CHAPTER 16

# The Nitrogen Cycle in the North Pacific Trades Biome: An Evolving Paradigm

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## 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,

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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 interconnections 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.

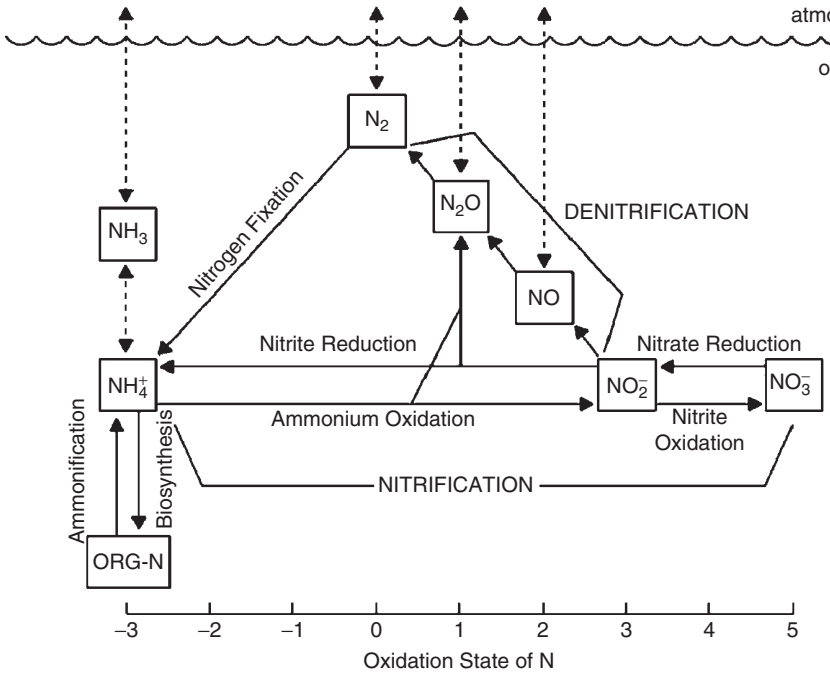


**Figure 16.1** Schematic view of the marine N-cycle showing major pools and transformations. Shown on the bottom is the oxidation state of N in each of the major pools. Changes in the valence state of N require (reductions) or release (oxidations) energy and are often coupled to metabolism. Adapted from Capone (1991) and Karl and Michaels (2001). ORG-N = organic N; $NH_2OH$, not shown, is the precursor for $NH_4^+$ oxidation to $N_2O$.

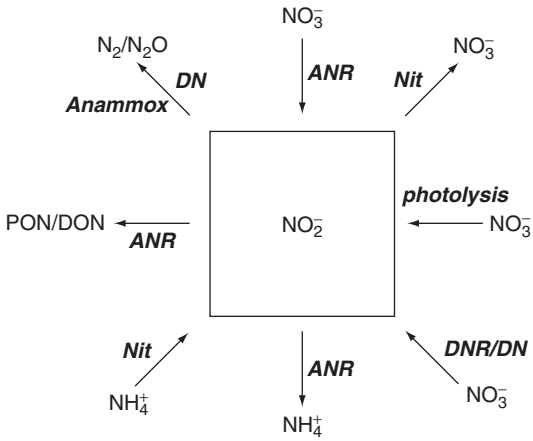


**Figure 16.2** The redox intermediate, nitrite ($NO_2^-$), occupies a central position in the marine N-cycle. Shown are the various processes that are either sources or sinks for $NO_2^-$. Abbreviations include (clockwise from top): ANR = assimilatory nitrate reduction, Nit = nitrification, photolysis = 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

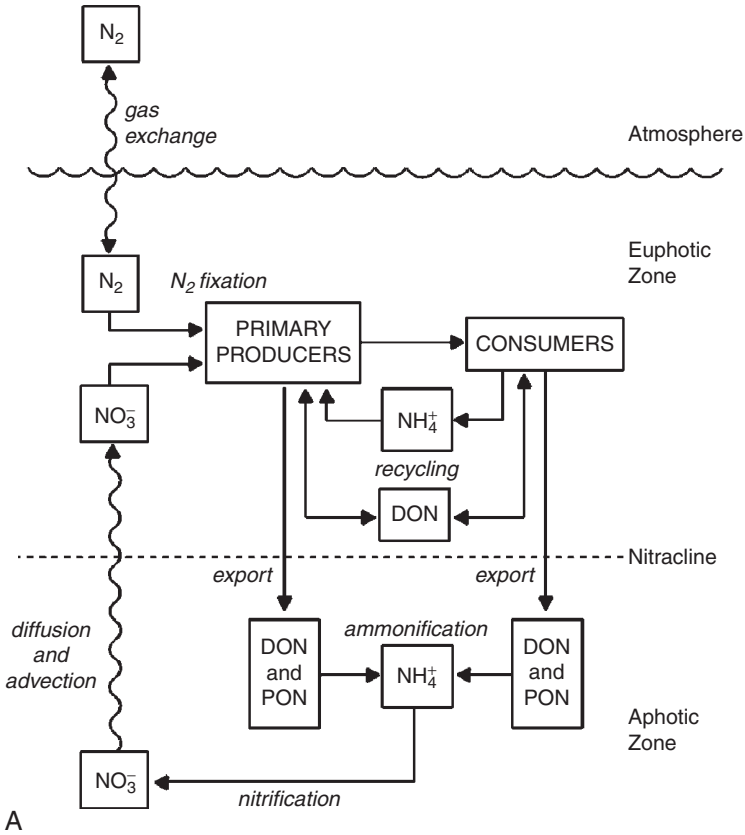


**Figure 16.3** Microbial food web processes sustain the marine N-cycle in the North Pacific trades biome. Shown are: (A) a schematic view of the various sources, transformations and sinks for key N pools and

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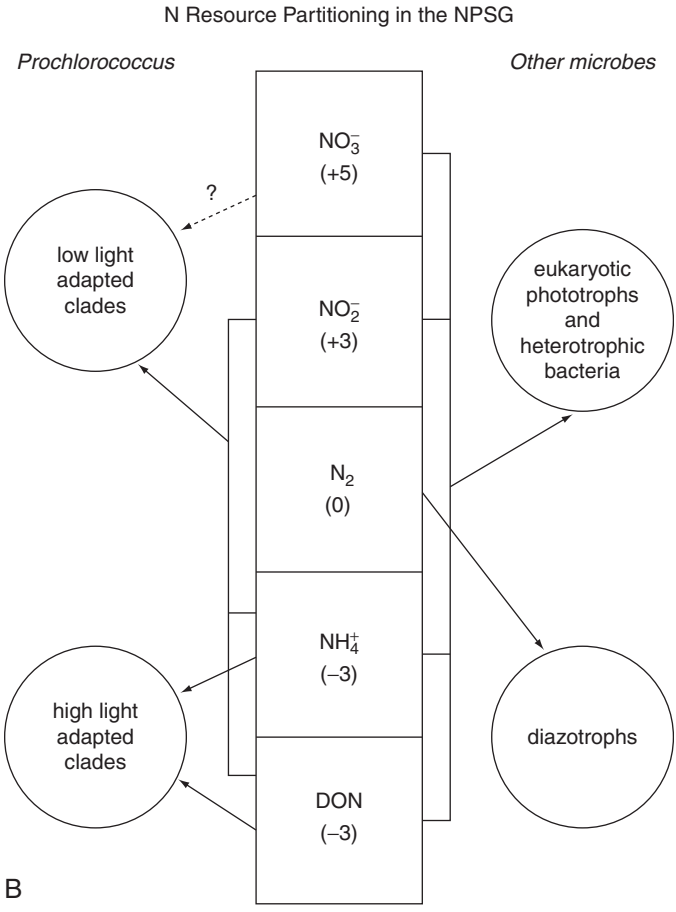



**Figure 16.3 cont'd** (B) a hypothetical microbial N-resource partitioning in the euphotic zone among the various N pools and components of the microbial assemblage.

