Nitrogen Cycle of the Open Ocean: From Genes to Ecosystems

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Abstract

The marine nitrogen (N) cycle controls the productivity of the oceans. This cycle is driven by complex biogeochemical transformations, including nitrogen fixation, denitrification, and assimilation and anaerobic ammonia oxidation, mediated by microorganisms. New processes and organisms continue to be discovered, complicating the already complex picture of oceanic N cycling. Genomics research has uncovered the diversity of nitrogen metabolism strategies in phytoplankton and bacterioplankton. The elemental ratios of nutrients in biological material are more flexible than previously believed, with implications for vertical export of carbon and associated nutrients to the deep ocean. Estimates of nitrogen fixation and denitrification continue to be modified, and anaerobic ammonia oxidation has been identified as a new process involved in denitrification in oxygen minimum zones. The nitrogen cycle in the oceans is an integral feature of the function of ocean ecosystems and will be a central player in how oceans respond during global environmental change.

INTRODUCTION

The oceans are a central feature of the biosphere, with biogeochemical links to the land and atmosphere. Because the oceans cover almost three quarters of the Earth's surface, the chemical reactions within the oceans, both biotic and abiotic, have profound effects on the gas composition of the atmosphere. The microorganisms in seawater maintain the fertility of the oceans by catalyzing reactions that provide nutrients for growth of higher organisms and result in production and consumption of greenhouse gases. Many key variables control these ecosystem properties, and nitrogen (N), the fourth most abundant element (after hydrogen, oxygen, and carbon) in organic matter, is one of them. The N cycle is a critical component of the biogeochemical cycles of the water column of the ocean (**Figure 1**) because N is often in short supply relative to other nutrients needed for growth and, thus, is often a major limiting nutrient.

Nitrogen, the seventh element in the periodic chart, has an atomic mass of 14 and exists in redox states ranging from −3 to +5. It is commonly found as amine or amide groups in organic matter but is readily oxidized or reduced and, thus, has an additional significance in marine systems as both an electron acceptor and donor for energy metabolism. It is this complexity in microbial metabolism that results in the formation and consumption of different chemical forms involving N atoms, and this complexity drives the biogeochemical cycle of N in the sea (and on Earth, in general). Clearly, N is a central nutrient for terrestrial and aquatic systems (Vitousek & Howarth 1991) and is a key component in global environmental change. Anthropogenic influences on the biogeochemistry of N have resulted in major changes in the Earth's N cycle (Galloway et al. 2004, Howarth 1988),

