, 2002). Biomass levels increased and macronutrients (nitrate and phosphate) were greatly reduced after enrichment, providing strong support
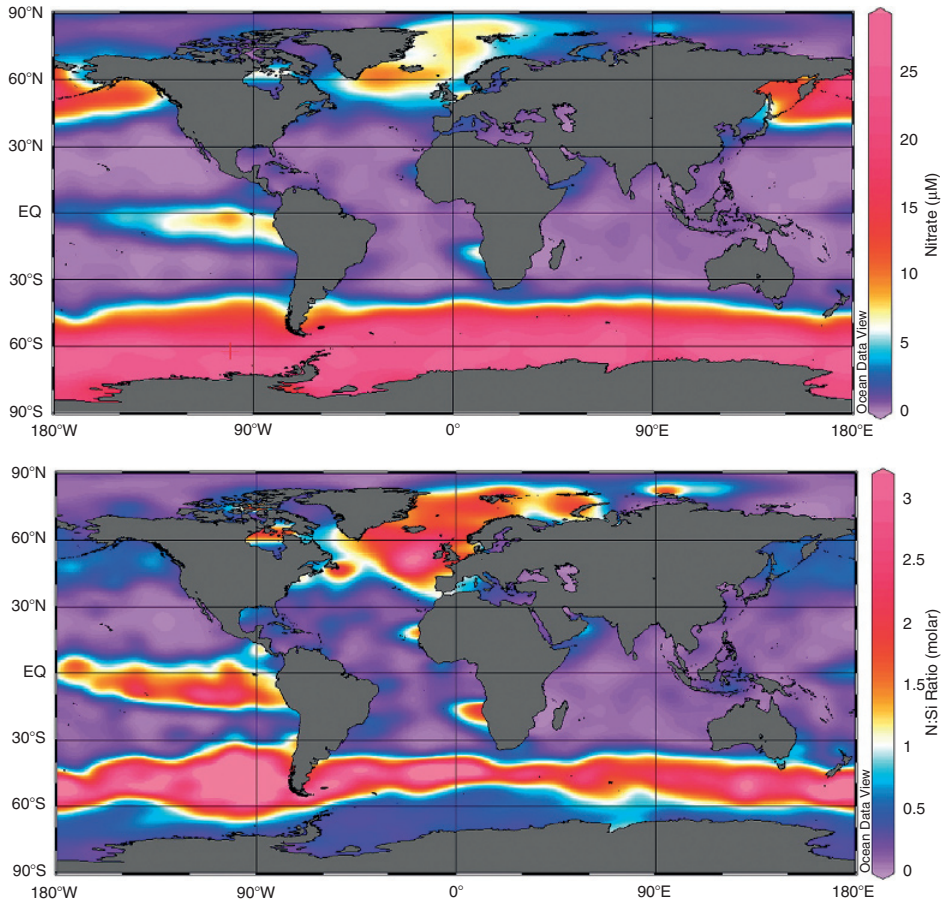
Figure 37.5 A global map of nitrate (top) and the Si:N ratio (bottom) for surface waters of the world's oceans. The elevated surface N values (top) roughly correspond to the world's Fe-limited HNLC regions (Minas *et al.*, 1986); in contrast, the elevated N:Si ratio in the Antarctic Polar Front and equatorial Pacific suggest that these areas might be better classified as HNLSiLC waters (Dugdale *et al.*, 1995). The North Atlantic and African upwelling systems, while exhibiting elevated nitrate and a low N:Si ratio, are regions of elevated chlorophyll. Data source: World Ocean Atlas, 2001 (Schlitzer, R., Ocean DataView, http://www.awi-bremerhaven.de/GEO/ODV, 2005).

for the idea that Fe limits phytoplankton growth in HNLC areas. These findings have been confirmed and greatly extended with the subsequent development of mesoscale Fe-enrichment experiments in all of the major HNLC regions (with the de Baar and Boyd, 2007).

Despite the extensive confirmation of Fe-limitation in HNLC waters, and subsequent dominance by diatoms when Fe-limitation is removed, not all HNLC waters are identical, as one would expect (see review by de Baar and Boyd, 2000), and there are some HNLC regions that are also strongly regulated by silicic acid (Fig. 37.5). Partially to address this issue, (Dugdale et al., 1995) invoked the concept of a "High Nitrate, Low Silicate, Low Chlorophyll" (HNLSiLC) system. According to this classification scheme, the Si:N ratio in some HNLC regions (notably the eastern equatorial Pacific upwelling regime and parts of the Southern Ocean; (Brzezinski et al., 2005; Dugdale and Wilkerson, 1998; Dugdale et al., 1995) is regulated in a chemostat-like fashion, where silicic acid concentrations provide a "set point" for diatom production, which in turn limits the drawdown of nitrogen. This occurs because biogenic silica is rapidly and preferentially exported from the euphotic zone relative to nitrogen; this is referred to as the "silicate pump" (Dugdale et al., 1995), or more correctly, the "silica pump", since it is biogenic Si and not silicate, that is being exported (Brzezinski et al., 2003b).

The silica pump model is based in large part on the observation that while silicic acid accumulates at depth along the path of the deep-water conveyer belt (Broecker and Peng, 1982) and is typically found in ratios significantly greater than 1:1 (Si:N) at mid-water depths and deeper (Rageuneau et al., 2000), the Si:N ratio is often <1:1 in near-surface waters. According to the original silica pump hypothesis, little or no silicic acid is recycled in the euphotic zone, making Si dynamics strikingly different from N (or P) dynamics (Dugdale et al., 1995), and leading to the preferential loss of Si relative to N from the euphotic zone. As a result, the Si:N ratio of waters in the near-surface, including the euphotic zone, are often less than the optimal stoichiometric ratio of diatoms. This is hypothesized to ultimately lead to Si-limitation of diatoms, and a shift from large cells to smaller, grazer-controlled phytoplankton, resulting in the development of HNLSiLC conditions, despite the abundance of Si at depth. One necessary prerequisite for the silica pump model to be valid is that biogenic Si is preferentially exported relative to particulate organic C and N, or conversely that very little Si regeneration (dissolution) occurs in the surface ocean. Since seawater is always undersaturated for Si, biogenic Si will always dissolve when exposed to seawater, largely dependent on the thermodynamics of the rate reaction (i.e., to first order, dependent on temperature). Very few studies have directly quantified what fraction of Si production is maintained by the regeneration of biogenic silica in the near-surface; however, work by Nelson et al. (1995) and Brzezinski et al. (2003b) suggest that the global average may be as high as 50%. If so, then Si production would (as an annual average) behave much more like C and N; recent estimates of global f-ratios provide an annual average of ~20% export of net photosynthesis (Laws, 2000), comparable to the ca. 50% regeneration rate for silicic acid.

Despite the potentially high global average for the ratio of Si production to dissolution, Brzezinski et al. (2003) argue that this average is driven by mean non–bloom ratios that are elevated, while the ratio during diatom blooms, expected

to account for the majority of flux out of the euphotic zone, exhibit much lower ratios. Thus, although it is not strictly true that there is little or no silicic acid recycling in surface waters (e.g., Beucher *et al.*, 2004; Bidle and Azam, 1999; Brzezinski *et al.*, 2003b; Fujii and Chai, 2005; Nelson *et al.*, 1995), this makes a reasonable approximation, and models based on this assumption have been used to evaluate the role of diatom production in the modern and paleo-ocean (e.g., Chai *et al.*, 2002; Dugdale *et al.*, 1995, 2004; Moore *et al.*, 2002; Mongin *et al.*, 2003; Pondaven *et al.*, 1998).

In the silica pump scenario, Fe is thought to control the set point for Si:N drawdown, by modulating the growth of diatoms (with a high Fe requirement) relative to other, non-siliceous phytoplankton (e.g., Franck *et al.*, 2003). This has been attributed to the higher Fe-requirement for large diatoms relative to pico-plankton (e.g., Boyd *et al.*, 1996; Coale *et al.*, 1996; Franck *et al.*, 2005; Hudson and Morel, 1990; Price *et al.*, 1994), although there is also a differential Fe requirement among diatoms, related to the occurrence of coastal versus oceanic species (Bruland *et al.*, 2001, 2005). As discussed above, Si-uptake will occur so long as there is ambient silicic acid in the environment, even when N or Fe-limitation inhibits cell division. As a result, Fe-stressed cells exhibit markedly higher Si:C and Si:N ratios compared to Fe-replete cells (Boyle, 1998; Hutchins and Bruland, 1998; Takeda, 1998). In almost all Fe-enrichment studies to date, whether in enclosures or as mesoscale enrichments, diatoms are the dominant group of phytoplankton respond-ing to the enrichment (Boyd *et al.*, 2000; Coale *et al.*, 1996, 2004; Smetacek, 2001; Tsuda *et al.*, 2003). When combined with the concept of a silica pump, the interrelationships between Si and Fe-limitation and stoichiometry have the potential to profoundly influence the bigeochemical cycling of carbon and nitrogen, particu-larly over glacial/interglacial time scales. Although Si:C:N:Fe interactions can potentially occur throughout the world's oceans, the Southern Ocean is of particular importance, because of its role in regulating global climate.

3.3. The southern ocean

The Southern Ocean is thought to be one of the most important areas of the world in regulating global climate (Anderson, 1993). Model simulations (Knox and McElroy, 1984; Sarmiento and Le Quéré, 1996; Sarmiento and Toggweiler, 1984; Sarmiento *et al.*, 2004; Siegenthaler and Wenk, 1984) suggest that, over time scales of 10^2–10^5 years, atmospheric carbon dioxide is controlled largely by conditions in the polar oceans and that the largest CO_2 fluxes to the ocean occur south of 40° (Sarmiento *et al.*, 1992). These Southern Ocean fluxes demonstrate high sensitivity to changes in primary productivity and global warming (Sarmiento and Le Quéré, 1996; Sarmiento *et al.*, 2004), which arises in part because large amounts of the major plant macronutrients remain unused, providing the potential for much greater primary production than occurs in the modern ocean. These regions are also the source of abyssal water in all the ocean basins, and under present conditions, these regions are the main routes by which the huge reservoir of dissolved carbon in the deep sea exchanges with the atmosphere. As a consequence, Southern Ocean open waters have a disproportionately large influence on atmospheric carbon

dioxide even though they represent only 10–20% of the oceanic surface area. Sarmiento and Toggweiler's model further suggests that complete utilization of the macronutrients in the Southern Ocean HNLC regions would result in a decrease in the (pre-anthropogenic) steady state atmospheric CO_2 on the order of 80 ppm (Sarmiento and Toggweiler, 1984), equal to the drawdown during glacial periods.

Previous studies in HNLC regimes including the Southern Ocean have revealed that despite high ambient NO_3 concentrations, autotrophic N utilization is usually met by regenerated NH_4 and urea from within the euphotic zone (review by Dugdale and Wilkerson, 1992; Chapter 12 by Cochlan, this volume). Brzezinski et al. (2003a) have demonstrated that under Fe-limited conditions in the Southern Ocean, diatoms will increase utilization of regenerated N compounds to maintain a quasi-Redfield cellular composition while growing, but that Si:N export of particles is increasingly enriched in Si, consistent with the silica pump hypothesis. As a result, diatoms in Fe-limited HNLC regions precipitate silicate at ratios of ca. 4:1 (molar) for Si:N (e.g., Brzezinski et al., 2005; Firme et al., 2003; Franck et al., 2005; Hutchins and Bruland 1998; Pondaven et al., 2000; Takeda, 1998) rather than the expected ratio of ca. 1:1 for Fe-replete diatoms (Brzezinski, 1985). With the release of Fe-limitation, the stoichiometric composition of the particulate material again approaches 1:1 (Bruland et al., 2005) while the f-ratio increases, resulting in a drawdown of excess nitrate (Brzezinski et al., 2002; Coale et al., 2004).

Because the subantarctic mode water, formed in the Southern Ocean, is the main source of nutrients for the world's oceanic thermocline (Sarmiento et al., 2004), any changes in the drawdown ratio of Si:N in the Antarctic will have a direct impact on phytoplankton at lower latitudes. The subantarctic intermediate and mode water ratio of Si:N is <1:1 in the modern ocean (Dugdale and Wilkerson, 2001); if there were major changes in the availability of Fe, and therefore a shift in the Si:N drawdown ratio in the Southern Ocean, it has been proposed that there would be a subsequent "silicic acid leakage" northward (Matsumoto et al., 2002). This leakage to northern latitudes would potentially favor diatoms relative to coccolithophores (see review by Archer et al., 2000), and is consistent with the higher opal content in the glacial subantarctic (Chase et al., 2003; François et al., 1997). More recently, Dugdale et al. (2004) have argued that the opal mass accumulation rate in the Antarctic and equatorial Pacific covary, casting doubt on the regulation of low latitudes by changes in the Si:N source ratio. Higginson and Altabet (2004) confirmed the increase in coccolithophore:diatom production in Peru margin sediments using class-specific phytoplankton biomarkers from sediment cores during glacial/interglacial periods (18.0–15.5 Ka), but also demonstrated that there are inconsistencies in this record if the sole source of variability was silicic acid leakage from the Southern Ocean. Another alternative hypothesis has been proposed by Harrison (2000), who suggested that direct changes in silicic acid concentration could be caused by dissolution of silica deposited on the surface ocean by dust events, based on increased Aeolian transport during glacial periods. This "silica hypothesis" would result in a 40% decrease in coccolithophores with a 2–8× increase in dust concentrations.

Although the precise mechanisms for the changes in pCO_2 during glacial periods is unclear, it is evident that changes in Si:N export (the biological and silica pumps),

possibly driven by variability in Fe availability, and the subsequent changes in nutrient ratios for the worlds oceans can have profound effects on the phytoplankton composition over millennial time scales, resulting in oscillating patterns of diatom versus coccolithophore dominance. Climate-driven shifts in terrestrial ecosystem diversity are now well documented (Korner, 2003; Walther *et al.*, 2002), but similar experimental evidence for marine environments is scarce or nonexistent (see review by Riebesell, 2004). This information is essential to parameterize more realistic models that can predict future changes in climate regimes and biogeochemical fluxes (e.g., Boyd and Doney, 2002), and the interplay of Si:N ratios in the ocean are a critical component.