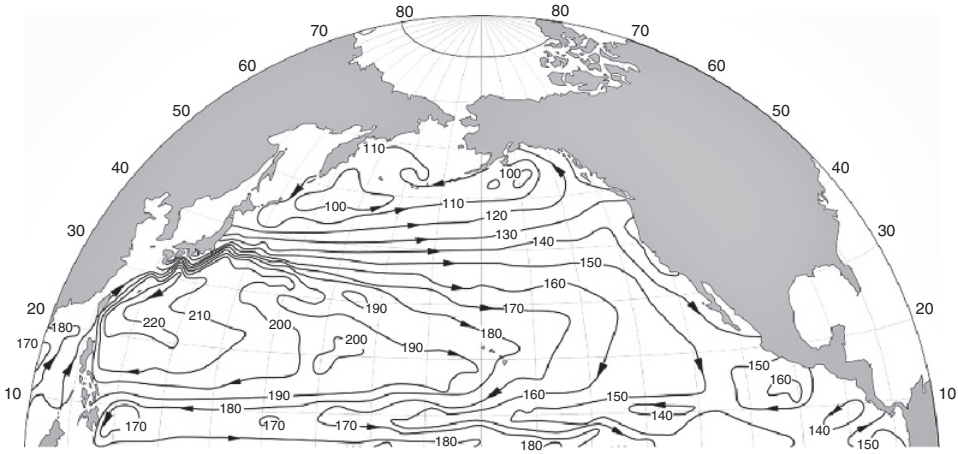
appear to be four primary biomes in the ocean: a coastal boundary biome and three oceanic zones (polar, westerlies and trades; Longhurst, 1998). Biological processes in both the polar and westerlies regions are characterized by strong seasonal cycles that are established by changes in upper water column 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.

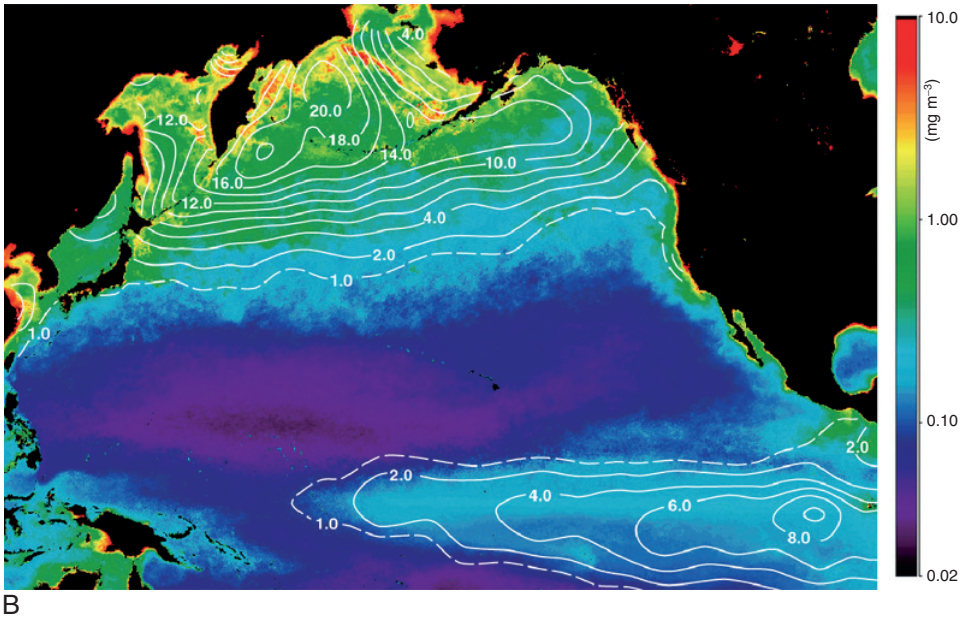
The dynamics of the N-cycle in the trades biomes is distinct from those in other oceanic regions because gross primary production is supported largely by locally recycled $NH_4^+$ and DON, with a subsidy via local $N_2$ fixation and stochastic nutrient entrainment events. Due to the rapid and efficient 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




A




B

**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 $m^{-3}$).

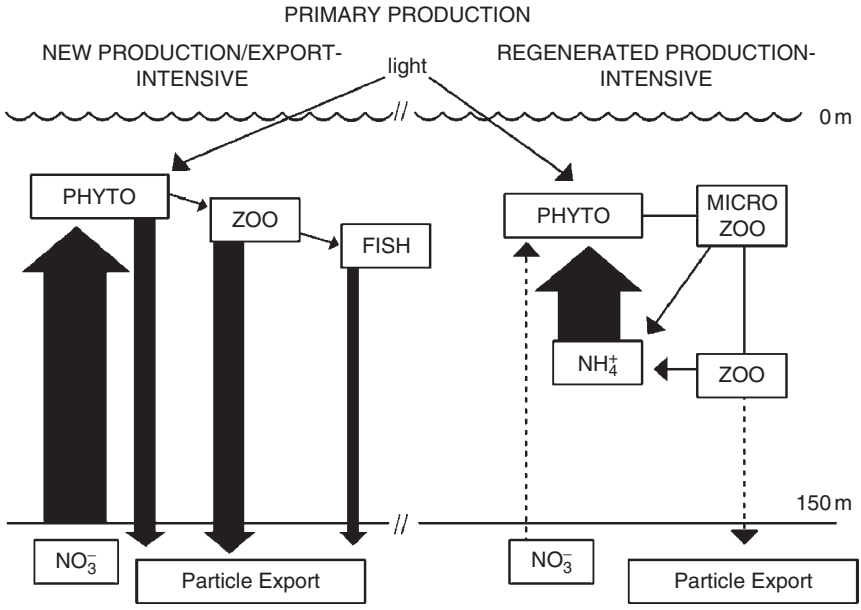



**Figure 16.5** A conceptualized view of the new versus regenerated N model based on the classic work of Dugdale and Goering (1967). Shown are two contrasting marine ecosystems: (Left) an upwelling habitat where allochthonous $NO_3^-$-supported new production dominates total primary productivity, and (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<br>*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

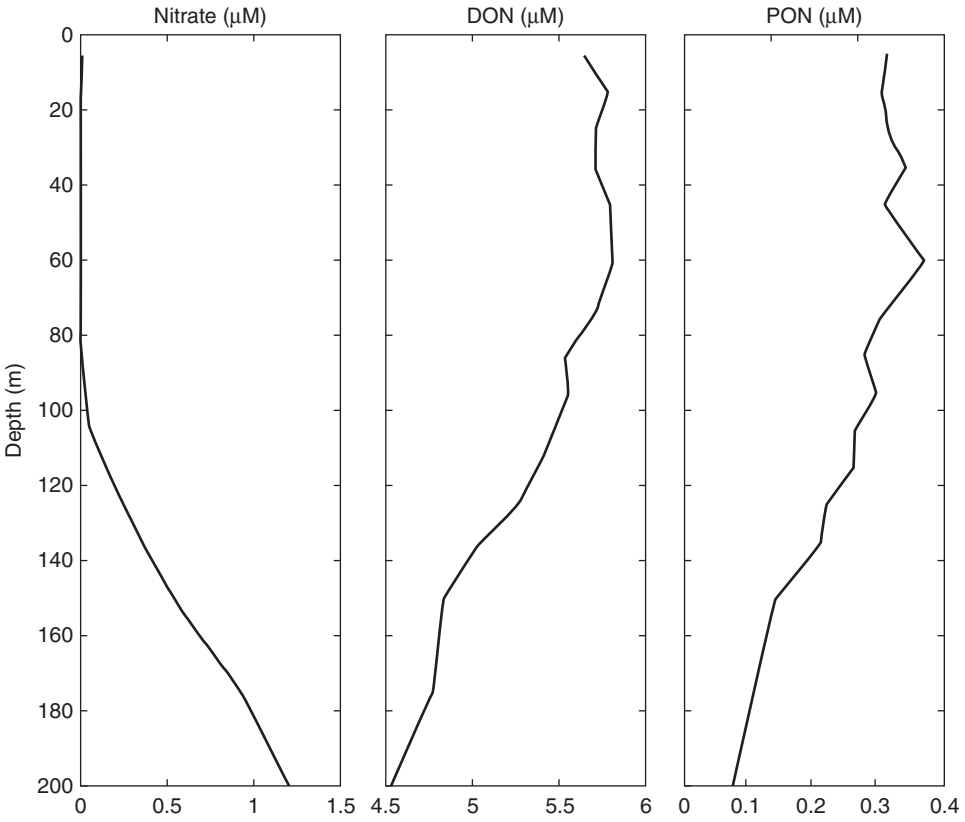



**Figure 16.6** Representative upper ocean profile of $NO_3^-$, DON and PON at Station ALOHA based on 17-year time-series observations. Note accumulation of reduced N, especially DON in near-surface and decreases with depth. In the near-surface waters at Station ALOHA, DON accounts for nearly 95% of 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).

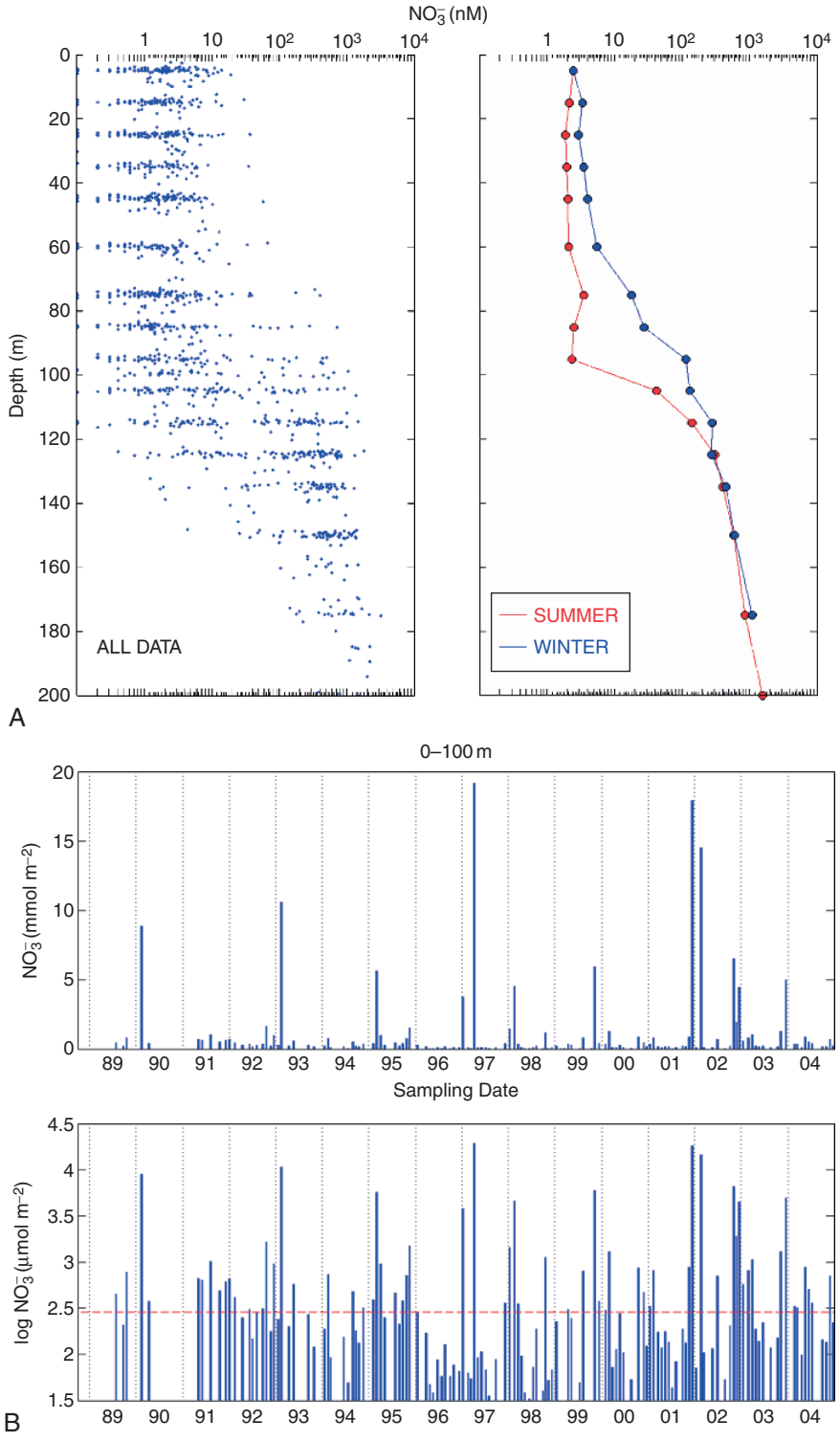



**Figure 16.7** Seasonal and interannual variations in $NO_3^-$ concentrations at Station ALOHA. Shown are: (A) the 16-year data set on $NO_3^-$ (nM) in the upper 200 m as well as the summer vs. winter climatologies. Note the log scale in both graphs. (B) integrated (0–100 m) inventories of $NO_3^-$ 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)*

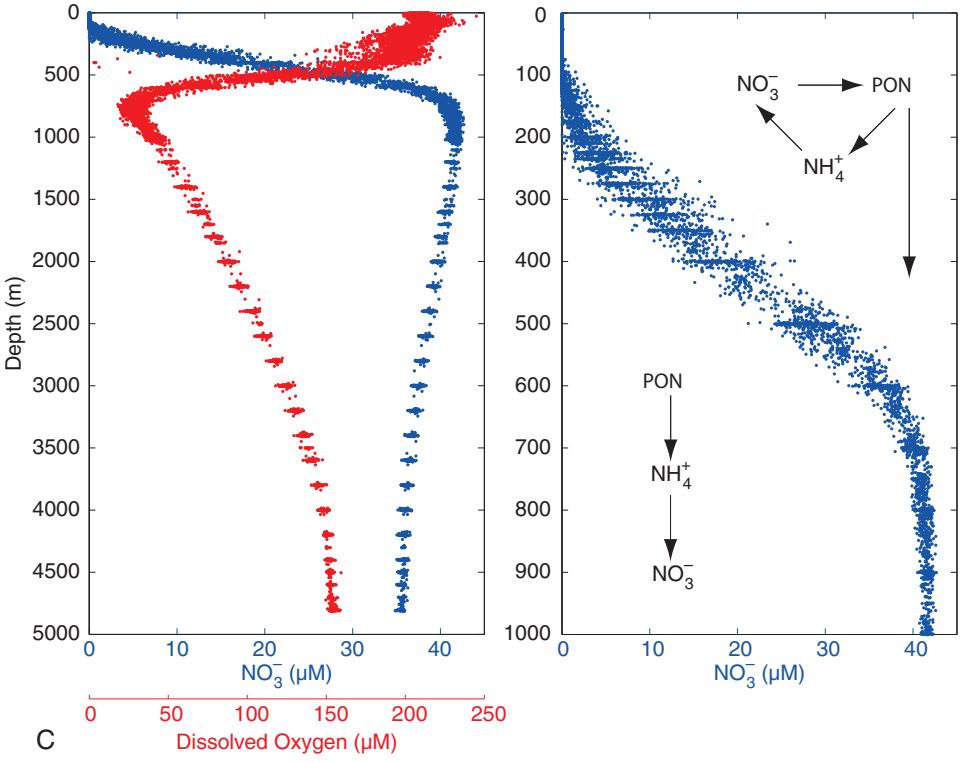



**Figure 16.7 cont'd** (C) full water column $NO_3^-$ and dissolved oxygen profiles showing the relationships between the two pools. On the right hand plot is an enlargement of the upper 1000 m where most of the N transformations take place.