4. ANTHROPOGENIC CHANGES IN SILICA TO NITROGEN RATIOS

4.1. Causes and consequences of silica to nitrogen ratios changes

For much of the world's oceans, silicic acid reaches the euphotic zone via upwelling, and the upper limit for biological production by diatoms is set by the ratio of Si to N (Dugdale and Wilkerson, 1998; Dugdale *et al.*, 1995) modulated by Fe-availability (e.g., Leynaert *et al.*, 2004). The source of this dissolved Si is ultimately from the chemical weathering of the continental crust, with subsequent input to the coastal ocean by rivers (*ca.* 84% of dissolved Si input to the oceans; Tréguer *et al.*, 1995). The second largest source, Aeolian transport, is substantially lower (*ca.* 7.5%; Tréguer *et al.*, 1995), although there is evidence that Aeolian deposition of Si can have a great impact on biological productivity on time scales greater than about 10,000 years (Harrison, 2000). Coastal waters play a disproportionately important role in marine productivity; although these regions represent only 10% of the oceanic area and less than 0.5% by volume, coastal marine ecosystems produce nearly 95% of the annual global production of marine biomass (Sherman, 1991), while shelf and delta-influenced sediments account for ca., 80% of the organic carbon sequestration in the modern ocean (Berner, 1982; Jahnke *et al.*, 1990; Walsh, 1991), and riverine inputs to the coastal ocean have been shown to impact the annual global carbon budget (Smith and Hollibaugh, 1983).

Despite the high silicic acid load associated with rivers (discharge-weighted average silicic acid concentrations have been estimated at 150 μM; Tréguer *et al.*, 1995), there is ample evidence for biological removal in estuaries and coastal waters. Nelson and Dortch (1996) demonstrated biological removal of 80–99% of the silicic acid in the Mississippi River plume. Milliman and Boyle (1975) reported a 25% drawdown of silicic acid within the Amazon River estuary, with further depletion in the nearshore ocean. Kimmerer (2005) demonstrated *ca.* 85% drawdown of silicic acid within San Francisco Bay, directly related to diatom production. In contrast, silicic acid concentrations are rarely less than 0.5 μM in much of the world's oceans (*cf.* Nelson and Dortch, 1996), despite the prevalence of diatoms and the dominance of diatom productivity in export flux.

Given the importance of riverine input to Si cycling in the oceans, any changes in the source or nutrient ratios could have an impact on diatom production and

ecological structure in the oceans. Inputs of reactive N have increased globally by a factor of 20 since 1860, due to a combination of fossil fuel combustion, rice and legume production, and fertilizers; today, anthropogenic N fixation exceeds natural terrestrial fixation (Galloway and Cowling, 2002). Accompanying this increase in reactive N, phosphorous, also from fertilizers, has increased as well; the net result is that N and P loading to the coastal zone from rivers has increased by a factor of 2.5 and 2.0, respectively (Humborg *et al.*, 2000). In contrast, the global flux of riverine Si has been steady or decreasing (Humborg *et al.*, 1997, 2000; Turner *et al.*, 2003b), since human activities for the most part have not increased the use of Si in the terrestrial environment. The net result is that the elemental ratio of Si:N:P in these rivers has also decreased globally, with *ca.* 7.3% and 21% of the world's population now living in watersheds with a Si:N ratio of 1:1 and 2:1, respectively (Turner *et al.*, 2003b).

There is no a priori reason to expect a reduction in dissolved Si to have a direct impact on marine primary production, since a change in the Si:N:P ratio should simply select against diatoms and for non–siliceous organisms (e.g., Egge and Aksnes, 1992). However, as discussed above, diatoms are associated with enhanced productivity in the marine environment, and one might expect at minimum, a change in the ecological structure of coastal food webs as the Si:N ratio approaches the optimal 1:1 requirement of diatoms (Brzezinski, 1985). As the Si:N ratio is reduced, there will be a corresponding decrease in efficiency of Si–transport relative to that of N transport (i.e., the ratio of the ambient Si concentration to the K_s for Si is constant or decreasing, while the ratio of the ambient N concentration to the K_s for N is constant or increasing, favoring N–utilization and inhibiting Si–utilization). This is not a new concept, and the potential role of Si:N dynamics in modulating cultural eutrophication has been of interest for several decades (Conley *et al.*, 1993; Officer and Ryther, 1980). The first well publicized evidence for widespread changes in ecological function related to shifts in Si:N ratios was provided by Schelske and colleagues, in a series of papers documenting shifts in phytoplankton community composition for the Laurentian Great Lakes in response to anthropogenic changes in land use and water quality (Schelske and Stoermer, 1971, 1972; Schelske *et al.*, 1983).

Since those first studies, there have been dramatic effects reported in the marine environment directly related to changes in the Si:N:P ratios of riverine inputs to coastal waters, due to both increased loading of N and P, and decreases in Si (Conley *et al.*, 1993; Ittekkot *et al.*, 2000; Turner *et al.*, 2003b). Some of these changes have been directly attributed to cultural eutrophication (e.g., Turner and Rabalais, 1991, 1994) while other regions have been heavily influenced by hydrological changes related to the building of large dams (e.g., Humborg *et al.*, 1997, 2000; Ittekkot *et al.*, 2000; Liu *et al.*, 2003; Milliman, 1997). Although dams potentially reduce all nutrients (N, P, Si, Fe) by trapping them in reservoirs and/or promoting freshwater blooms (Humborg *et al.*, 1997), N and P are largely replaced downstream of the dam due to increased anthropogenic loads, while Si is not. The net ecological result can be difficult to predict, since the marine community may respond to increased nutrient loads (more diatoms) as well as to decreased Si:N ratios (fewer diatoms), as well as selection for and against specific species of phytoplankton (see reviews by

Conley *et al.*, 1993; Humborg *et al.*, 2000; Officer and Ryther, 1980). The Black Sea provides a particularly clear example of these potential consequences. The River Danube, which provides 70% of the freshwater input to the Black Sea, was dammed ("The Iron Gates") in 1970–1972, resulting in a decrease in the Si:N ratio from *ca.* 42 to 2.8 (Humborg *et al.*, 1997; Ittekkot *et al.*, 2000). In the decade following this hydrological alteration, diatoms increased by a factor of 2.5, but non-siliceous organisms, including dinoflagellates, the Prymnesiophyte *E. huxleyi*, the facultative toxic genera *Chromulina*, and the Euglenophyte *Eutreptia lanowii*, increased by a factor 6. While Si-limitation did not eliminate diatoms from the community, there was a dramatic shift in the overall phytoplankton composition, as expected (Humborg *et al.*, 1997).

4.2. Harmful algal blooms

Harmful algal blooms (HABs) appear to be increasing in frequency and intensity worldwide (Glibert *et al.*, 2005a). While the causes for this are potentially complex, one oft-cited rationale for this trend is the concomitant change in nutrient loading, and the stoichiometric shift in Si:N:P ratios (Anderson *et al.*, 2002; Glibert *et al.*, 2005b; Smayda, 1990). Officer and Ryther (1980) suggested that as Si:N ratios in marine waters approached unity, an alternative phytoplankton community (most likely flagellates) would replace diatoms as the dominant organism, as was seen in the Black Sea. Smayda (1990) argued that the apparent increase in HABs worldwide could be directly related to a shift in the Si:N ratio. Anderson *et al.* (Anderson *et al.*, 2002) concurred, although they pointed out that shifts in community structure related to HABs were often associated with changes in the N:P ratio as well as changes in the N:Si ratio.

There are many regional examples of HAB proliferation attributed to changes in the N:Si ratio to corroborate the apparent global patterns. In addition to the increase in *Chromulina* spp. associated with the damming of the Danube (Humborg *et al.*, 1997), Maestrini and Granéli (1991) attributed the exceptional bloom ($>10^7$ cells L^{-1}) of *Chrysocromulina polylepis* in the Skagerrak in 1988 to a series of environmental conditions favoring this flagellate. Specifically, Maestrini and Granéli proposed that high, N-rich runoff in the spring was followed by strong vertical stratification; this was followed by two large *S. costatum* (diatom) blooms, reducing the Si and P concentrations, but further enriching the waters in N. The subsequent weak turbulence, strong stratification, and elevated N:P and N:Si ratios favored the formation of the *C. polylepis* bloom, which subsequently inhibited grazers and other organisms via toxin production. Noxious blooms of the Prymnesiophyte *Phaeocystis pouchetii* in the North Sea have similarly been associated with selection against diatoms resulting from changes in the Si:N ratio (Anderson *et al.*, 2002; Radach *et al.*, 1990).

The greatest impacts from cultural eutrophication and hydrological modifications may have yet to be realized. Bouwman *et al.* (2005) predict a global increase in river N from 1970 to 2030 of 46% for the Indian, 38% for the Pacific, and only 6% for the Atlantic oceans, because of the predominance of developing countries in watersheds draining into the Indian and Pacific basins. These authors predicted a

corresponding increase in P loads, but no change or decreases in Si loads. Zhao *et al.* (2005) report that Jiaozhou Bay, on the eastern side of the Yellow Sea (China), has exhibited a shift from N–limitation towards P– and Si–limitation from 1960 to 1990, related to increasing anthropogenic loading. This shift in nutrient ratios is correlated with a decrease in phytoplankton biodiversity, and an increasing dominance of the phycotoxin–producing diatom *Pseudo-nitzschia*.

China also began development of the Three Gorges Project (TGP) in the upper region of the Changjiang (Yangtze River) in 1994, which when completed in 2010, will be the world's largest dam system (Zhang *et al.*, 1999). The Changjiang is the largest river in Asia, and is third in length, fourth in sediment discharge, and fifth in water discharge worldwide (Liu *et al.*, 2003). Changes in nutrient loading and stoichiometric ratios related to the TGP are already evident (Liu *et al.*, 2003), with N:Si and N:P ratios increasing relative to pre–dam periods. Chen *et al.* (2003) report that prior to the TGP project, the East China Sea was dominated by diatoms (*S. costatum*). With the shift in nutrient ratios, the predominant phytoplankton species have shifted to a suite of HAB organisms, including the dinoflagellates *Prorocentrum dentatum, Noctiluca scintillans*, and *Alexandrium* spp. (the latter produces paralytic shellfish poisoning). These changes in both nutrients and algal species are expected to continue to shift towards non–diatom production once the TGP is completed.

4.3. *Pseudo-nitzschia*: A case study

In 1987, the first confirmed case of a naturally occurring neurotoxic diatom bloom was reported in Prince Edward Island, Canada, later confirmed to be caused by the production of domoic acid from the pennate diatom *Nitzschia pungens*, now renamed *Pseudo-nitzschia multiseries* (Bates *et al.*, 1989; Subba Rao *et al.*, 1988). Since this first outbreak, many (but not all) species of *Pseudo-nitzschia* have been confirmed to produce domoic acid (as well as some species of *Amphora* and now *Nitzschia* (Kotaki *et al.*, 2000); see Bates, 1998, 2000, for a summary). These potentially toxic species include *P. multiseries, P. pseudodelicatissima, P. delicatissima, P. seratia, P. australis, P. pungens*, and *P. fraudulenta*, although it is apparent that not all species are toxic in all locations, nor are individual strains toxic under all conditions.

Some ecosystems are either intermittently or borderline Si limited, which allows diatoms with low Si requirements to dominate, rather than resulting in a shift to non–diatoms (e.g., Nelson and Dortch, 1996). One of the few direct examples of direct stimulation of a HAB organism by eutrophication can be found on the Louisiana shelf, related to the changing Si:N ratios of the Mississippi outflow. Blooms develop in the spring when river flow and nutrient loading is highest (Dortch *et al.*, 1997; Pan *et al.*, 2001). *Pseudo-nitzschia* is characterized as a lightly-silicified genus (Parsons and Dortch, 2002) and appears to be well adapted to low light and moderately low Si:N ratios (Sommer, 2004), and is tolerant to a wide range of salinities, although optimal growth is achieved at higher rather than lower salinity ranges (Thessen *et al.*, 2005). Abundances of *Pseudo-nitzschia* determined from sediment cores demonstrate a marked increase beginning in the 1950s, coincident with changes in nutrient loading of the Mississippi (Parsons and Dortch, 2002),

strongly suggesting that anthropogenic changes in Si:N ratios have directly selected for this cosmopolitan diatom. The recent evidence from China (Zhao *et al.*, 2005) suggests that these patterns are not isolated to the Louisiana shelf.

Multiple factors have been shown to trigger the production of domoic acid by the genus *Pseudo-nitzschia* (*cf.*, reviews by Bates, 1998, 2000; Bates *et al.*, 1998), but the most thoroughly characterized are macro-nutrient limitation by either phosphate or silicate (Pan *et al.*, 1996a,b,c). Despite the general trend for decreasing Si:N ratios to favor non-siliceous organisms, *Pseudo-nitzschia* has previously been associated with eutrophication and a reduction in these ratios. Freshwater runoff (which typically exhibits decreased N:Si ratios) has been implicated in triggering DA events (*cf.* review by Bates *et al.*, 1998) and there is circumstantial evidence that the massive domoic acid poisoning event which occurred in Monterey Bay, California in 1998 was similarly triggered by post-El Niño runoff (Scholin *et al.*, 2000). Thus, cultural eutrophication might have the unanticipated consequence of both selecting for *Pseudo-nitzschia* and promoting toxin production in this organism.

In addition to the apparent stimulation of *Pseudo-nitzschia* by riverine runoff, this organism also consistently blooms in response to Fe-additions to HNLC waters. Iron limitation directly modulates Si:N ratios in diatoms (see above), and has been linked to domoic acid production in *Pseudo-nitzschia*, which may serve as an Fe-acquisition mechanism (Maldonado *et al.*, 2002; Rue and Bruland, 2001); either directly, or through the stimulation of a Cu-mediated transport system (Wells *et al.*, 2005). Although domoic acid has not been measured in most of the Fe-enrichment experiments performed to date, potentially toxic *Pseudo-nitzschia* were stimulated in bottle experiments from the Pacific northwest (Boyd *et al.*, 1996), coastal California (Hutchins and Bruland, 1998; Hutchins *et al.*, 1998); and coastal Peru (Hutchins *et al.*, 2002). The toxigenic diatom was also identified in many of the mesoscale Fe-enrichment experiments, including Iron Ex II in the equatorial Pacific (Landry *et al.*, 2000), EisenEx (Gervais *et al.*, 2002) and SOFeX (Coale *et al.*, 2004) in the Southern Ocean, and in the subarctic Pacific as part of SERIES (Marchetti and Harrison, 2004). Based on these findings, it appears that anthropogenic changes in nutrient ratios, both due to Si:N changes in riverine runoff, and more recently due to direct manipulation of Fe-concentrations in the coastal and open ocean, may have the unintended consequence of selecting for the toxigenic diatom *Pseudo-nitzschia*.