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 ± 0.6‰) and tropical Pacific (5.4 ± 0.8 ‰) Oceans, and showed little correlation with sources or concentrations of N supporting

primary production. However, the $\delta^{15}N$ of the dissolved protein fraction was consistently $\delta^{15}N$-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 $\delta^{13}C$ 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 ($\leq$0.1 in the

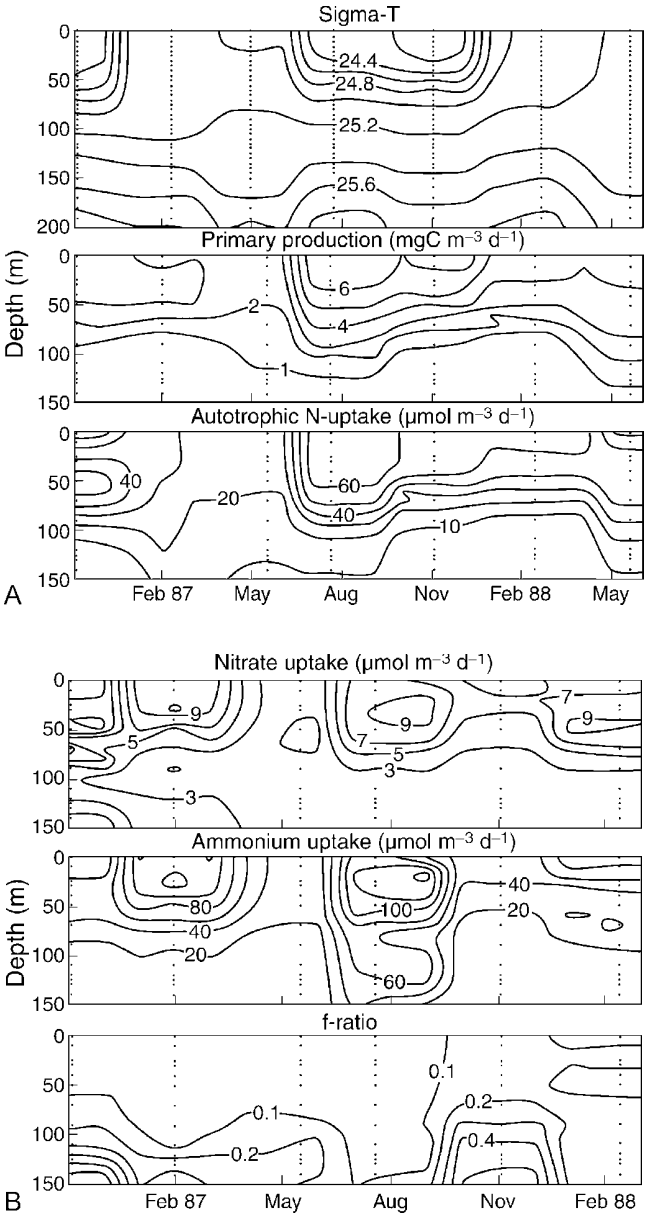



**Figure 16.8** N assimilation data from the VERTEX program time-series station at 33°N, 139°W (adapted from Harrison *et al.*, 1992). Shown are: (A) Upper water column density, expressed as "sigma-T" showing changes in stratification of the water column, $^{14}C\text{-}HCO_3^-$-based primary production, autotrophic N-uptake based on the $^{14}C\text{-}HCO_3^-$ 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<br>$NO_3^-$: 0<br>Urea: 0.8–19<br>$N_2$: 0.03–0.7 | Mague *et al.* (1977) |
| 18°44′N, 157°W Feb 1980 | *''Net'' Total microbial production:* Conversion of $^3H$-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)<br>$NH_4^+$: 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<br>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 $^3H$-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 $\delta^{15}N$ mass balance of exported PON for period 1990–2000 | $N_2$-based: mean = 1.89<br>$NO_3^-$-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 (±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 chemolithoautotrophic $NH_4^+$-oxidizing marine archaeon has been isolated documenting

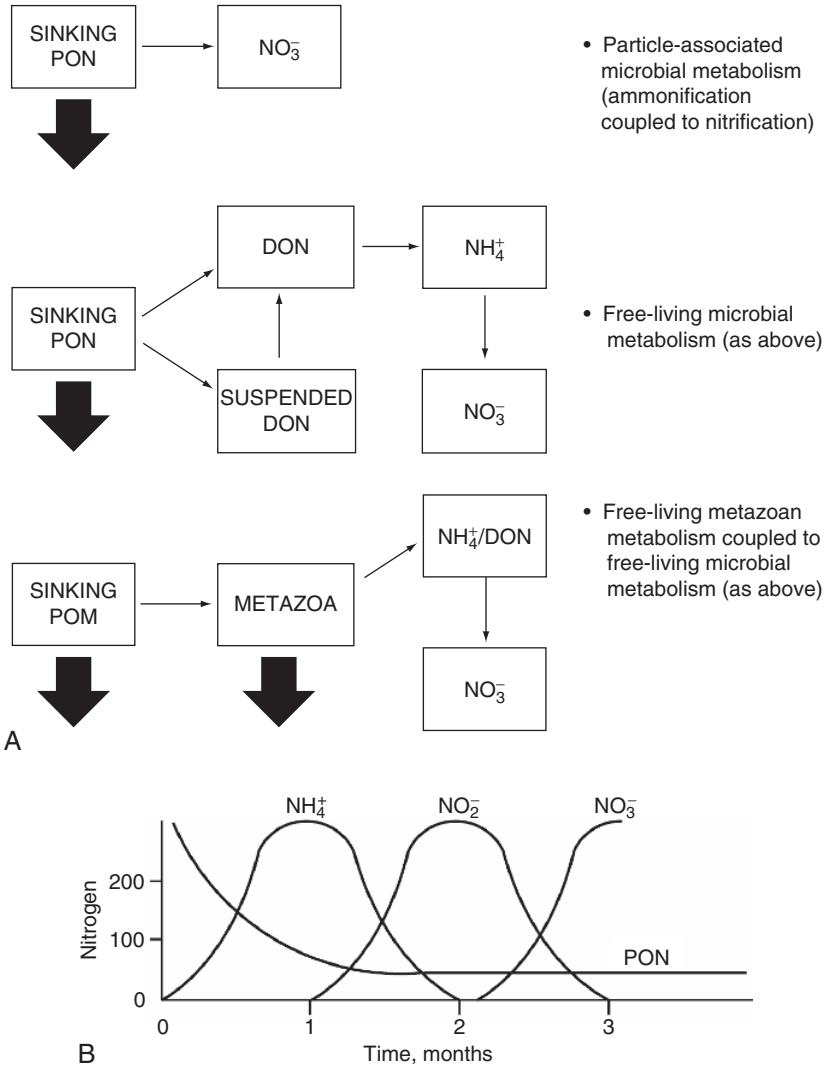



**Figure 16.9** Coupled euphotic zone PON export and mesopelagic zone remineralization processes. (A) Shown are three conceptual models to account for the net decomposition of sinking POM and coupled nitrification showing the various and, potentially independent, roles of micro- and macroorganisms in 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).

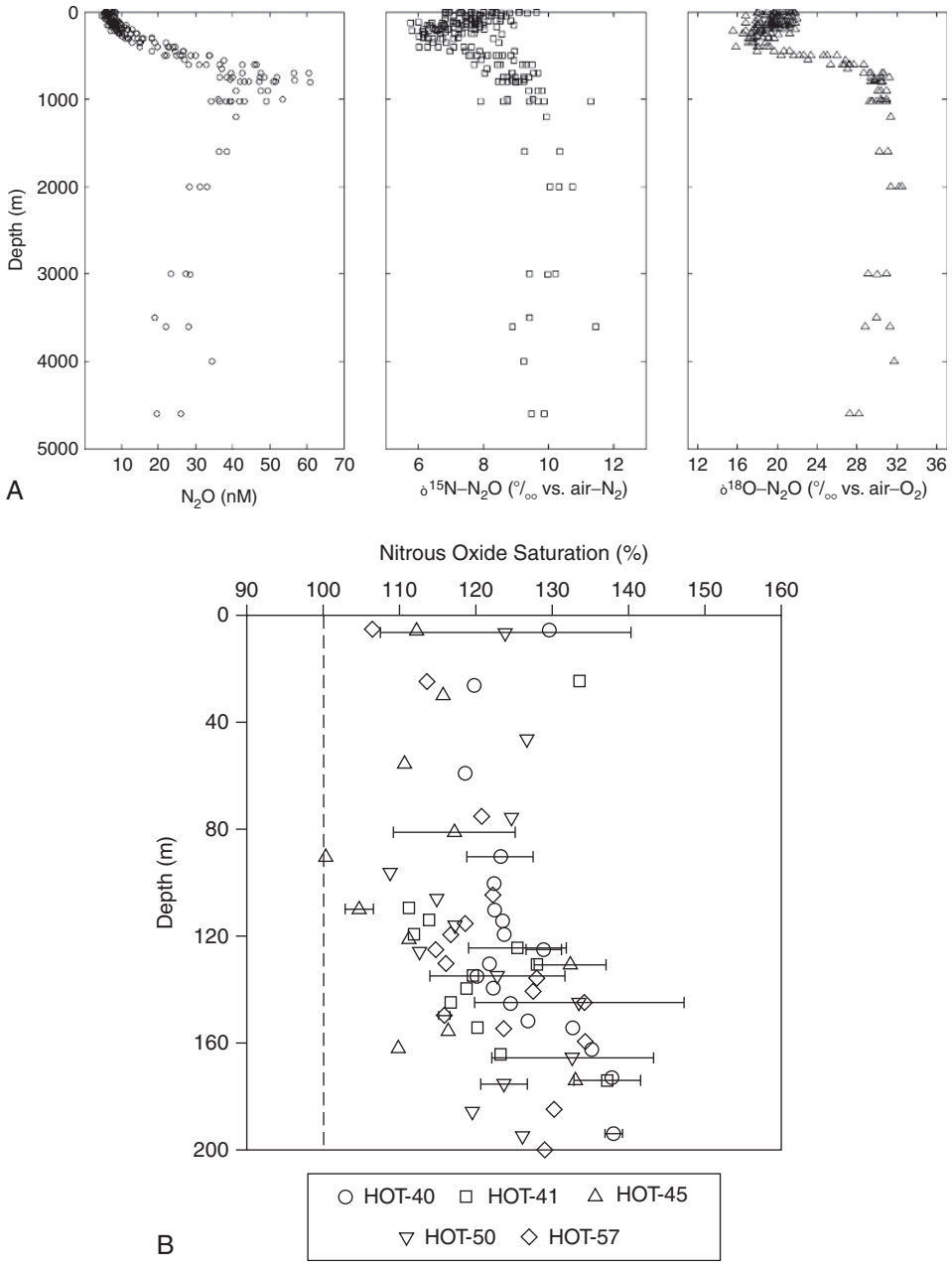



**Figure 16.10** (A) Nitrous oxide ($N_2O$) concentrations and isotopic composition for water samples collected at Station ALOHA. [Left] Depth profile of $N_2O$ showing a distinct mid-depth maximum of ~60 nM coincident with the dissolved oxygen minimum. [Center] $^{15}N$ isotope composition of $N_2O$. [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 $3.7 \times 10^{-5}$ $m^2$ $s^{-1}$ (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 $\delta^{15}N$ and $\delta^{18}O$ 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 $0.4 \pm 0.2$ 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 $\delta^{18}O$ of $H_2O$ and dissolved $O_2$ (Ostrom *et al.*, 2000), the $\delta^{15}N$ 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 ($1.1 \pm 0.7$ 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

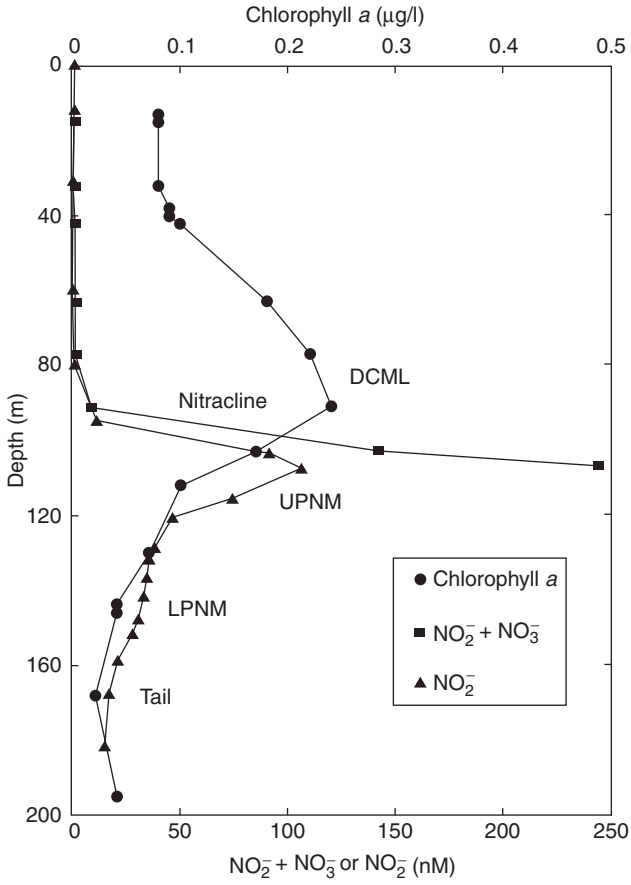



**Figure 16.11** Vertical distributions of Chlorophyll *a*, $[NO_2^- + NO_3^-]$, and $[NO_2^-]$ for the water column at Station ALOHA in October 1992. The conspicuous $NO_2^-$ maximum beginning at approximately 100 m is positioned below the deep Chlorophyll *a* maximum layer (DCML) and is coincident 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 $\approx$ 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.

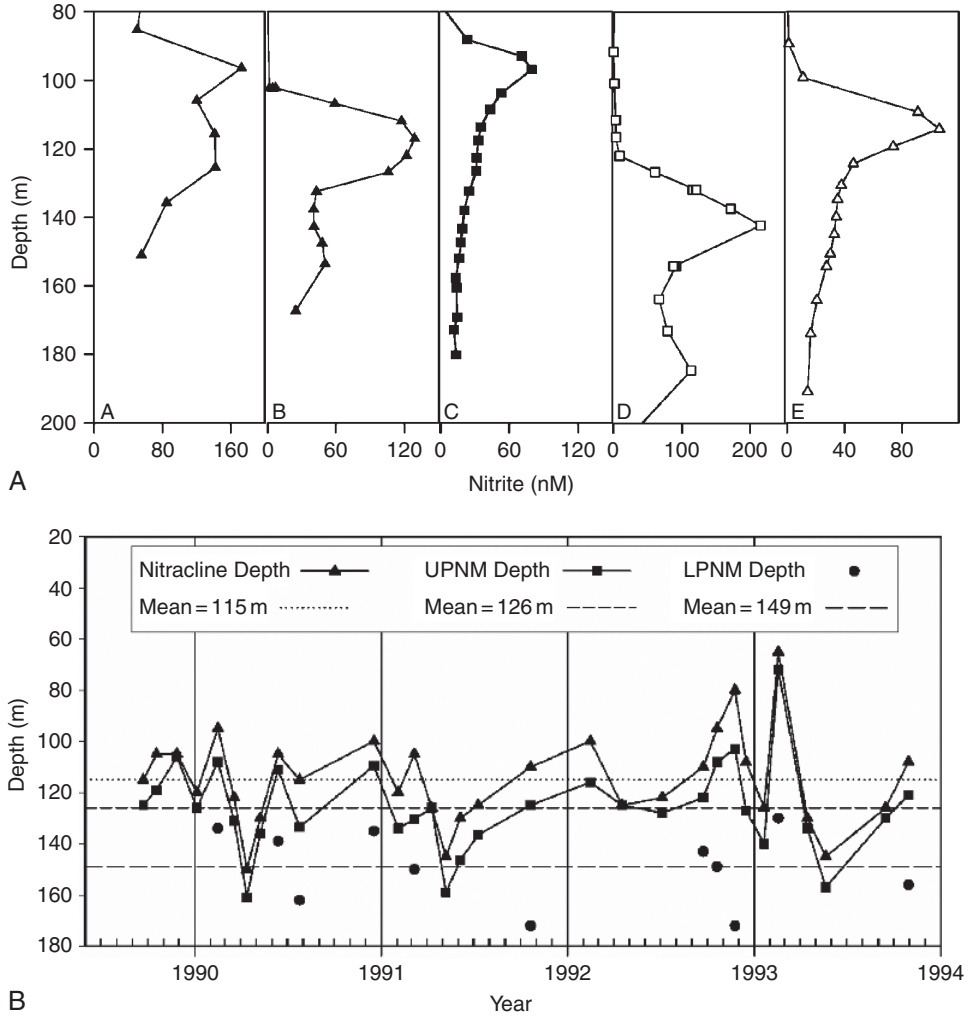



**Figure 16.12** Temporal and spatial (depth) variations in the primary nitrite maximum layer at Station ALOHA. (A) Shown are five representative profiles of $NO_2^-$ between 80 and 200 m from February 1990 to September 1992 displaying the two-peaked structure (U = UPNM and L = LPNM) of the general feature. 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