4.4. Impacts on higher trophic levels

In addition to direct impacts of Si:N interactions in the marine environment, such as a shift towards flagellates or a promotion of potentially harmful algae, there are also indirect impacts on higher trophic levels. The world's fisheries are largely dependent on the formation of large diatom blooms in upwelling and temperate waters (Ryther, 1969). Copepods are generally considered to be the dominant grazers of diatom blooms (Irigoien *et al.*, 2002), although food quality and grazer deterrence by diatoms is also important (Harris, 1996; Jones and Flynn, 2005; Miralto *et al.*, 1999). Both Officer and Ryther (1980) and Conley *et al.* (1993) speculated that changes in the biogeochemical cycling of Si and N due to anthropogenic influences could lead to drastic changes in the food web structure of freshwater and marine systems due to the shift from diatoms to non-siliceous organisms, with subsequent increases in the

microbial food web (Sommer *et al.*, 2002) and a reduction in carbon transport to higher trophic levels (Cebrian, 2002, 2004; Sommer *et al.*, 2002).

In the Gulf of Mexico, changes in the Si:N ratio have led to increasing diatom species diversity (e.g., Nelson and Dortch, 1996) but also to a restructuring of the mesozooplankton populations (Turner *et al.*, 1998). Turner *et al.* reported a decrease in copepod abundance from >75% of the mesozooplankton population to <30% as the Si:N ratio decreases from 1:1 to 0.5:1. These food web shifts can in turn impact the occurrence and impact of bottom–water hypoxic events (Rabalais, 2002; Rabalais *et al.*, 2002; Turner and Rabalais, 1994; Turner *et al.*, 1998). In San Francisco Bay, Kimmerer (2005) attributes changes in the number and species composition of pelagic consumers to the decline of diatoms, again due to a shift in the Si:N ratio. Turner *et al.* (Turner *et al.*, 2003a) suggest that this shift from Si-enriched to N and P enriched river discharge is a global phenomenon, and that we may expect to see alternative, if not harmful, phytoplankton communities in an increasing number of coastal waters.

5. Conclusions

Despite the dominant role of nitrogen in the marine environment, the interplay of silica and nitrogen clearly has a strong modulating effect on ecological processes, from cellular metabolism to global climate. In the modern ocean, diatoms are the key organisms that regulate this process, because of their absolute requirement for silicon (Lewin, 1962) and their dominance in the biogeochemical cycling of this element (Treguer *et al.*, 1995). Despite the importance of Si:N coupling, we still know relatively little about the interactions of these elements at the cellular and genetic level (Armbrust *et al.*, 2004; Martin–Jézéquel *et al.*, 2000), although the rapid advances in genomic information and techniques will undoubtedly shed light on these processes (Armbrust *et al.*, 2004; Grossman, 2005).

The importance of diatom production for both the modern ocean (e.g., Ryther, 1969) and as a regulator of climate (Ragueneau *et al.*, 2000; Tréguer and Pondaven, 2000) and global productivity (Sarmiento *et al.*, 2004) has also been recognized recently. As a result, significant advances have been made in modeling Si:N interactions at both the cellular level (Flynn and Martin–Jézéquel, 2000) and in coupled biogeochemical ecosystem models (Chai *et al.*, 2002; Dugdale and Wilkerson, 2001; Dugdale *et al.*, 1995; Mongin *et al.*, 2003; Moore *et al.*, 2002; Yool and Tyrrel, 2003), while the importance of Fe as a modulating factor on Si:N dynamics is increasingly well documented (e.g., Brzezinski *et al.*, 2002; de Baar and Boyd, 2000; Dugdale *et al.*, 2004; Harrison, 2000).

There is also increasing evidence for fundamental changes in ecosystem structure in response to shifts from diatoms to other organisms as previously predicted (Conley *et al.*, 1993; Officer and Ryther, 1980). While these shifts in ecosystem structure are not necessarily negative (e.g., Nelson and Dortch, 1996), the long-term consequences of anthropogenic influences on Si:N dynamics are likely to be an overall decrease in fisheries production (Sommer *et al.*, 2002) and an increase in noxious and harmful algal species (Anderson *et al.*, 2002; Glibert *et al.*, 2005b; Smayda, 1990).

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Linking the Oceanic Biogeochemistry of Iron and Phosphorus with the Marine Nitrogen Cycle

David A. Hutchins *and* Fei-Xue Fu

Contents

1. Introduction

Modern biological oceanography has evolved from an emphasis on single limiting nutrients to a perspective that includes multiple, interacting limiting factors. It is no longer necessary to consider solely an "either/or" scenario when discussing algal nutrient limitation. In fact, the single Liebig–limiting nutrient paradigm does not adequately describe many of the limitation processes that are important for primary producers in the ocean. Both physical factors (e.g., light, temperature, and carbonate chemistry) and biological factors (e.g., competition, grazing and viruses)

can strongly modulate the effects of nutrient limitation, and vice versa. The growth, biomass and productivity of particular algal taxa can be influenced to different degrees by several of these factors simultaneously. Likewise, multiple limiting nutrients can also interact in numerous ways to help determine phytoplankton production and community structure. Experimental and observational biological oceanographers have been aware of the implications of multiple resource limitation for quite some time; unfortunately, much of the theoretical ecological literature is still based on simple single nutrient Liebig limitation models.

Arguably, phosphorus (P) and iron (Fe) are the two nutrients that most impact the global marine nitrogen (N) cycle. Over the past 15 years a proliferation of new work on Fe limitation has left no doubt that this micronutrient profoundly influences nitrogen biogeochemistry over perhaps the majority of the ocean's surface. Recent work has also brought a renaissance of renewed attention to P limitation, and to its reciprocal interactions with nitrogen.

In this chapter, we synthesize the state of the discipline with regards to Fe and P interactions with the N cycle. First we briefly review what is currently known about the cycles of Fe and P, with concurrent comparisons and contrasts with the N cycle. Then, we consider how these two elements individually and together may impact dinitrogen (N_2) fixation in the ocean. Along with presenting new evidence and discussing long-standing or emerging paradigms, we attempt to point out areas that still remain uncertain and questions that still need to be answered.

2. The Marine Iron Cycle

In recent years the Fe biogeochemical cycle has probably received more intensive scrutiny than that of any other element in the ocean. Despite this huge international investment of research effort, crucial parts of the Fe cycle remain speculative and unquantifiable. For some major Fe pools, fluxes, and rate processes, even the general chemical species involved are still relatively unknown. In large part these persistent uncertainties are directly related to the analytical difficulties associated with low-level measurements of Fe speciation and biogeochemical transformations. Another reason is that a great deal of the community research emphasis has been on diagnostic evaluations of biological Fe limitation, rather than on obtaining a realistic understanding of the unperturbed, *in situ* Fe cycle (Boyd *et al.*, 2005).

Despite this, we have made major forward progress in conceptual and even quantitative modeling of Fe cycling (Bowie *et al.*, 2001; Fung *et al.*, 2000; Moore *et al.*, 2002, 2004; Parekh *et al.*, 2004, 2005; Moore and Doney 2007; Krishnamurthy *et al.* 2008; Tagliabue *et al.* 2008). Trace-metal clean measurement and manipulation methods that were once the exclusive preserve of a few elite laboratories are now in relatively common use by many research groups all over the world. The advent of new technologies has also greatly advanced the field, notably *in situ* manipulative experiments, molecular methodology, and advances in analytical chemistry and computer modeling capabilities. This array of novel tools has allowed a much more in depth picture of Fe biogeochemistry than has ever been available in the past. A conceptual diagram of our current picture of the marine Fe biogeochemical cycle is presented in Fig. 38.1; many of these processes are discussed in more depth in the following sections.
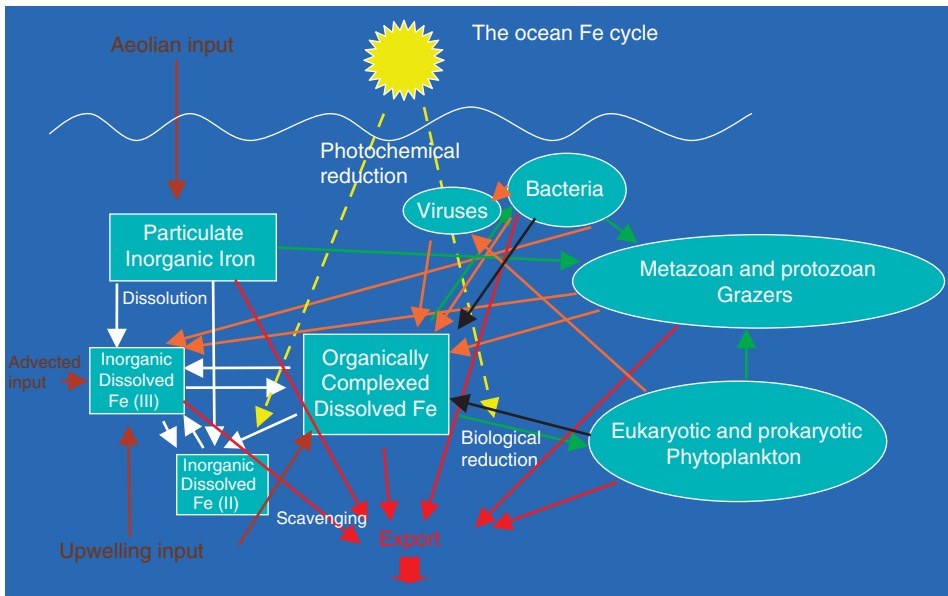
Figure 38.1 A conceptual diagram of important pools and fluxes in the marine Fe cycle. *Non-living pools* (rectangles) include particulate inorganic Fe (suspended lithogenic material or colloids), dissolved inorganic Fe (III) and Fe (II), and dissolved organically complexed Fe. *Living pools* (ovals) include prokaryotic and eukaryotic phytoplankton, protozoan and metazoan grazers, and bacteria and viruses. *New inputs* (brown text and arrows) include upwelled dissolved Fe (inorganic or organically bound), aeolian deposition (dust particles) and advected sources (largely from shelf sediments). *Transformations between non-living pools* (white arrows) include dissolution of dust to dissolved Fe(III) or Fe(II) (in the ocean or in the atmosphere prior to deposition), organic complexation and dissociation of dissolved Fe(III), redox cycling between dissolved inorganic Fe(III) and Fe(II), and photochemically-mediated reduction of ligand-bound Fe(III) (yellow arrow). *Biological uptake pathways* (green arrows) include assimilation of organically bound Fe by phytoplankton and bacteria, including photochemical (yellow arrow) and biological reduction fluxes. Direct uptake of dissolved inorganic Fe(III) and Fe(II) is assumed to be negligible as they comprise <1% of the total dissolved Fe pool. Also shown is trophic transfer of cellular Fe via grazing and possible direct uptake of particulate Fe by ingestion of dust particles or colloids by grazers. *Regeneration pathways* (orange arrows) include recycling of biological Fe by grazers, viruses and bacteria as dissolved inorganic Fe and/or organically bound iron. *Production of organic ligands* (black arrows) includes siderophore production by bacteria and cyanobacteria, and possible release of intracellular chelators by eukaryotic phytoplankton. *Export pathways* (red arrows) include direct sinking of lithogenic and biological particulate iron (PFe); although arrows are shown from all living pools, larger phytoplankton and grazers are likely to be dominant sources. Another important export pathway is scavenging of dissolved inorganic Fe(III); export of organically bound Fe is also depicted, although the importance of scavenging or downwelled export of this Fe species has not been established.

2.1. Iron limited regimes

The three classic oceanic high nutrient, low chlorophyll (HNLC) regimes are now universally recognized by oceanographers as being Fe-limited. A plethora of deck-board and open ocean Fe addition experiments have conclusively demonstrated

that Fe is a limiting nutrient for phytoplankton growth in the Equatorial Pacific, the subArctic Pacific, and throughout most of the vast Southern Ocean (Boyd, 2002; Boyd *et al.* 2007; Coale *et al.*, 2004; Martin *et al.*, 1990, 1991). Recent work has extended the scope of potentially Fe-limited waters to include the coastal upwelling areas off California (Firme *et al.*, 2003; Hutchins *et al.*, 1998; King and Barbeau 2007) and Peru (Hare *et al.*, 2005; Hutchins *et al.*, 2002), the Bering Sea (Leblanc *et al.*, 2005; Aguilar-Islas *et al.* 2006), parts of the Northeast Atlantic (Blain *et al.*, 2004), and even some estuaries (Lewitus *et al.*, 2004; Zhang, 2000). In addition, broad areas of the oligotrophic oceans traditionally considered to be major nutrient–limited are probably actually co-limited by both Fe and major nutrients (Behrenfeld and Kolber, 1999; DiTullio *et al.*, 1993). Iron limitation is now recognized as a global scale phenomenon, and modeling work suggests that as much as 50% of the world ocean exhibits evidence for Fe limitation of algal growth and production (Moore *et al.*, 2002).

2.2. Iron limitation and marine food webs

It is now an accepted paradigm that relieving Fe limitation in HNLC areas fosters the growth and productivity of diatoms (reviewed in Wells, 2003, Fig. 38.2). In most cases, these have been pennate species (Boyd *et al.*, 2000; Cavender-Bares *et al.*, 1999; Coale *et al.*, 2004; Gervais *et al.*, 2002; Hutchins *et al.*, 2002; Martin *et al.*, 1991; Price *et al.*, 1991), although centric diatoms have also bloomed after Fe enrichments in coastal California (Firme *et al.*, 2003; Hutchins and Bruland, 1998) and in the SEEDS open ocean experiment in the western subarctic Pacific (Tsuda *et al.*, 2003). These diatom blooms are not necessarily followed by commensurate
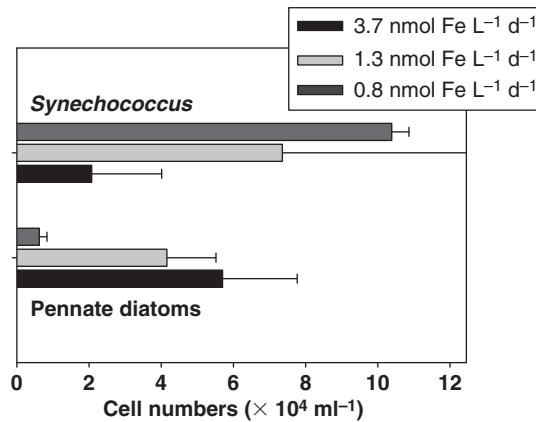
Figure 38.2 A progressive shift from a *Synechococcus*-dominated community to a diatom-dominated assemblage with increasing Fe supply in an experiment in the Peru Upwelling region. A shipboard continuous culture system was used to supply a continuous flow of Fe-amended sea-water at a dilution rate of $1\,day^{-1}$, simulating upwelling of water with three different Fe concentrations. Iron supply rates used were 0.8, 1.3, or 3.7 nmol Fe $L^{-1}\,day^{-1}$. Values are the means and error bars are the standard deviations of triplicate incubation bottles. Redrawn from Hare *et al.* (2005), *Aquatic Microbial Ecology* 38: 269–282.

drawdown of silicate, however, as the relationship between Fe availability and silicate utilization is complex (Hutchins and Bruland, 1998, see Chapter 7 by Kudela, this volume). It is widely believed that Fe-mediated increases in the contribution of large diatoms to community biomass also increase the potential for carbon (C) and N export (Boyd *et al.*, 2004; Buesseler *et al.*, 2005).