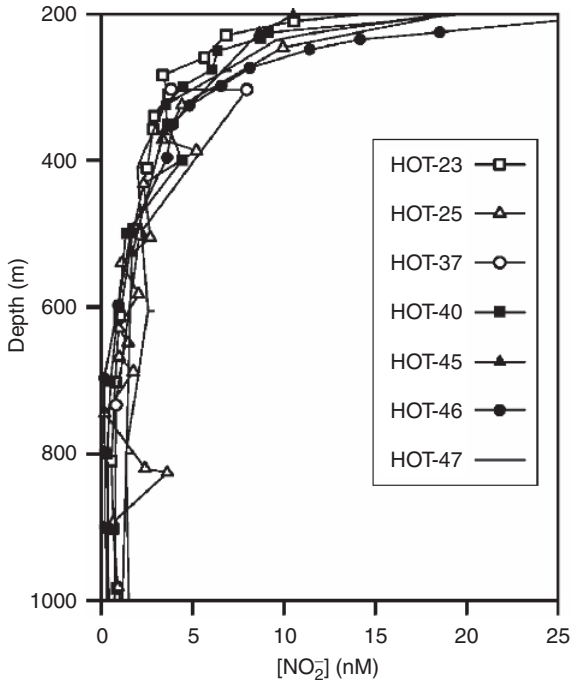



**Figure 16.13** Mesopelagic zone profiles of nitrate from Station ALOHA during 7 cruises from February 1991 to May 1993. From Dore and Karl (1996b).

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

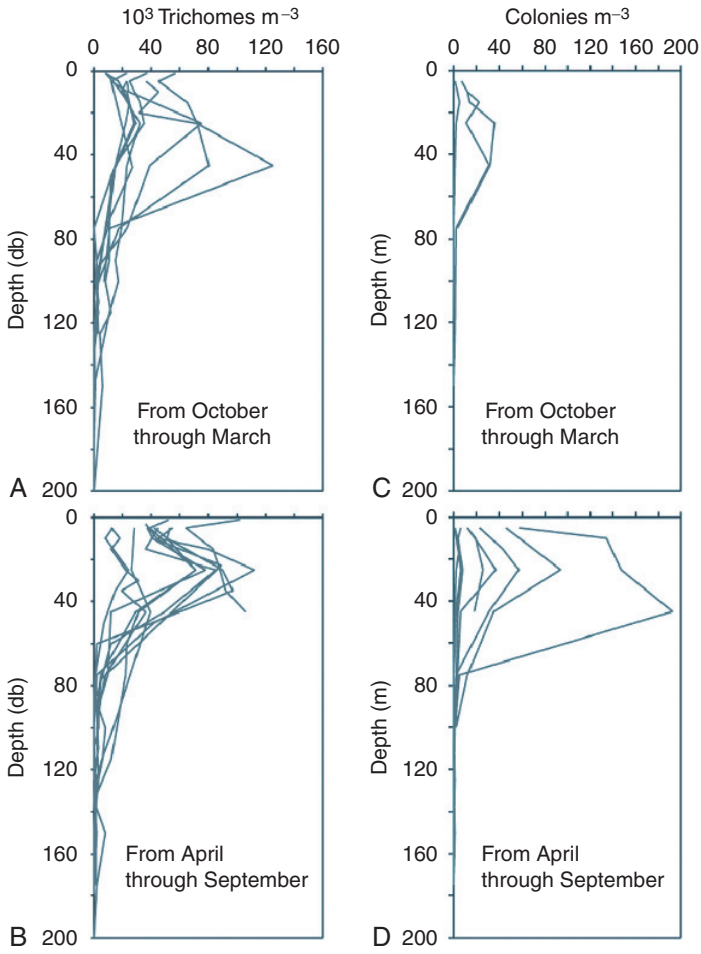



**Figure 16.14** *Trichodesmium* spp. vertical distribution of single trichomes (A, C) and colonies (B, D) at Station ALOHA between October 1989 and December 1992. From Letelier and Karl (1996).

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 $\times$ $10^4$ trichomes $m^{-3}$, and from 0.02 to 1.4 $\times$ $10^2$ 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

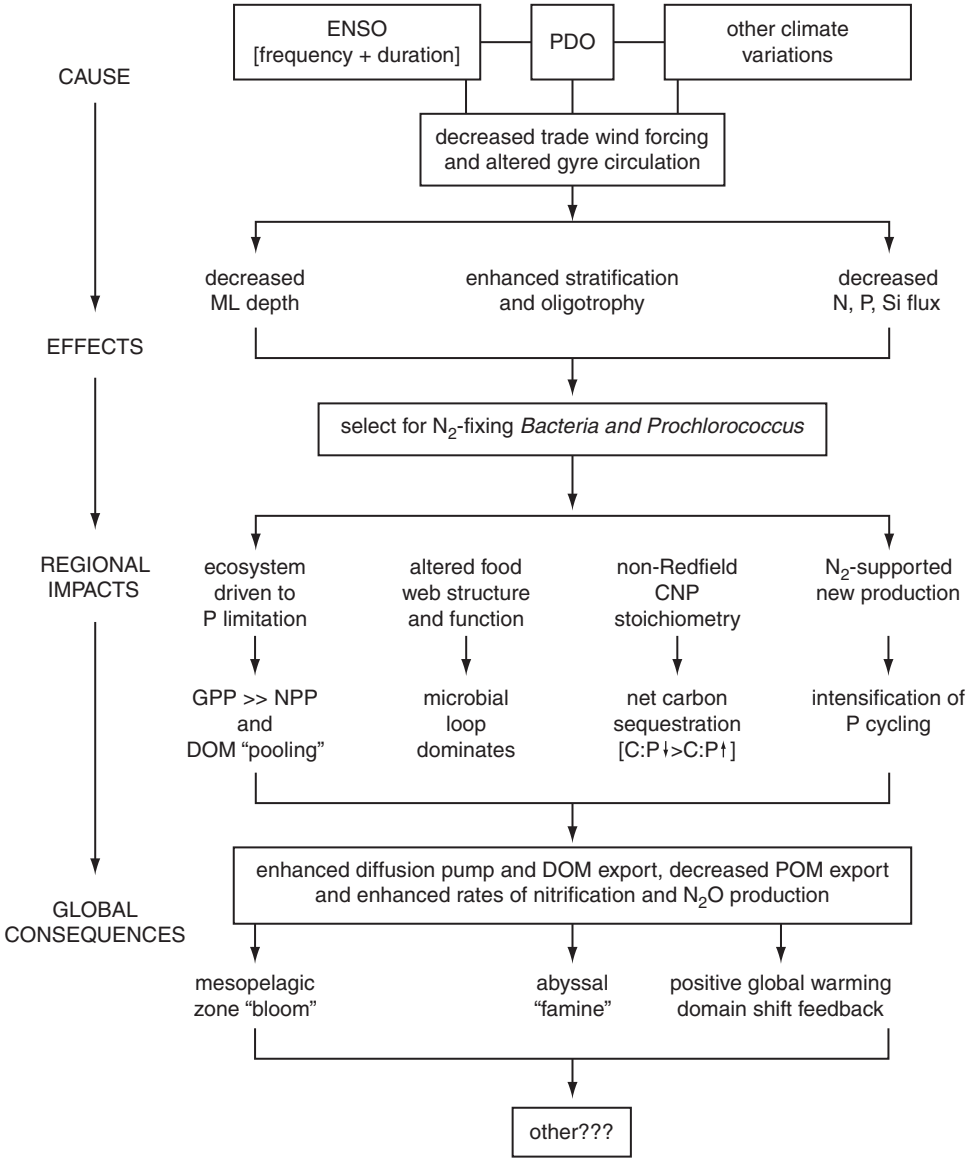



**Figure 16.15** Hypothetical view of the effects of climate variability on ecosystem structure and function in the NPSG based, in part, on results obtained during the decade-long HOT research program. Changes in the stratification of the surface ocean have affected nutrient and trace element budgets 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 $2 \times 10^5$ *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