However, diatoms are not the only phytoplankton group that responds to Fe additions. Cavender-Bares *et al.* (1999) showed that cellular size and fluorescence of all phytoplankton groups, including *Prochlorococus*, *Synechococcus*, and ultra- and nano-eukaryotes increased during the Equatorial Pacific IronEx II experiment. Similarly, eukaryotic nanoplankton have shown rapid initial increases in other addition experiments such as the Southern Ocean SOIREE experiment (Boyd *et al.*, 2000), usually followed by decreases as the diatom bloom progresses. Like diatoms, these smaller algal groups are also clearly Fe-limited in HNLC areas, but tight coupling with their micrograzers usually keeps large biomass increases in check after fertilization.

Heterotrophic bacterial production and sometimes abundance have also been shown to increase following Fe enrichments, although there is no consensus about whether this represents a response to increased phytoplankton-derived dissolved organic carbon (DOC) supplies, or a direct release from Fe limitation (Arrieta *et al.*, 2004; Church *et al.*, 2000; Cochlan 2001; Hutchins *et al.*, 1998; Kirchman *et al.*, 2000; Oliver *et al.*, 2004; Pakulski *et al.*, 1996). There has been little work to examine Fe effects on bacterial community structure, but one study (Hutchins *et al.*, 2001) concluded that shifts in bacteria assemblages are probably less dramatic and pronounced than those seen in the algal community. A recent study, however, documented significant changes in bacterial diversity across a natural Fe gradient in the Southern Ocean (West *et al.* 2008). Culture studies also show that protozoan grazer growth rates can be limited by the Fe content of their bacterial prey (Maranger *et al.*, 1998; Nodwell and Price, 2001). No work to date, however, has examined whether metazoan grazers and other higher trophic levels are also subject to Fe constraints on their growth.

2.3. Sources of iron

The broad outline of the marine Fe cycle is analogous to the N cycle, in that regenerated forms are thought to support the bulk of biological production while new inputs determine the potential for export (Fung et al., 2000; Hutchins *et al.*, 1993; Landry *et al.*, 1997). As with N, vertical advection is an important source of new Fe, and new fluxes to the large oceanic HNLC areas of the polar Southern Ocean and the Equatorial Pacific are heavily dominated by upwelling sources (Coale *et al.*, 1996; de Baar *et al.*, 1995). In the subArctic Pacific HNLC region and throughout most of the oligotrophic ocean, however, aeolian input is the most important new iron source (Boyd *et al.*, 1998; Jickells and Spokes 2001; Johnson *et al.*, 2003). Calculations suggest that even "extraterrestrial Fe" from meteorite deposition could be quantitatively significant in the open ocean (Johnson, 2001).

In coastal regimes or downcurrent from island platforms, sedimentary sources play a predominant role in supplying new iron (Blain *et al.* 2007; Bruland *et al.*, 2001, 2005; Bucciarelli *et al.*, 2001; Croot and Hunter, 1998; Croot *et al.*, 2004a; Hutchins

and Bruland 1998; Johnson *et al.*, 1999, 2001; Planquette *et al.* 2007). The influence of sedimentary Fe was previously thought to be limited to surface waters in close proximity to the coast, but recent evidence suggests that Fe from this source can penetrate far into the interior of the ocean along isopycnal lines. Evidence to support this process is presented in Elrod *et al.* (2004), who coupled measurements of benthic Fe fluxes with water column measurements showing strong onshore/offshore gradients in Fe (Fig. 38.3). Thus, Fe from continental shelf sediments may contribute a much larger share of total new Fe inputs to the open ocean than has been generally realized (Lam *et al.* 2006).

Iron has been shown to be transferred rapidly between algal size classes (Hutchins *et al.*, 1993) and plankton trophic levels (Twining and Fisher, 2004), and regenerated Fe is believed to support up to 90% of total production in oceanic regimes (Landry *et al.*, 1997). Gordon *et al.* (1997) calculated that new Fe inputs could only support between 4% and 20% of biological demand in the equatorial Pacific. Direct experimental comparisons suggest that recycling efficiencies for Fe are slightly lower (∼25%) than those of N (Hutchins and Bruland, 1995). Direct field comparisons of Fe and N recycling in the ocean are lacking, but one study found that mixed layer residence times (presumably a proxy for recycling efficiency) are about twice as long for N as for Fe (Frew *et al.*, 2006, see below).

The processes responsible for Fe turnover and recycling are also likely the same as those that drive Fe cycling. Grazing by both protozoan and metazoan zooplankton has been shown to rapidly make biological and colloidal Fe available for algal uptake (Barbeau *et al.*, 1996; Chen and Wang 2001; Hutchins and Bruland 1994; Hutchins *et al.*, 1995; Maranger *et al.*, 1998; Wang and Dei, 2001). Viral lysis of phytoplankton and bacteria has also been shown to be potentially important in Fe recycling (Gobler *et al.*, 1997; Poorvin *et al.*, 2004), and calculations suggest that this process could even be a dominant regeneration source in prokaryote-dominated HNLC areas (Poorvin *et al.*, 2004).
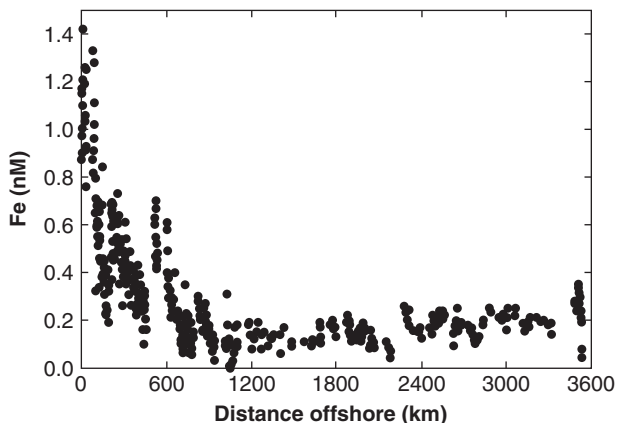
Figure 38.3 Iron concentrations in surface waters versus distance offshore on a transect from central California to Hawaii. Elrod *et al.* (2004) used benthic flux measurements and these water column data to make a case for transport of Fe from shelf sediments out into the open ocean gyre. Elrod *et al.* (2004), *Geophysical Research Letters* 31: L12307.

Despite similarities between the general outlines of the N and Fe cycles, the details of their biogeochemistry are strikingly different. Our ability to quantify particular new and regenerated fluxes of Fe lags far behind the well developed methodology available for N. The chemical species involved in new and regenerated Fe inputs remain speculative, and consequently realistic uptake measurements cannot be performed. Current methodology also does not allow the detection of Fe uptake using isotopic methods at true tracer levels, and all attempts at measuring rate parameters or f-ratios for the Fe cycle are thus compromised by potential artifacts. As our knowledge of Fe chemical speciation and our ability to precisely measure various Fe species advances, a crucial objective of future research will be to definitively quantify the relative importance of new and regenerated Fe in marine regimes.

2.4. Iron export and residence time

In contrast to N, surprisingly little information is available on export rates of Fe from the surface ocean, despite the obvious importance of this parameter to biogeochemical models. Partly, this is due to the difficulties associated with making trace-metal clean measurements of sinking fluxes out of the mixed layer. Two published estimates from relatively nearshore waters present quite high export estimates of 2 μmol m^{-2} day^{-1} (in the Drake Passage, Martin *et al.*, 1990) and <10–140 μmol m^{-2} day^{-1} (in the Baltic Sea, Pohl *et al.*, 2004).

Frew *et al.* (2006) used trace-metal clean sediment trap methods developed by Kremling *et al.* (1996) in subAntarctic HNLC waters to obtain much lower export estimates of 218–548 nmol m^{-2} day^{-1} (Fig. 38.4A). This flux roughly corresponds to published estimates of aeolian deposition rates in this region (Jickells and Spokes, 2001) and represents a daily loss of about 1% of the mixed layer Fe inventory. In the same trap arrays, daily fluxes of particulate organic C (POC), N (PON) and P (POP) were <0.5% and BSi (biogenic silica) fluxes were ~1.9% of the total mixed layer inventories of these elements. Patterns of Fe export between duplicate arrays and with depth were qualitatively similar to those of PON (Fig. 38.4B) and POP (Fig. 38.4C), despite orders of magnitude differences in absolute amounts. In this study sinking particulates exhibited dramatically increasing Fe:C and Fe:N ratios below the mixed layer, suggesting preferential remineralization of C and N relative to Fe and/or additional scavenging of Fe by particles during sinking (Frew *et al.*, 2006).

Compared to N and other nutrient elements the residence time of Fe in both the surface and deep ocean is quite short, much shorter than the overall mixing time-scales of the ocean. This is largely due to the high biological demand for Fe relative to its supply, and to rapid removal by its scavenged chemical behaviour. Recent data compilations suggest that there are inter-ocean differences in deep-water Fe concentrations (Parekh *et al.*, 2004, 2005), contrary to earlier suggestions (Johnson *et al.*, 1997). These differences between the Atlantic and Pacific are not, however as large as for other nutrient-type elements and are likely related to distance gradients from major source terms such as Saharan dust inputs (Boyle, 1997; Watson *et al.*, 2000), rather than to differences in accumulated remineralization
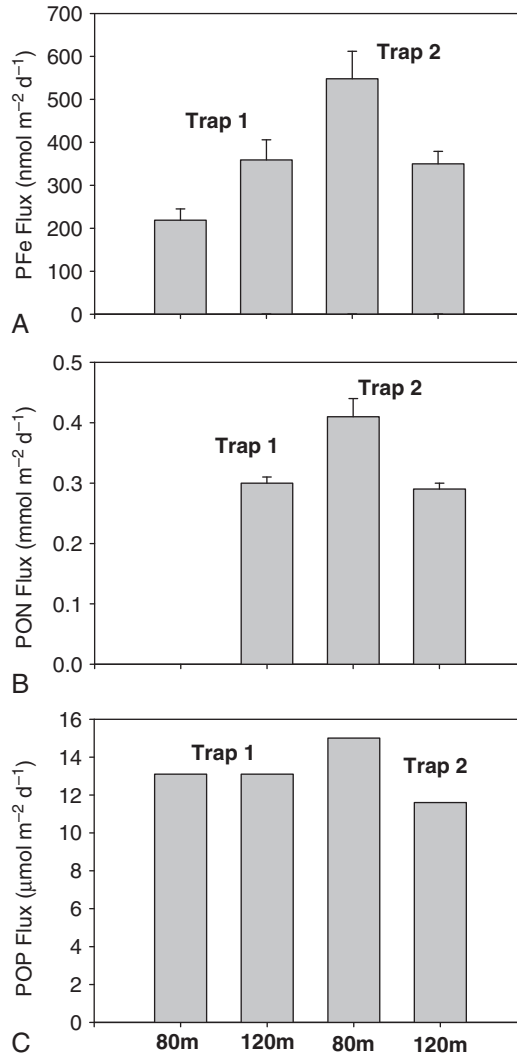
Figure 38.4 Export fluxes out of the mixed layer measured in unperturbed subAntarctic HNLC waters near New Zealand during the FeCycle experiment for (A) PFe, (B) PON, and (C) POP. Samples were collected at 80 and 120 m using two trace metal-clean drifting sediment trap arrays deployed for 7 days. Values are the means of four (PFe), two (PON), or one (POP) collection cylinders at each depth, after subtraction of procedural blank values (deployed but unopened cylinders). Error bars represent standard errors. PON samples were lost from the 80 m depth of Trap 1. Adapted from Frew *et al.* (2006), Global Biogeochemical Cycles 20: GB1S93.

fluxes as for N and P. Estimates of Fe residence times in the mixed layer range from 6–62 days (Croot *et al.*, 2004b) to 100 days or so (Frew *et al.*, 2006). On larger spatial scales, Moore *et al.* (2004) suggest an upper ocean residence time of 1–10 years, and Boyle *et al.* (2005) estimated a surface ocean residence time in the Pacific of about half a year.

Johnson et al. (1997) calculated an overall oceanic residence time for Fe of about 100 years. The order-of-magnitude difference between this estimate and recent oceanic residence time calculations for fixed N (roughly 1500–3500 years, Codispoti et al., 2001) is due almost entirely to iron's scavenged chemical behaviour. In most oxygenated waters below the euphotic zone, nitrate (NO_3^-) is probably minimally impacted by biological demand, and relatively unreactive chemically. Although little is known about biological demand for Fe below the euphotic zone, it is also likely small. Relatively rapid particle scavenging throughout the water column (compared to species like NO_3^-), however, reduces the turnover time of Fe in the ocean to mere decades.

2.5. Dissolved and particulate iron pools

Iron is the only nutrient element for which particulate concentrations are typically higher than dissolved levels (de Baar and de Jong, 2001). Despite this, considerably more research attention has focused on measuring dissolved pools than on the particulate fraction. The literature on total dissolved Fe concentrations is now far too extensive to comprehensively review here, but most analysts report oceanic surface concentrations of <0.1–0.5 nM (de Baar and de Jong, 2001; Johnson et al., 1997), and deep-water levels ranging from 0.3 to 0.7 nM (Parekh et al., 2005). Some differences in reported dissolved Fe levels could be due to analytical artifacts, since various methods used by different groups may measure different fractions of dissolved and/or colloidal and particulate pools. Methods intercomparisons are currently underway that could help to clarify these issues.

Reported surface PFe values are more variable (Croot et al., 2004a,b; Gordon et al., 1998; Martin et al., 1990; Tovar-Sanchez et al., 2003; Weinstein et al., 2004), but are generally also less than 1 nmol L^{-1} in HNLC areas. Much of the interest in PFe has been directed towards understanding the solubility of lithogenic Fe from dust (Spokes and Jickells, 1996), although biogenic Fe particulate concentrations are often equivalent or higher in many areas (Frew et al., 2006; Tovar-Sanchez et al., 2003). Various estimates of dust Fe solubility range from 1–10% (Jickells and Spokes, 2001), but Boyle et al. (2005) arrived at an estimate of 30–40% in the Pacific near Hawaii. Johnson et al. (2003) also calculated a possible dissolution value of about 20% for this region, and suggested that the process is likely far more complex than is recognized by current simple dissolution models.

Frew et al. (2006) also suggest that an exclusive emphasis on understanding abiotic dust dissolution processes may be misplaced. Their results show that as much as 53% of suspended lithogenic Fe in the euphotic zone is transformed to biogenic Fe on a timescale of weeks, prior to export with sinking particle fluxes. Efficient biological transformation pathways (such as bacterially-mediated dissolution or grazer ingestion of dust particles) probably result in much higher bioavailability of aeolian Fe inputs than simple chemical solubilization calculations would suggest. It seems that dust dissolution is not a simple, short term process governed by thermodynamics, but is instead a kinetic process where biological influences can substantially shift the equilibrium.

Biogenic PFe actually consists of two distinct pools, intracellular or biological Fe and surface-adsorbed or scavenged Fe (Hutchins, 1995). The latter has usually not been considered to be a true component of the cellular Fe quota, and a titanium (Ti) wash method (Hudson and Morel, 1989) has therefore been commonly employed to remove surface-bound Fe in radiotracer studies (Eldridge *et al.*, 2004; Hutchins *et al.*, 1999; Poorvin *et al.*, 2004; Sunda and Huntsman, 1997; Sunda *et al.*, 1991). However, the titanium reagent is itself heavily contaminated with Fe and so its use is questionable for non-radioactive measurements.

Tovar-Sanchez *et al.* (2003) developed a trace-metal clean oxalate-based reagent which can be used to evaluate surface-bound and internal pools of Fe in natural samples. Using this method, they showed that scavenged Fe in suspended particulates in several parts of the Southern Ocean comprised 16–86% of the total particulate concentrations. Results obtained using the oxalate wash have been shown to be quite comparable to those obtained from the Ti wash (Tang and Morel, 2006). Methods like this one hold great promise to actually quantify and compare relative biological uptake and particle scavenging rates *in situ*, crucial parameters for estimating dissolved Fe removal processes in Fe biogeochemical models (Fung *et al.*, 2000; Moore *et al.*, 2002).

2.6. Organic complexation and redox chemistry

Chemists have been aware for at least a decade that the dissolved speciation of Fe is heavily dominated by organic chelates, with typically 99% of total dissolved Fe bound in these complexes. The development of several cathodic stripping voltammetry electrochemical methods has allowed measurements of conditional stability constants for iron-binding by these natural ligands (Croot and Johansson, 2000; Gledhill and van den Berg, 1994; Rue and Bruland, 1995; Wu and Luther, 1995), and these analyses have now been carried out in surface waters of virtually every ocean basin and on a growing number of depth profiles (reviewed in de Baar and de Jong, 2001; Johnson *et al.*, 1997).

The picture these data present is remarkably consistent. Iron ligands are frequently placed into two classes that are operationally defined by their electrochemical analytical detection "windows," a weaker "L2" class with a typical concentration of 0.2–1.5 nmol kg^{-1} and a stronger "L1" class with a lower concentration of 0.3–0.4 nmol kg^{-1} (Bruland and Lohan, 2004). Because the L1 class has conditional stability constants at least an order of magnitude higher than those of L2, however, most of the dissolved Fe is bound in L1-type complexes. Electrochemical techniques however have limitations, partly because the specific analytical window of each particular method precludes measurement of potential ligand classes outside these limits, and partly because ligands already bound to Fe *in situ* must be indirectly estimated by difference since only "free" or unbound ligands can be detected directly. Nevertheless, electrochemistry does yield unambiguous information about the uncomplexed ligand concentrations available to bind new Fe inputs.

The chemical structures and biological sources and functions of these Fe-binding ligands remain mysterious. Witter *et al.* (2000) point out that electrochemically-derived conditional stability constants can give little useful

information about ligand structure, since a wide variety of completely unrelated Fe-binding functional groups yield very similar values in seawater. It seems clear that L1 and L2 ligands do not represent single compounds, but instead are mixtures of broad classes of organic molecules with similar binding constants but probably quite heterogeneous structures.

Among the candidates for organic ligands in seawater are siderophores, high-affinity Fe-binding compounds with various functional moieties that are produced by Fe-stressed prokaryotes as an uptake strategy (Butler, 1998). Many marine bacteria and cyanobacteria can produce diverse siderophores in culture (Granger and Price, 1999; Wilhelm and Trick, 1994). Using column concentration methods, Macrellis *et al.* (2001) identified siderophore-type functional groups in bulk DOM isolated from seawater, and known siderophores have been detected in Baltic seawtare using capillary electrophoresis (Kosakowska *et al.*, 1999; Mucha *et al.*, 1999). Electrospray mass spectrometry also offers a promising avenue for determining the actual structures of unknown siderophores in seawater (McCormack *et al.*, 2003), and a method combining high performance liquid chromatography with electrospray mass spectrometry has detected a number of siderophores in coastal seawater incubations enriched to promote bacterial growth (Gledhill *et al.*, 2004). Some intracellular biomolecules such as porphyrins are also very strong Fe chelators (Rue and Bruland, 1997; Witter, 2000), and are likely released to the water by grazing or cell lysis (Hutchins *et al.*, 1999).

A number of studies have looked at bioavailability of Fe chelated to various "model" Fe ligand complexes (Fig. 38.5, Achilles *et al.*, 2003; Guan *et al.*, 2001; Hutchins *et al.*, 1999; Kuma *et al.*, 2000; Maldonado and Price, 1999; Weaver *et al.*, 2003). It has been suggested that different taxonomic groups of marine plankton may have preferences for Fe bound to particular types of ligands (Guan *et al.*, 2001; Hutchins *et al.*, 1999). Biological Fe uptake from various colloidal species has also been investigated (Kuma and Matsunaga, 1995; Nishioka and Takeda, 2000; Nodwell and Price, 2001; Wang and Dei, 2003). Colloids may include a substantial fraction of organically complexed Fe, and also could represent an intermediate form in conversions between the lithogenic and biogenic pools. Despite these advances, the chemical nature and biological implications of Fe organic complexation remain largely obscure, and this uncertainty persists as a surprisingly intractable barrier to a full understanding of the biogeochemistry of this element.

Like N, Fe has multiple redox states and can cycle between oxidized Fe(III) and reduced Fe(II) chemical species. Under oxic conditions, the ferric ion is by far the dominant form, but Fe(II) in aerobic waters can be produced transiently through photochemical interactions with DOM (Rose and Waite, 2003; Waite and Morel, 1984) or by biological uptake system reductive processes (Shaked *et al.*, 2004). Barbeau *et al.* (2003) suggest that some organic complexing ligands such as particular types of siderophores can act as electron donors in the photochemically-mediated reduction of chelated Fe(III), and thereby perhaps increase its bioavailability. Appreciable Fe(II) concentrations were observed in the fertilized patches for an extended period of time during the SOIREE and EisenEx open ocean Fe addition experiments in the Southern Ocean (Croot *et al.*, 2001, 2005). Although Fe(II) oxidation kinetics are slower at cold temperatures, this situation is highly
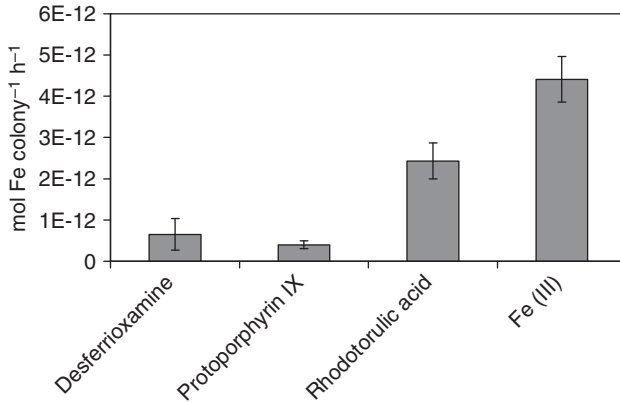
Figure 38.5 Short term Fe uptake rates from model iron/ligand complexes by *Trichodesmium* colonies collected in the subtropical Atlantic Ocean. Shown are uptake rates from Fe complexed to a trihydroxamate siderophore (desferrioxamine), a dihydroxamate siderophore (rhodoturulic acid), and a porphyrin (protoporphyrin IX). For comparative purposes, uptake rates were also measured using added inorganic Fe (III). Achilles *et al.* (2003), Limnology and Oceanography 48: 2250–2255.

thermodynamically unfavorable. The persistence of high Fe(II) levels thus suggests either continual replenishment through ongoing biological or photochemical reduction processes, or that the ferrous ion was stabilized with respect to oxidation by an (unknown) Fe(II) chelator.

2.7. Iron and nitrogen acquisition

From the earliest shipboard addition experiments in the late 1980s (Martin *et al.*, 1991), an empirical relationship between Fe availability and NO_3^- drawdown has been recognized. Deckboard Fe addition experiments in HNLC areas invariably demonstrate increased utilization of NO_3^-, relative to control treatments, and NO_3^- is always consumed much faster in unenclosed Fe-enriched mesoscale patches than in surrounding unfertilized waters (Fig. 38.6, Boyd 2002; Coale *et al.*, 2004). It is well known that algal enzymatic systems for oxidized N assimilation contain Fe, and that Fe-intensive photosynthetic electron transport systems must supply reductant for NO_3^- reduction and uptake (Raven, 1988). Consequently, nitrate-grown cells have higher Fe uptake rates (Maldonado and Price, 1996) and intracellular Fe quotas (Wang and Dei, 2001) than those growing on ammonium (NH_4^+). Adequate Fe supplies are thus an absolute prerequisite to growth on nitrate.

Laboratory work suggests, however, that Fe limitation probably does not directly inhibit NO_3^- uptake by compromising nitrate reductase activity (Milligan and Harrison, 2000). Instead, Fe limitation may block the NO_3^- reduction pathway further downstream, by preventing nitrite (NO_2^-) reduction to NH_4^+ due to insufficient supplies of photosynthetically-produced reductant. This study showed that Fe-deficient diatom cells continue to take up and reduce NO_3^- to nitrite.
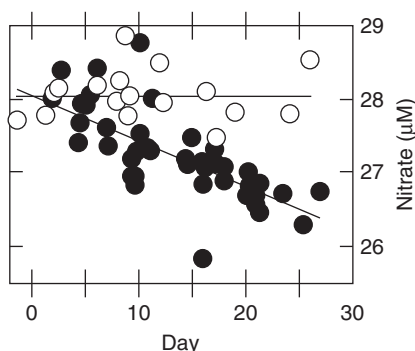
Figure 38.6 Effects of Fe enrichment on NO_3^- drawdown in the South Patch (66.45°S, 171.8°W) during the SOFeX Southern Ocean mesoscale iron addition experiment. Shown are NO_3^- concentrations versus time in the iron-fertilized patch (closed circles) compared to nearby unfertilized control waters (open circles). Coale et al. (2004), Science 304: 408–414.

Since nitrite reductase activity is impaired, however, the cells then excrete much of the "excess" accumulated NO_3^- back to the water. Iron/light co-limitation of phytoplankton near the base of the euphotic zone (Boyd *et al.*, 2001; Maldonado *et al.*, 1999; Sunda and Huntsman, 1997) could contribute to the persistent subsurface NO_3^- maximum zones that commonly occur in many parts of the ocean, along with other sources such as bacterial denitrification or ammonium oxidation (Keifer *et al.*, 1976).

Iron interactions with N sources are not limited to phytoplankton. Kirchman *et al.* (2003) found that the growth rate, respiratory electron transport system activity, and growth efficiency of a marine gamma proteobacterium (*Vibrio harveyi*) were much lower in Fe-limited cultures grown with NO_3^- than when NH_4^+ or amino acids were supplied as N sources. They suggested that these results may help to explain why natural bacterial communities in nitrate-rich, iron-poor HNLC areas also typically exhibit reduced growth rates and efficiencies.

Another critical N cycle enzyme which requires large quantities of Fe is nitrogenase, which catalyzes the fixation of atmospheric N_2 to NH_4^+ by some prokaryotes. Consequently, diazotrophs may be especially vulnerable to iron limitation and there has been a tremendous amount of recent research effort to understand how this could impact global marine N_2 fixation (see Chapter 4 by Carpenter and Capone, this volume). Nitrogenase is a complex enzyme, with the most common form containing two proteins termed the molybdenum iron (MoFe) protein (dinitrogenase) and the Fe protein (dinitrogenase reductase) (Paerl and Zehr, 2000). The Fe use efficiency of nitrogenase, calculated as the rate of enzymatic substrate turnover per mole of Fe, is the lowest among all of the Fe-containing enzymes involved in N metabolism. In addition to this large enzymatic Fe requirement, N_2-fixing organisms also require a large amount of Fe to facilitate electron transfer reactions (Kustka *et al.*, 2003; Raven, 1988).

N_2 fixation can be affected by the availability of Fe through either direct effects on nitrogenase synthesis (Berman-Frank *et al.*, 2001; Küpper *et al.* 2008) or indirectly

through effects on photosynthesis and growth (Fu and Bell, 2003a). Berman-Frank *et al.* (2001) observed that only 11% of Fe-depleted cultured *Trichodesmium* cells contained nitrogenase, whereas 20% of Fe-replete cells tested positive for nitrogenase activity. Western blots of nitrogenase reductase also showed higher levels of this protein in their Fe-replete cultures. They concluded that the synthesis and expression of nitrogenase by *Trichodesmium* is directly controlled by Fe availability.

3. THE OCEAN PHOSPHORUS CYCLE

Phosphorus was traditionally considered not to be limiting on ecologically relevant timescales in the marine environment (Ryther and Dunstan, 1971) because N usually became limiting before P was depleted. However, evidence is accumulating for P limitation in several coastal systems and estuaries (Thingstad *et al.*, 1998; Yin *et al.*, 2000), the oligotrophic Atlantic Ocean (Cotner *et al.*, 1997; Sañudo-Wilhelmy *et al.*, 2001; Wu *et al.*, 2000), the North Pacific central gyre (Björkman *et al.*, 2000), and in the eastern basin of the Mediterranean Sea (Krom *et al.*, 1991, 1993).