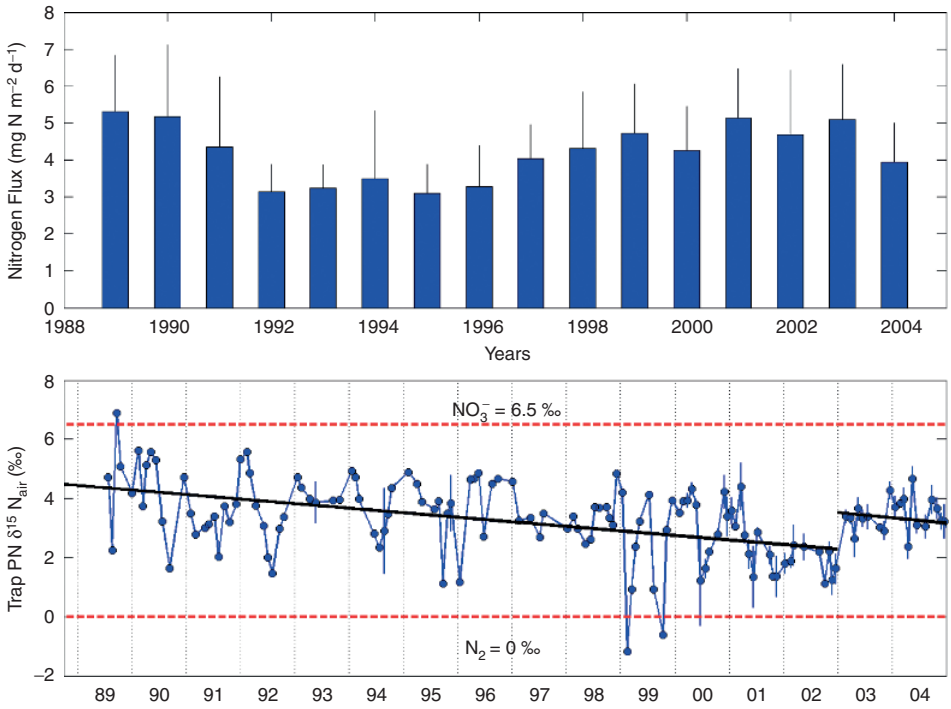



**Figure 16.16** Time series of particulate nitrogen (PN) export and isotopic composition determined from 150-m floating sediment trap collections at Station ALOHA. (Top) PN flux (μmol N $m^{-2}$ $d^{-1}$). Error bars represent ±SE of three to six individual trap measurements. (Bottom) Stable nitrogen isotopic composition ($\delta^{15}N$) of trap-collected material (‰ vs. air $N_2$). Error bars, where shown, represent ±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‰) or entirely by dinitrogen gas (0 ‰). 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).

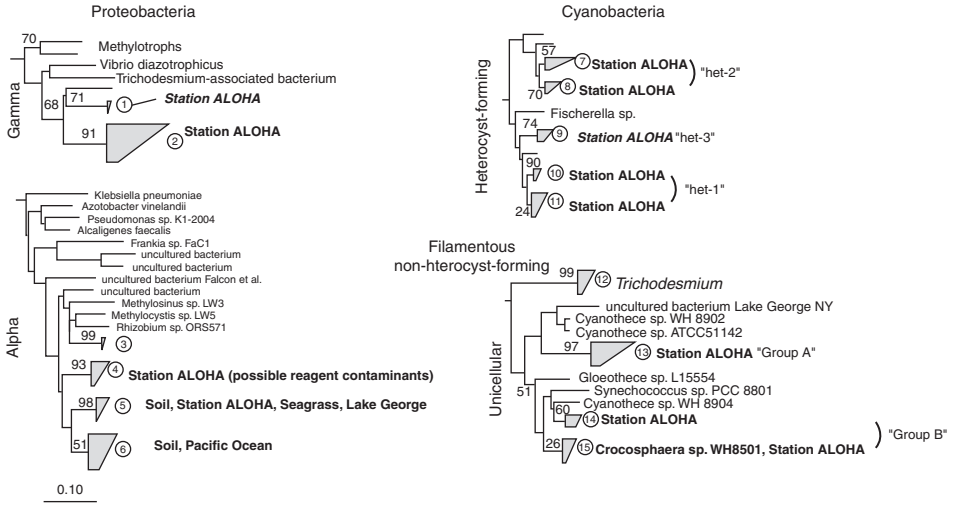



**Figure 16.17** Phylogenetic tree showing major *nifH* DNA sequence phylotypes found at Station ALOHA. Cyanobacterial lineages include three heterocyst-forming groups that are associated with diatoms, free-living filamentous nonheterocyst forming *Trichodesmium*, and the two unicellular cyanobacterial 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 $C_2H_2$ reduction) assays or to reconcile the results of short- ($C_2H_2$) 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.

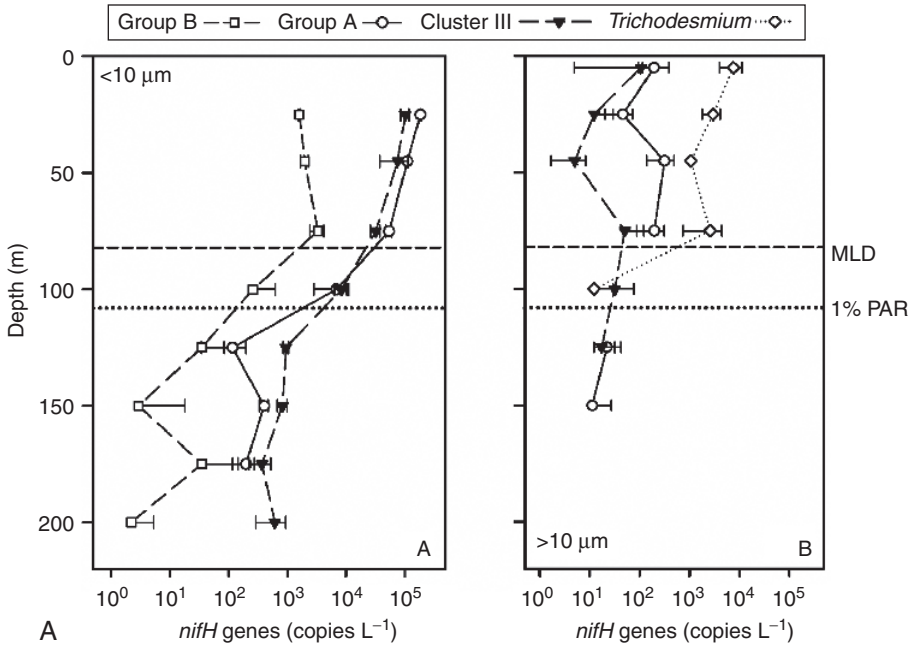



**Figure 16.18** Distribution, abundance and temporal dynamics of $N_2$ fixing bacteria at Station ALOHA. (A) Vertical profiles of <10 μm (left) and >10 μm (right) *nifH* phylotypes in December 2002 relative to upper mixed-layer depth (dashed line) and 1% surface radiance isopleth (dotted line). Error bars are ±1 SD of triplicate QPCR (45 cycles) reactions. From Church *et al.* (2005a).