Compared to N and Fe, the P cycle is relatively simple. Phosphorus lacks the gas phase that is so important for N, and unlike either N or Fe, is unaffected by redox cycling. The marine P cycle consists of a series of transformations between dissolved inorganic, particulate organic, and dissolved organic forms, presented diagrammatically in Fig. 38.7. The three main pathways of dissolved P delivery from the continents to the ocean are via riverine, atmospheric and volcanic sources. Supply is driven largely by continental weathering, but because P is only a minor component of most crustal rocks, its delivery is restricted relative to more abundant lithogenic elements like silicon (Cosgrove, 1977).

By far the dominant transport mode of P to the ocean is through river runoff. Pre-human riverine P loading to the oceans has been estimated at $2.6–3.3 \times 10^{11}$ mol P year^{-1}, and current anthropogenic inputs have elevated this input to $7.4–15.6 \times 10^{11}$ mol P year^{-1}(Froelich *et al.*, 1982; Howarth *et al.*, 1995). The atmospheric delivery of P from the continents to the ocean was first considered by Graham and Duce (1979), who estimated that a net flux of $\sim 3.2 \times 10^{10}$ mol P year^{-1} is transferred via atmospheric dust inputs. Chadwick *et al.* (1999) and Rea (1994) estimated an aeolian P flux rate ranging from $0.2–1.4 \times 10^{10}$ mol P year^{-1} using geochemical tracers of continental crust. Mills *et al.* (2004) also made a convincing case for the importance of aeolian P supply in the Atlantic. This supply of phosphate from the delivery of airborne dust is significant (Föllmi, 1996), because 21–51% of total aeolian phosphate (PO_4^{3-}) is bioavailable once deposited in the ocean (Duce *et al.*, 1991; Graham and Duce, 1982). However, this value could be underestimated by as much as 50% since it does not include acid soluble and high temperature soluble P fractions (Benitez-Nelson, 2000). The contribution of localized volcanic sources to ocean P delivery on a global scale is probably minor (Benitez-Nelson, 2000), although a few studies have suggested that volcanoes could be potential P sources to the ocean (Resing, 1997; Yamagata *et al.*, 1991).

Figure 38.7 A conceptual diagram of important pools and fluxes in the marine P cycle. *Non-living pools* (rectangles) include particulate inorganic P (suspended lithogenic material), dissolved inorganic P (phosphate), and dissolved organic P. *Living pools* (ovals) include prokaryotic and eukaryotic phytoplankton, protozoan and metazoan grazers, and bacteria and viruses. *New inputs* (brown text and arrows) include upwelled dissolved PO_4^{3-}, aeolian deposition (dust particles), and riverine sources. *Transformations between non-living pools* (white arrows) include dissolution of dust as PO_4^{3-}, and hydrolysis of dissolved organic P (DOP) to yield PO_4^{3-} by biological enzymatic activity. *Biological uptake pathways* (green arrows) include assimilation of phosphate (probably the dominant pathway in coastal and upwelling areas) and DOP (probably the dominant flux in oligotrophic regimes) by phytoplankton and bacteria. Also shown is trophic transfer of cellular P via grazing. *Regeneration pathways* (orange arrows) include recycling of cellular P as dissolved inorganic PO_4^{3-} (by viruses and bacteria) and DOP (by all organisms). *Export pathways* (red arrows) include direct sinking of lithogenic and biological particulate P; although arrows are shown from all living pools, larger phytoplankton and grazers are likely to be dominant sources. Another recently recognized pathway that could be important is scavenging of dissolved PO_4^{3-}; export of DOP is also depicted, although the importance of scavenging or downwelled export of this P species has not been established.

Sedimentary burial is the only important removal process for P from the ocean (Benitez-Nelson, 2000). In the marine system, almost all bioavailable PO_4^{3-} is taken up by primary productivity in the euphotic zone and incorporated into organic matter for export by the biological pump. Approximately 95% of the P is released as dissolved inorganic PO_4^{3-} during the oxidation of sinking organic particles, and will be reintroduced to the photic zone by upwelling processes and reused by the marine biota (Föllmi, 1996). The other 5% is removed from the ocean by sedimentary burial either with organic matter, as inorganic PO_4^{3-} absorbed on $CaCO_3$ shells and metal oxyhydroxides, or by incorporation of PO_4^{3-} into authigenic minerals such as apatites and phosphorites (Benitez-Nelson, 2000; Berner *et al.*, 1993). New evidence suggests that export and burial of intracellular P in

diatoms (as polyphosphates) may be a significant removal process in the biogeo-chemical cycle of this element (Diaz *et al.* 2008). The estimated residence time of P in the ocean ranges from 20,000–80,000 years based on P sources, but is reduced to 9300–29,100 years relative to P sinks (Benitez-Nelson, 2000; Ruttenberg, 1993).

3.1. Speciation of dissolved phosphorus

Dissolved P in the ocean is present as inorganic orthophosphates and as P-containing organic compounds. Bioavailable PO_4^{3-} comes from the continental weathering of phosphate-bearing igneous and sedimentary rocks (Föllmi, 1996), from remineraliz-ation of organic P by bacteria and algae through the extracellular enzymes alkaline phosphatase (Björkman and Karl, 1994; Li *et al.*, 1998) and $5'$ nucleotidase (Ammerman, 1991; Ammerman and Azam, 1985, 1991a,b; Tamminen, 1989), or from the viral lysis of bacteria (Scanlan and Wilson, 1999). Orthophosphates exist as three free ions, $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}, and have a tendency to make ion pairs with bivalent metals such as Ca^{2+}, Mg^{2+}, and Fe^{3+} ions at neutral pH (Cosgrove, 1977; Lobban and Harrison, 1994). The major form of dissolved inor-ganic P at a seawater pH of 8 is HPO_4^{2-}, dominating ~87% in DIP (Benitez-Nelson, 2000). The ongoing acidification of the ocean through anthropogenic CO_2 emissions (Caldeira and Wickett, 2003) will tend to shift the phosphate buffer system incrementally towards $H_2PO_4^-$. The consequences of this process for future P biogeochemistry are unknown.

Like other dissolved organic nutrients, the chemical composition of organic P is relatively unknown. Marine dissolved organic phosphorus (DOP) composition has been mostly identified as particular compound classes such as monophosphate esters, phosphonucleotides, nucleic acids, phospholipids, phosphonates and polyphosphates (Benitez-Nelson, 2000). Kolowith *et al.* (2001) found that monophosphate esters and phosphonates are major components in the Pacific Ocean, the Atlantic Ocean and the North Sea. ATP (adenosine triphosphate), ubiquitous in all living cells, has been used as a model DOP compound in radiotracer experiments to obtain infor-mation on P sources and fluxes in natural environments (Björkman *et al.*, 2000; Karl and Bossard, 1985) and cyanobacterial cultures (Fu *et al.*, 2006).

DOP phosphorus is often present in concentrations up to an order of magnitude higher than DIP in oligotrophic regimes (Karl *et al.*, 2002). There is good evidence that phytoplankton in these areas rely heavily on DOP to support growth (Jackson and Williams, 1985; Li *et al.*, 1998); this literature is reviewed for *Trichodesmium* below. Alkaline phosphatase activity measurements are frequently used to detect DOP utilization in natural communities (Mulholland *et al.*, 2002; Stihl *et al.*, 2001; Yentsch *et al.*, 1972). For instance, there is a strong correlation between alkaline phosphatase activity and *Synechococcus* abundance in the Red Sea (Li *et al.*, 1998).

3.2. Surface-bound phosphorus

New evidence suggests that, like Fe, the cycling of P can be substantially affected by particle reactivity and scavenging. Although oxyanions such as PO_4^{3-} have not generally been considered by biologists to be scavenged chemical species,

geochemists have long recognized that PO_4^{3-} is removed from seawater by adsorption onto sediment and hydrothermal plume particles (Berner, 1973; Bjerrum and Canfield, 2002; Wheat *et al.*, 1996). Sañudo-Wilhelmy *et al* (2004) showed that ~30–50% of the total P associated with Atlantic *Trichodesmium* colonies was easily washed off the cells with an oxalate wash procedure that was originally developed to remove surface-bound trace metals (Tovar-Sanchez *et al.*, 2003). Correlations between adsorbed Mn oxides and surface-bound P suggested that P scavenging could be mediated by ligand/ligand complexation reactions between surface-bound metal cations and the PO_4^{3-} oxyanion (Sañudo-Wilhelmy *et al.*, 2004), but this proposed mechanism will require further investigation.

Both cultures and natural estuarine blooms of a wide variety of eukaryotic and prokaryotic phytoplankton other than *Trichodesmium* also have a large fraction of their total cell-associated P in surface-adsorbed pools (Fig. 38.8A, Fu *et al.*, 2005a). This study found that P scavenging was a unique property of inorganic phosphate, and DOP-grown phytoplankton did not exhibit any surface-adsorbed P. P-starved cultures were able to internalize some of their surface-bound P over the course of several days, suggesting that this pool could in effect serve as a cellular P reserve. The evident potential importance of scavenging in the biogeochemical cycle of P raises questions about the definition of cellular P quotas and kinetic
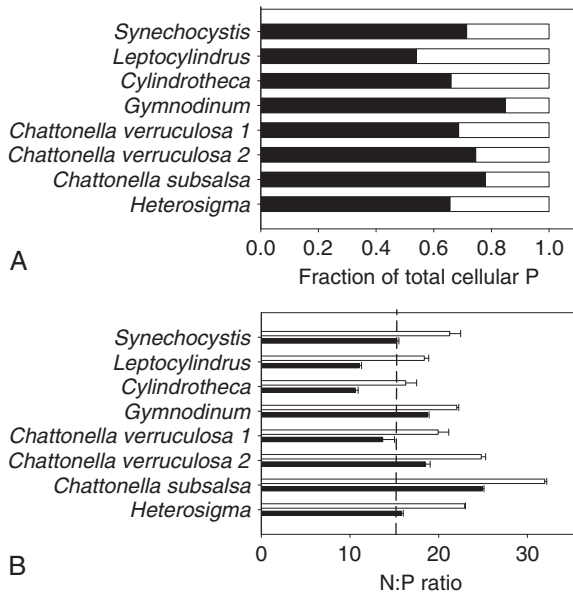
Figure 38.8 Effects of PO_4^{3-} scavenging on cellular partitioning of P and N:P ratios in eight natural estuarine bloom samples of prokaryotic and eukaryotic algae. (A) Fractions of total cell-associated P in the intracellular (filled bars) and surface-adsorbed (open bars) pools; (B) Nitrogen to phosphorus (N:P) ratios calculated using total cellular P (filled bars) and intracellular P pools after removal of surface-adsorbed P with the oxalate reagent (open bars). Error bars represent the standard deviations of triplicate samples, and the dashed lines indicate the Redfield ratio. Fu *et al.* (2005a), Limnology and Oceanography.

uptake constants, the interpretation of Redfield ratios (see below), and the possi-
bility that this process could be an important but currently unrecognized removal
mechanism for dissolved P in the ocean (Fu *et al.*, 2005a).

3.3. Phosphorus versus nitrogen as proximate and ultimate limiting nutrients

There are two viewpoints on which nutrient, P or N, controls primary production
in the ocean: the geochemical view and the biological view (Tyrell, 1999). The
geochemists think that P limits phytoplankton growth in most marine ecosystems.
The scenarios that have been proposed include: (1) N can be acquired via N_2
fixation from atmospheric N_2 and reduced to bioavailable NH_4^+. This of course
assumes that N_2 fixation can keep pace with demands of the photoautotophs (see
below); (2) There is no atmospheric or other alternative reservoir for P, and most is
supplied to the ocean from continental weathering and river inputs; and (3) the
residence time of P in the ocean is significantly longer than that of N and so the rate
of P supply is much lower than N. Finally, this assumption has been demonstrated
experimentally in freshwater environments (Hecky and Kilham, 1988; Howarth
et al., 1988; Smith, 1983) but not in marine ecosystems (Tyrell, 1999).

However, biologists have demonstrated that N is more likely to control phyto-
plankton productivity in the coastal and open oceans through nutrient enrichment
experiments and observations of nutrient distributions (Falkowski *et al.*, 1998).
Typically, NO_3^- runs out slightly before PO_4^{3-} when nutrients become depleted
in the surface waters (Fig. 38.9, GEOSECS 1996, Tyrell and Law, 1998). It has long
been known that additions of NO_3^- to natural samples collected from oligotrophic
surface ocean waters usually stimulate phytoplankton growth, whereas the addi-
tion of PO_4^{3-} usually does not (Codispoti 1989; Ryther and Dunstan, 1971).
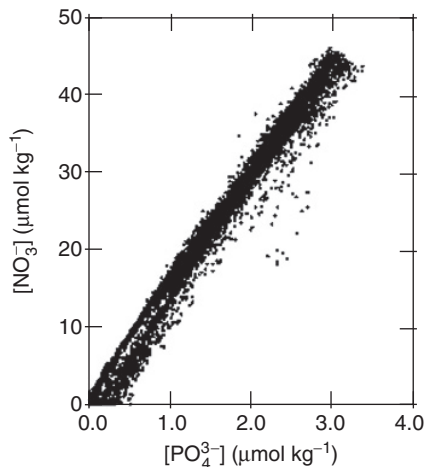
Figure 38.9 Surface ocean NO_3^- versus PO_4^{3-} scatter plot from the GEOSECS global data set.
The intercept suggests that there is often PO_4^{3-} remaining when NO_3^- is fully depleted. Tyrell
1999, Nature 400: 525–531.

Although N_2 fixation certainly provides a significant source of new N to the ocean, diazotrophy may be limited by factors such as Fe limitation (see below). Hence the concentration of fixed N is variable (Falkowski, 2000).

The wide distribution across prokaryotic taxa and the close genetic homologies of N_2 fixation systems suggests that fixed inorganic N in the primeval ocean was likely scarce (Falkowski, 1997), whereas inorganic PO_4^{3-} was probably abundant (Van Cappellen and Ingall, 1996). Finally, due to denitrification, the ratio of sinking particulate organic N to particulate P is less than the ratio of dissolved inorganic N to P. For this reason, throughout most of the world's oceans dissolved inorganic N rather than P has been suggested to limit primary production on geological time-scales (Dugdale 1967; Falkowski, 1997; McElroy 1983; Ryther and Dunstan, 1971).

Tyrell (1999) produced a model showing that the proximate limiting nutrient in the ocean is NO_3^-, while the ultimate limiting nutrient is PO_4^{3-}. Mills and colleagues (2004) confirmed that N is the proximate limiting nutrient in the eastern tropical North Atlantic with nutrient addition experiments. N limitation or P limitation is not always constant temporally and spatially. Hence Karl *et al.* (2001) suggested that when considering nutrient limitation in the oceans, the exact time and spatial scale should be defined.

For example, an extensive 9-year data set from ALOHA indicates that the bulk dissolved matter N:P ratio is variable. In the subtropical North Pacific Ocean, N_2 fixation may supply up to half of the new N to sustain the rate of the annual particulate N export from the euphoric zone. A recent increase in this source of new N appears to have shifted the North Pacific subtropical gyre from N limitation to P limitation (Karl *et al.*, 1997). Hence in the oligotrophic ocean, N_2 fixation could lead to a decoupling of N and P pool dynamics and an alternation of N versus P control of primary production (Karl *et al.*, 2001). Similarly but on a shorter time-frame, in the central Baltic Sea the development of N_2-fixing cyanobacterial blooms can drive the system from N limitation to P limitation between May to September (Nausch *et al.*, 2004).

3.4. Variability in biological and dissolved nitrogen:phosphorus stoichiometry

Redfield (1958) first described the average composition of marine organic matter based on Fleming's data for the average elemental composition of marine organisms. The classical Redfield N:P stoichiometry of 16:1 is often regarded as an upper limit for differentiating N limitation from P limitation. Ratios less than 16 are assumed to indicate N limitation, and ratios above 16 are assumed to denote P limitation. The 16:1 ratio is accepted by biologists (Goldman *et al.*, 1979), whereas many geochemists prefer to use an N: P stoichiometry of 15 based on the covariation of nitrate and phosphate in deep seawater (Broecker and Peng, 1982).

The precise Redfield ratio values have, however, been widely challenged recently (Broecker and Henderson, 1998) with some best estimates being ~25 mol N: 1 mol P. Falkowski (2000) questioned whether the biologists could support or refute this value. Geider and LaRoche (2002) re-examined the observed range of N:P ratios. They found that algal N:P ratios range from 5 to 19 under

optimal nutrient–replete growth conditions, with most observations below the Redfield ratio (Fig. 38.10). Taxonomic trends are also evident, with cyanobacteria presenting high ratios and more recently evolved groups like diatoms characterized by lower values (Bertilsson *et al.*, 2003; Fu *et al.*, 2005a; Heldal *et al.*, 2003; Ho *et al.*, 2003; Quigg *et al.*, 2003). The critical N:P differentiating N limitation and P limitation for phytoplankton growth is significantly higher than Redfield, ranging from 20–50 mol N:mol P (Fig. 38.11). Contrary to the variability in the elemental composition of marine organisms, the inorganic N:P ratio is constant close to the Redfield ratio in most of the deep ocean. This constancy results from feedback mechanisms including nitrogen fixation and denitrification (Redfield, 1958).
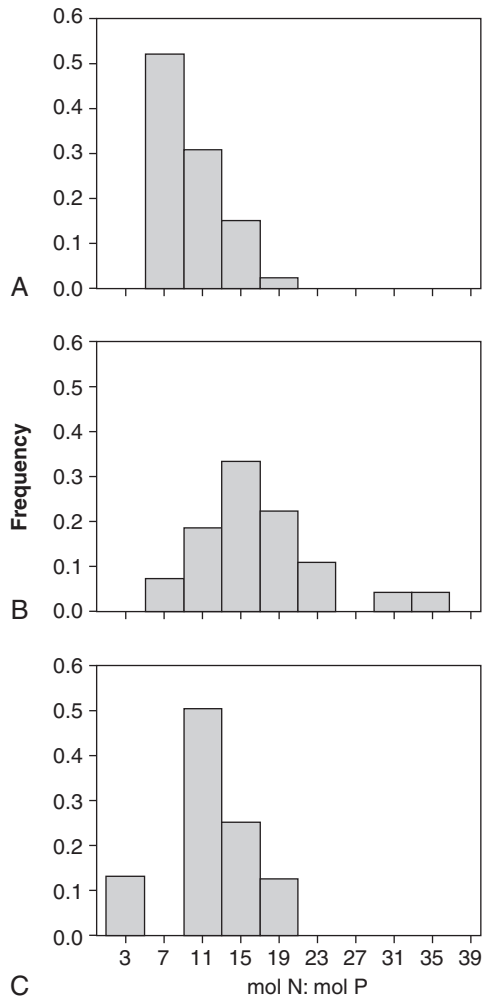
Figure 38.10 N:P ratios of (A) nutrient-replete marine microalgal and cyanobacterial cultures; (B) marine particulate matter and (C) nutrient draw-down during phytoplankton blooms. Adapted from Geider and LaRoche (2002), European Journal of Phycology 37:1–17.
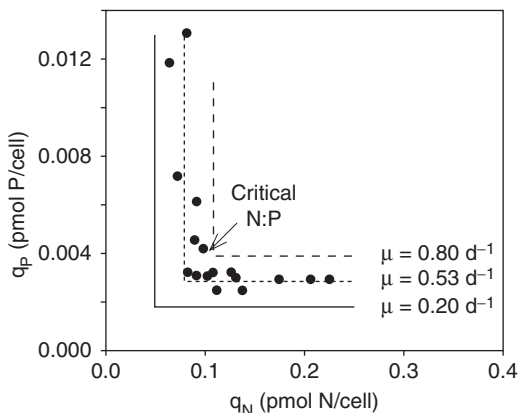
Figure 38.11 Contour plot of cell N quota versus P quota at three growth rates for the green alga *Scenedesmus* sp., showing the critical N:P threshold value for N versus P limitation. Adapted from Geider and LaRoche (2002), *European Journal of Phycology* 37:1–17.

The newly recognized reactivity of PO_4^{3-} with algal cell surfaces (Fu *et al.*, 2005a; Sañudo-Wilhelmy *et al.*, 2004) throws another variable into the Redfield mix. If the scavenged PO_4^{3-} on algal cells is not considered to be part of the cell quota, as it traditionally has not been for other particle-reactive species like Fe (Hudson and Morel, 1989; Tovar-Sanchez *et al.*, 2003), then true algal ''biological'' elemental ratios are probably often considerably higher than Redfield. Because P is used as a normalizer in the Redfield ratio, removing the surface-bound pool can easily result in calculated intracellular N:P ratios that are 50% or more above 16:1 (Fig. 38.8B, Fu *et al.*, 2005a). It appears that overall Redfield ratios integrate the scavenged and internal P pools of phytoplankton, as both together (the total cell-associated P) usually yield Redfield values (Fig. 38.8B). Oceanographers should agree on a common convention for particle-reactive nutrients such as Fe and PO_4^{3-}. When considering overall particulate ratios, such as in vertical flux estimates, it is probably most appropriate to include both interior and surface-bound pools. When discussing nutrient limitation and biological cell quotas, the use of intracellular values makes more sense. In general for any study involving Fe or P, it is necessary to carefully consider and specifically define what subset of the total cell-associated element is to be considered.

4. PHOSPHOSRUS AND IRON AS ALTERNATIVE OR CO-LIMITING CONTROLS ON N_2 FIXATION

There is a limited amount of evidence for direct control of N_2 fixation by P availability. In the subtropical and tropical Atlantic, *Trichodesmium* N_2 fixation rates were correlated to cellular P rather than to colony iron content, suggesting that P limits N_2 fixation in this area (Sañudo-Wilhelmy *et al.*, 2001). Their observations support the hypothesis that P could be limiting N_2 fixation in the North Atlantic

Ocean, due to extremely low dissolved inorganic phosphate levels (Wu *et al.*, 2000). Karl *et al.* (1997) found that diazotrophy increased in the subtropical North Pacific Ocean over seven years of time series observations in parallel with a decrease in soluble reactive P levels, suggesting a shift from N limitation towards P limitation. At some locations in the Baltic Sea, phosphorus additions show a significant impact on N_2 fixation by cyanobacterial communities dominated by *Nodularia*, *Aphanizomenon*, and *Anabaena* (Fig. 38.12A). The level of observed stimulation of N_2 fixation by P addition alone was equal to the simulation by P addition combined with Fe (Moisander *et al.*, 2003).

These observations support the overall limitation of N_2 fixation by P, although it has been suggested that *Trichodesmium* could acquire inorganic phosphorus via vertical migration to the nutricline (Karl *et al.*, 1992; Villareal and Carpenter, 1990; 2003). In most marine ecosystems in which *Trichodesmium* (and probably other N_2-fixing organisms) are found, mixed layer dissolved inorganic phosphate concentrations are very low. Support for the idea that DIP is not the primary source
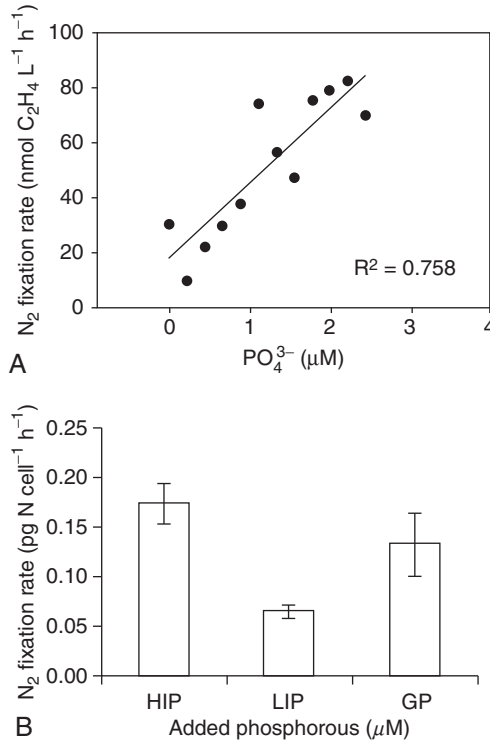
Figure 38.12 (A) Effect of phosphate addition on N_2 fixation by cyanobacterial blooms at a station in the Baltic Sea. Moisander *et al.* (2003), Marine Ecology Progress Series 262: 81–95. (B) Changes in N_2 fixation of the Great Barrier Reef isolate *Trichodesmium* GBRTRLI101during growth on inorganic and organic phosphorus. HIP: 3.5 μM PO_4^{3-}; LIP: 0.5 μM PO_4^{3-}; GP: 3.0 μM Na-glycerophosphate. Values are the means and error bars are the standard deviations of triplicate bottles. Fu and Bell (2003b), FEMS Microbiology Ecology 45: 203–209.

of P to diazotrophs comes from the observation that rates of N_2 fixation measured in many different ocean regions do not correlate with levels of dissolved inorganic P (Hood *et al.*, 2000).

Culture data on PO_4^{3-} growth and uptake kinetics in two strains of *Trichodesmium* from the North Atlantic and the Great Barrier Reef suggest habitat-related trends in P requirements. The strain from the oligotrophic Atlantic had lower phosphate uptake and growth half-saturation constants than the isolate from the higher P environment of coastal Australia. However, neither strain could achieve appreciable growth rates at realistic nanomolar levels of PO_4^{3-} such as those commonly found in the central gyres, with calculated doubling times at these concentrations ranging from one month up to several years (Table 38.1). This suggests that *Trichodesmium* in these areas must necessarily acquire most of their P from DOP sources (Fu *et al.*, 2005b).

Falcon *et al.* (2005) examined the PO_4^{3-} requirements of Atlantic and Pacific unicellular diazotrophic cyanobacteria isolates, and found that half-saturation constants for growth ranged from 0.06 to 0.25 μM. These are similar to the growth half-saturation constants of 0.14–0.22 μM reported for *Trichodesmium* by Fu *et al.* (2005b), suggesting that these unicellular N_2-fixers would also be quite unlikely to meet their cellular P demands by relying on PO_4^{3-} at the reported concentrations in the oligotrophic oceans (∼5–40 nM, Table 38.1).

A single-cell fluorescent assay for expression of alkaline phosphatase (Dyhrman and Palenik, 1999), has been used to show that *Trichodesmium* populations in the Atlantic are likely phosphate-deficient and largely growing on DOP sources (Dyhrman *et al.*, 2002). These results are supported by several other studies showing that *Trichodesmium* is capable of efficiently using DOP (Stihl *et al.*, 2001; Yentsch *et al.*, 1972). Mulholland

Table 38.1 Calculated specific growth rates (μ, day^{-1}) and cellular doubling times (T_d, d) of *Trichodesmium* strains GBRTRLI101 and IMS101 for phosphate-supported growth at typical PO_4^{3-} concentrations reported for surface waters of the North Atlantic Central Gyre, Northern Red Sea, South China, North Pacific Central Gyre, and the Great Barrier Reef lagoon, Australia

Regime and typical $[PO_4^{3-}]$ (μM)	GBRTRLI101		IMS101	
	μ	T_d	μ	T_d
North Atlantic Central Gyre[a] 0.0048	0.00039	2552	0.00082	1219
Northern Red Sea[b] 0.007	0.006	180	0.011	88
South China Sea[c] 0.013	0.01	99	0.02	49
North Pacific Central Gyre[a] mean 0.024 (9–40 nM)	0.018	56	0.035	28
Great Barrier Reef lagoon[d] mean 0.15	0.073	14	0.12	8

[a] Wu *et al.* (2000).
[b] Stihl *et al.* (2001).
[c] Wu *et al.* (2003).
[d] Mulholland *et al.* (2002).
Calculations are based on growth kinetics data obtained from laboratory culture experiments. Fu *et al.* (2005b), *Journal of Phycology* 41: 62–73.

et al. (2002) observed that alkaline phosphatase activities per colony were as much as 350 times higher in the very low PO_4^{3-} environment of the subtropical Atlantic than in a higher PO_4^{3-} regime in coastal Australia. Laboratory cultures grown on organic P (e.g., Na–glycerophosphate) exhibit significant N_2 fixation rates compared to phosphate–grown cells (Fig. 38.12B), suggesting that organic P is a good substrate for growth and diazotrophy by *Trichodesmium* (Fu and Bell, 2003b). New work suggests that unlike many other cyanobacteria *Trichodesmium* has the capability to utilize P from phosphonates, a component of the DOP pool that was previously believed to be relatively refractory (Dyhrman *et al.*, 2006).

In addition to the possibility of P limitation, Fe control of N_2 fixation has become increasingly apparent. Most studies on Fe limitation and N_2 fixation have examined the cyanobacterium *Trichodesmium* spp., since they are known as major contributors of new N to oligotrophic marine ecosystems. Rueter, (1988) and Paerl *et al.* (1994) suggested that natural populations could be Fe-limited and hence Fe would affect the input of new fixed N to the ocean. Later, laboratory culture work also demonstrated that the addition of Fe stimulates N_2 fixation (Berman–Frank *et al.*, 2001; Fu and Bell, 2003a; Kustka *et al.*, 2003). Berman–Frank and her colleagues, (2001) used seasonal maps of aeolian iron fluxes and model–derived maps of surface water total dissolved Fe to suggest that in 75% of the oligotrophic ocean, Fe availability limits N_2 fixation by *Trichodesmium*.