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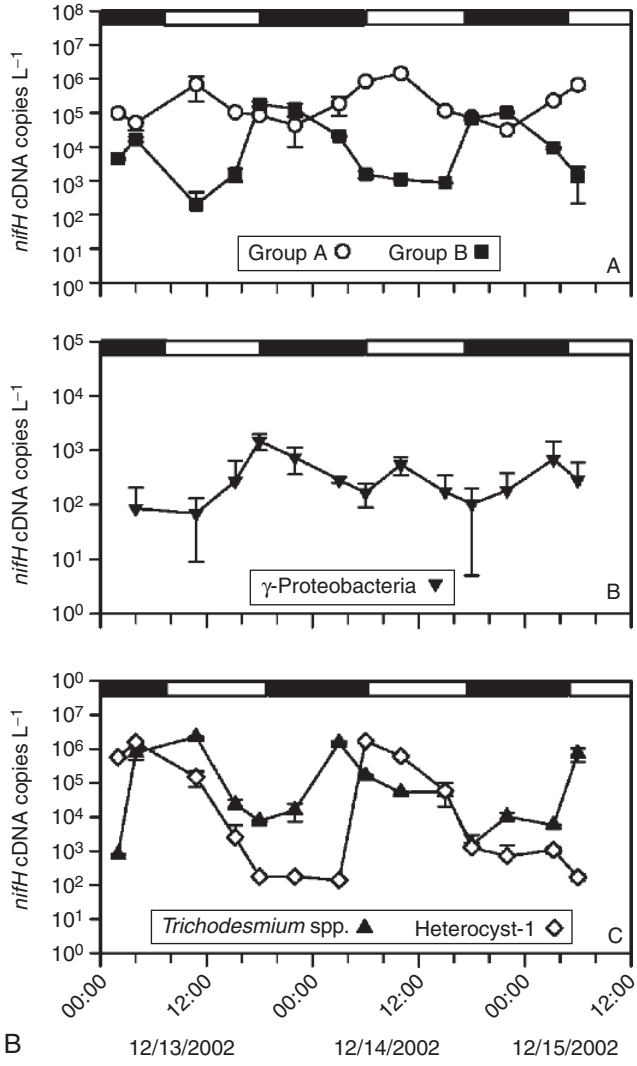


**Figure 16.18 cont'd** (B) Temporal patterns of *nifH* transcription (*nifH* cDNA copies per liter) by five unique phylotypes at a reference depth of 25 m in December 2002. Error bars are ± 1 SD of mean cDNA concentrations from triplicate QPCR reactions. From Church *et al.* (2005b).

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.

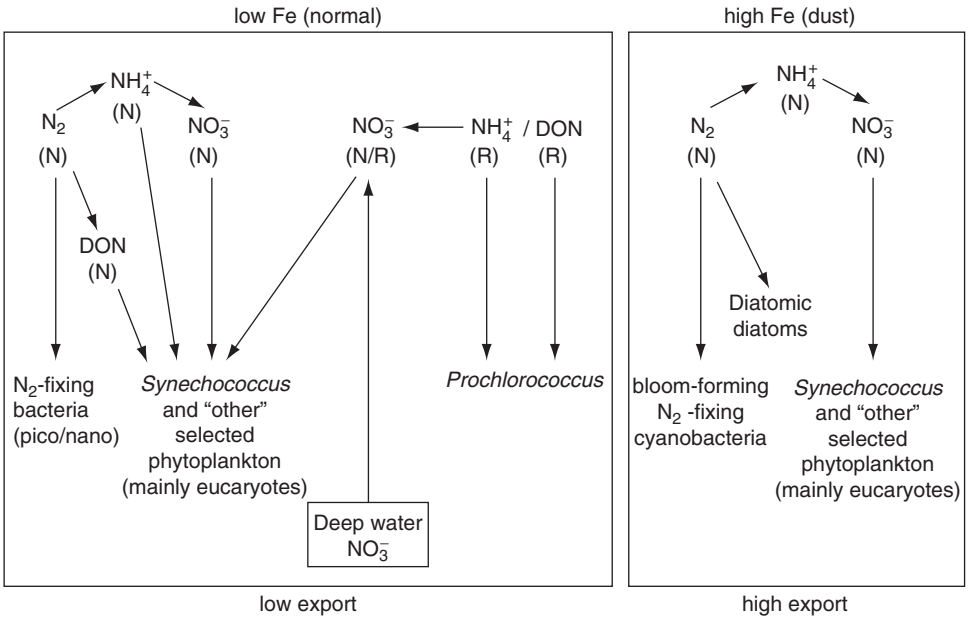


**Figure 16.19** Conceptual view of a revised version of the new vs. regenerated nutrient paradigm for primary production in the trades biome. On the left is the background or normal state (low Fe) where allochthonous inputs of N are dominated by upward diffusive flux of $NO_3^-$ from below with a variable 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 $2 \times 10^7$ $km^2$) 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

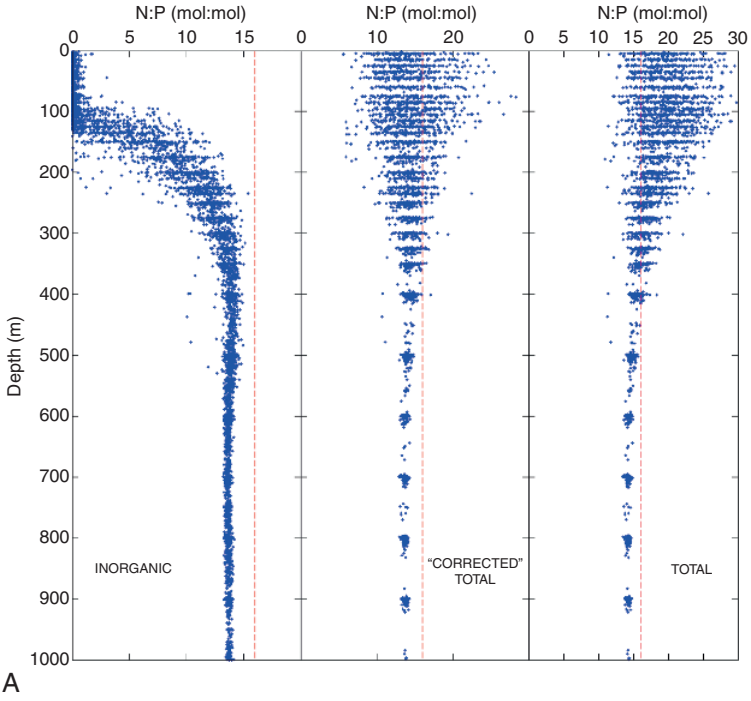


**Figure 16.20** Nitrogen-to-phosphorus (N:P) ratios at Station ALOHA. (A) Depth profiles of N:P for inorganic ($NO_3^-$:$PO_4^{3-}$) and total (TDN:TDP) pools showing fundamentally different depth trends relative to the 16N:1P Redfield ratio, which is shown as a vertical dashed line in each plot. The 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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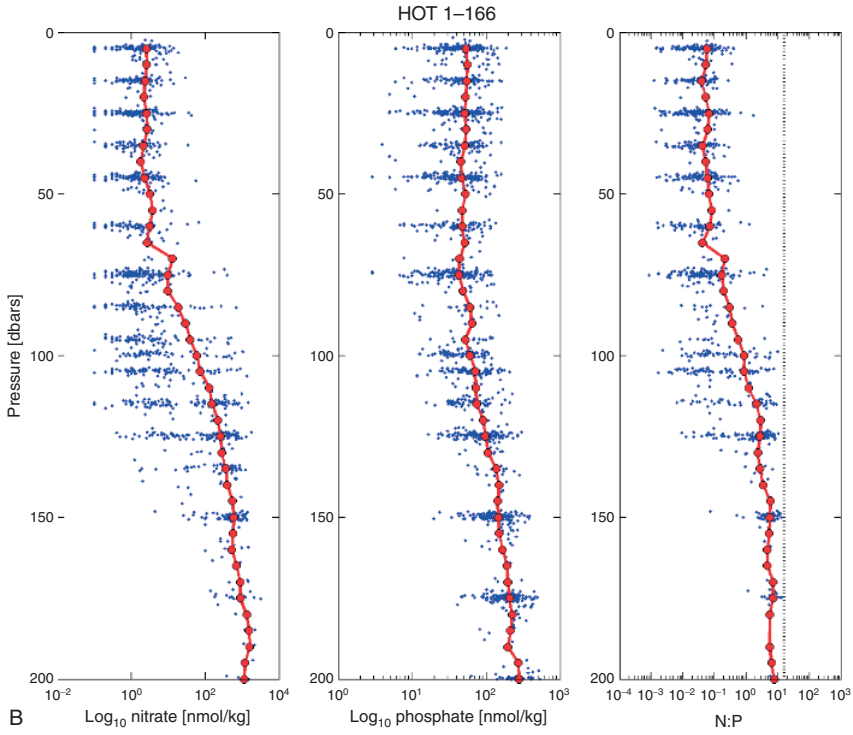


**Figure 16.20 cont'd** (B) Near-surface distributions ($log_{10}$ concentrations) of $NO_3^-$ and $PO_4^{3-}$ and the $NO_3^-$:$PO_4^{3-}$ ratios at Station ALOHA for the period 1989–2004. The large solid circles are the mean values observed and the smaller data points indicate all observations. The 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 $km^2$ 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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