There is both physiological and geochemical evidence showing that marine N_2 fixation may be Fe-limited. The nitrogenase in cyanobacteria contains 15 Fe atoms for each heterodimeric protein molecule (Berman–Frank *et al.*, 2001; Raven, 1988). Also, a high ratio of 24 photosystem I: 1 photosystem II partly explains the greater need for Fe by *Trichodesmium*, since each photosystem I complex contains 12 iron atoms (Falkowski *et al.*, 1998; Subramaniam *et al.*, 1999). Hence it is commonly thought that Fe requirements of marine N_2 fixers are higher than those of other phytoplankton (Raven, 1988). A recently revised estimate of Fe requirements suggests that N_2-fixing phytoplankton may have Fe requirements 2.5–5.2 times greater than NH_4^+ assimilating phytoplankton (Kustka *et al.*, 2003). Generally, 38–48 μmol Fe: mol C are required to sustain a diazotrophic growth rate of 0.1 day^{-1} in *Trichodesmium* (Berman–Frank *et al.*, 2001; Kustka *et al.*, 2002, 2003). N_2 fixation rates increase by 6.6 to 11-fold in Fe–replete *Trichodesmium* cultures compared to Fe-limited cultures (Berman–Frank *et al.*, 2001; Fu and Bell, 2003a; Kustka *et al.* 2002, Fig. 38.13A and B). New work on the Fe requirements of the unicellular N_2 fixer *Crocosphaera* suggests that it has lower Fe:C ratios than *Trichodesmium* ($\sim$27 μmol:mol at a growth rate of 0.1 d^{-1}, Fu *et al.* in press), and thus may be less likely to be Fe-limited *in situ* (Tuit *et al.* 2004).

Trichodesmium spp. probably rely on aeolian dust deposition and vertical mixing of Fe-rich subsurface water as Fe sources. Based on a regional correlation between *Trichodesmium* abundance and aeolian dust fluxes, Kustka *et al.* (2002) argued that *Trichodesmium* has a relatively high Fe demand. Although there is no direct evidence for stimulation of *Trichodesmium* by Fe contained in dust, Rueter *et al.* (1992) hypothesized that *Trichodesmium* may be able to intercept, adsorb and solubilize the Fe from dust and thus could possess a unique pathway for Fe acquisition. However, there has been little or no evidence for this uptake pathway presented in the years since.
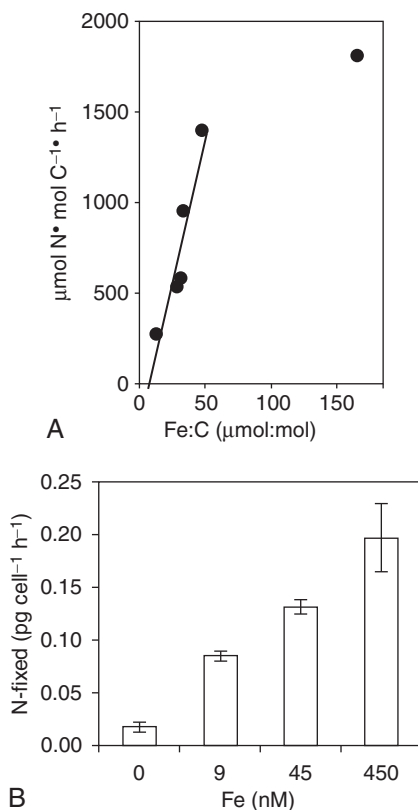
Figure 38.13 (A) N_2 fixation rates of the North Atlantic isolate *Trichodesmium* IMS101 relative to cellular Fe:C content. Fe:C<50 is considered to be Fe–limited, and Fe:C>150 is considered to be Fe replete. Kustka *et al.* (2002), Research in Microbiology 153: 255–262. (B) N_2 fixation rates by the Great Barrier Reef isolate *Trichodesmium* GBRTRLI101 relative to the addition of Fe. Fu and Bell (2003a), Biotechnology Letters 25: 645–649.

In the subtropical North Atlantic Ocean, trade winds blowing westwards from the Sahara desert bring some of the highest particulate dust fluxes in the ocean, with Fe inputs of up to 1000 mg m^{-2} year^{-1} (Duce and Tindale, 1991). This region of high dust flux corresponds reasonably well to the areas of highest abundance of *Trichodesmium* (Tyrrell *et al.*, 2003). Voss *et al.* (2004) observed a strong correlation between whole community N_2 fixation and Saharan dust supply in this regime, although they suggested that diapycnal phosphate inputs are potentially important as well. Lenes *et al.* (2001) suggested that the summer delivery of Fe in Saharan dust to the western Florida continental shelf fertilized the ocean with Fe, and was followed by massive *Trichodesmium* blooms. Similar *Trichodesmium* blooms were observed at the Bermuda–Atlantic Time Series station after dust input events, and the Fe concentration per colony increased approximately 3-fold during maximal dust deposition (Orcutt *et al.*, 2001). Capone *et al.* (1998) observed dense surface accumulations of *Trichodesmium* associated with low wind speed and high Fe in

atmospheric aerosols in the Arabian Sea. A *Trichodesmium* bloom was also observed at the Hawaii Ocean Times Series station in the North Pacific following a storm (i.e., deposition) event (Wells *et al.*, 1995). Tovar-Sanchez *et al.* (2006), however, found that metal ratios in *Trichodesmium* colonies from the tropical and subtropical Atlantic suggested that their Fe content originated primarily from South American and African rivers, rather than from Saharan dust.

Not all field evidence supports the limitation of *Trichodesmium* diazotrophy by Fe. Both Karl *et al.* (1997) and Sañudo-Wilhelmy *et al.* (2001) observed that median Fe concentrations <1 nM Fe did not seem to limit N_2 fixation in field collected samples in the subtropical North Pacific Ocean and the Central Atlantic. It has been observed that there is no close correlation between total dissolved Fe and *Trichodesmium* abundance in the North Atlantic Ocean when only open ocean stations are considered, and little difference between total Fe concentrations where *Trichodesmium* is abundant and where it is scarce (Tyrrell *et al.*, 2003). Moisander *et al.* (2003) noticed that in the Baltic Sea, Fe addition did not show a significant impact on N_2 fixation or biomass production by three other genera of N_2-fixing cyanobacteria.

In the South China Sea, vast inputs of Fe from aeolian deposition do not result in dense populations of N_2-fixing *Trichodesmium* or the diatom symbiont *Richelia* (Chen *et al.*, 2004). They found that P enrichment here also failed to stimulate algal growth. These researchers suggested that a shortage of organic ligands could reduce the solubility of Fe and make it unavailable to diazotrophs, despite massive airborne dust inputs. There have, however, been no field studies that have measured Fe speciation and N_2 fixation simultaneously, so this explanation remains speculative. In contrast to Chen *et al.* (2004), Wu *et al.* (2003) supported the Fe-limited N_2 fixation hypothesis in the South China Sea by noting that low populations of *Trichodesmium* co-occur with low concentrations of dissolved Fe in this marginal sea.

It is now evident that marine N_2 fixation can be limited by either P or Fe, although the relative importance of these two nutrients is still highly debatable. There may be an interaction between iron supply and P limitation of primary production through N_2 fixation. Recently, Mills *et al.* (2004) used a factorial nutrient addition experiment to show that Fe and P can co-limit N_2 fixation in the eastern tropical North Atlantic. They used trace-metal clean techniques with the addition of N, P, Fe, or Saharan dust individually or in combination to investigate which nutrient limits N_2 fixation. Their experiments demonstrated that at two out of three stations, N_2 fixation was enhanced by 2–3 times by the addition of P and Fe together, but the addition of either P or Fe alone had less effect (Fig. 38.14). Added dust also enhanced N_2 fixation, which they suggested could be due to mineral dissolution of both Fe and P.

Mills *et al.* (2004) suggested that future work should investigate whether Fe/P co-limitation is a widespread phenomenon. Because supplies of both Fe and P are patchy in space and time, it seems likely that limitation of N_2 fixation by either nutrient or by both together is possible at a particular site, and that this may vary seasonally or over even shorter timescales. Distinguishing between Fe and P limitation of diazotrophs may not be a simple question of one nutrient or the other, and consideration of both will be needed to fully understand what controls inputs of new N by cyanobacteria in the ocean.
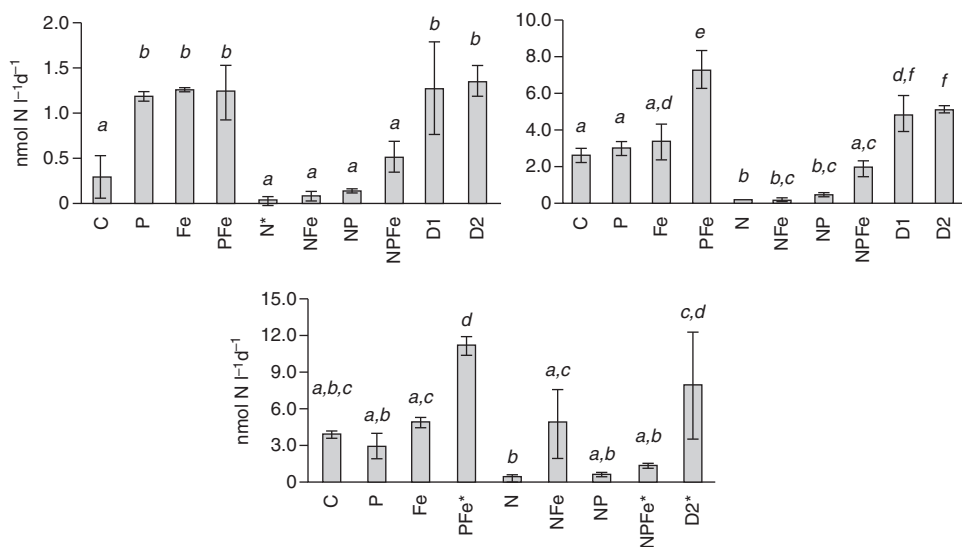
Figure 38.14 Effect of nutrient additions on N_2 fixation rates at three stations during bioassay experiments in the tropical Atlantic Ocean, illustrating Fe/P co-limitation effects. Treatments included controls (C), and nitrate/ammonium (N), phosphate (P), iron (Fe) and Saharan dust (D), added individually or in combination. Means that are not significantly different are labeled with the same letter. Mills *et al.* (2004), Nature 429: 292–294.

5. Conclusions and Future Directions

A tremendous amount of research effort in recent years has greatly expanded our knowledge of the ways that Fe and P affect marine N biogeochemistry. Significant gaps remain, however. For instance, our relative ignorance of the chemical composition of the organically bound Fe and DOP pools in the sea are major remaining obstacles that need to be overcome.

Biological oceanographers are also only now beginning to come to grips with the need to understand and predict anthropogenic global change influences on the ocean. Rapidly accelerating rising sea surface temperature and atmospheric CO_2, ocean acidification, and changes in stratification and circulation patterns will inevitably lead to profound changes in present day biogeochemical cycles over the next century (Bopp *et al.*, 2001; Riebesell *et al.*, 2000; Sarmiento *et al.*, 2004). How these factors will influence future dynamics between the Fe, P and N cycles and their control of ocean productivity is presently far from clear. However, new evidence suggests that N_2 fixation by *Trichodesmium* is C-limited at the present day atmospheric CO_2 concentration, and thus new N inputs by this genus could increase dramatically in the near future (Hutchins *et al.* 2007; Levitan *et al.* 2007; Ramos *et al.* 2007). These CO_2-mediated increases in diazotrophy occur regardless of P limitation in *Trichodesmium* (Hutchins *et al.* 2007), but are subject to control by Fe availability in *Crocosphaera* (Fu *et al.* in press). Thus rising CO_2 will undoubtedly interact strongly with both P and Fe limitation of oceanic N_2 fixation, but in different ways.

Little is known about Fe limitation of the recently discovered unicellular N_2 fixers, despite their abundance in oligotrophic regimes (Zehr et al., 2001) and their potentially large contribution to global new N inputs (Falcon et al., 2004; Montoya et al., 2004). The reason for this is that there is little published data on their Fe requirements, or on the potential for limitation of their N_2 fixation rates by this nutrient (Tuit et al. 2004; Fu et al. in press). Very little is known about their P requirements (Falcon et al., 2005) or their ability to use important P forms like DOP, either. Surprisingly, even the Fe requirements of many ubiquitous and well-studied phytoplankton groups such as Prochlorococcus are also not available in the literature. There is an obvious need to address these information gaps, as well as our ignorance of the Fe and P physiology of other plankton groups whose ecological significance has recently been recognized, such as the archaea (Karner et al., 2001).

Some of these questions will be answered using recently developed molecular and analytical approaches that are opening promising new avenues to explore the influences of Fe and P on the N cycle. Examples include the enzyme-labeled fluorescence (ELF) assay for cell-specific alkaline phosphatase activity (Dyhrman et al., 2002), the molecular marker PstS for P-limited Synechococcus (Scanlan and Wilson, 1999), and biomolecular indicators of Fe status like the IdiA (Webb et al., 2001) and IsiA (Michel and Pistorius, 2004) proteins, which are expressed by Fe-limited cyanobacteria. Powerful new analytical techniques are now becoming available such as synchrotron-based X-ray fluorescence, which can determine trace elemental stoichiometry in individual plankton cells (Twining et al., 2004). The increasing availability of entire microbial genome sequences will also facilitate studies of the regulation of critical biologically-mediated biogeochemical pathways (Armbrust et al., 2004; Dyhrman et al., 2006). Emerging technologies like metagenomic databases and genetic microarrays will also certainly find novel applications in studies of algal and bacterial Fe, P and N requirements in the ocean.

New biogeochemical processes like the recent discovery of surface-scavenged P pools in phytoplankton (Sañudo-Wilhelmy et al., 2004) or the co-limitation of diazotrophy by both Fe and P (Mills et al., 2004) also will continue to come to light as oceanographers delve deeper into the workings of marine nutrient cycles. One thing is already certain, however. The marine N cycle can never be considered realistically in isolation. Iron and P are two of the elements that are inextricably linked with the biogeochemistry of N in the sea, and future marine scientists will forever need to take their mutual interactions into account.

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