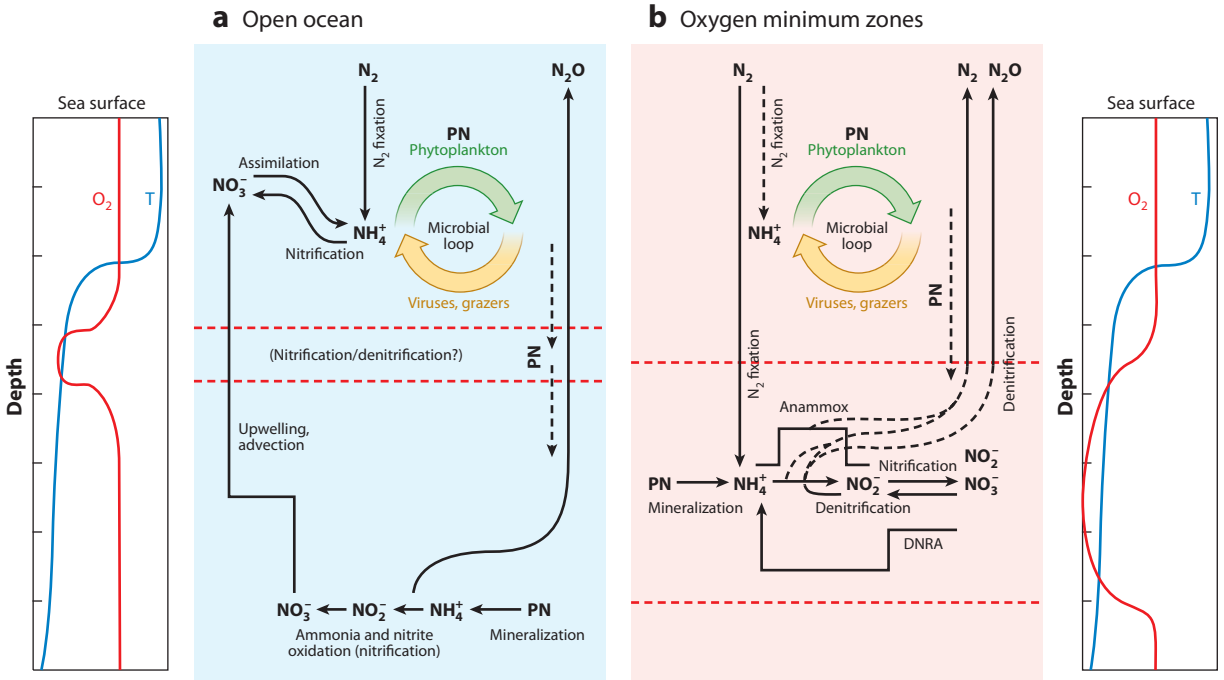

Figure 1

Conceptual diagram highlighting and comparing the major nitrogen-cycle components in (*a*) the typical oceanic water column to that in (*b*) oxygen minimum zones. The oxidation of ammonium to nitrate is called nitrification but includes the processes of ammonia oxidation and nitrite oxidation, catalyzed by different microorganisms. Abbreviations: DNRA, dissimilatory nitrate reduction to ammonia; PN, particulate nitrogen.

with implications for future-climate scenarios. The subject of this review is another intricacy of the marine N cycle: the changing perspective on the N cycle and the microorganisms involved in the open ocean and in the special case of oxygen minimum zones because of their significance to the N cycle. In recent years, new microorganisms have continued to be discovered, along with entirely new pathways in the N cycle. In the words of Lou Codispoti (pers. commun.), author of landmark assessments of the marine N budget, "While some consider the N-cycle to be fiendishly complicated, I prefer to think of it as deliciously complex."

The major challenges in understanding the nitrogen cycle in the ocean are the vast time and space scales of the global ocean. Recent advances in our understanding of the N cycle (see comprehensive reviews in Capone et al. 2008) span scales from genes and organisms to ocean basins. Nitrogen transformations are catalyzed by microorganisms and thus, genome organization, gene expression, and ecological selection can control N fluxes at biome scales. Genes, and more recently whole-genome sequences, provide information on the biological capabilities of the organisms involved in specific N cycle reactions, providing information on the ecological and evolutionary forces that shape the distributions and activities of these microorganisms and their influence on the N cycle. The purpose of this review is to juxtapose different perspectives and scales, to highlight the aspects of the N cycle that are relatively well understood, and to identify some of the knowledge gaps we still face in this "deliciously complex" system.

THE TWENTIETH-CENTURY NITROGEN CYCLE: A STAGE SET FOR DISCOVERY

In the marine environment, the distributions of chemical forms of nitrogen are maintained by chemical equilibria and redox states coupled with bioenergetic considerations. Briefly, concentrations of dissolved dinitrogen gas and dissolved organic nitrogen (DON) are generally the most abundant forms of N in the surface ocean (**Table 1**). Dinitrogen is available to only a relatively small but diverse set of Archaea and Bacteria who can fix N_2 into biologically available ammonium. In coastal waters, where deep water is brought to the surface, or where riverine inputs are significant, oxidized forms of fixed nitrogen, primarily nitrate, can be the dominant form of bioavailable N (**Table 1**). Ammonium, although often at low or undetectable concentrations, is the primary source of N for photoautotrophic carbon (C) fixation. Ammonium, which is fully reduced N (−3), is an energetically favorable N source for most plants and algae, as it is readily transported and assimilated into organic matter with minimal energy expenditure (**Table 1**). Ammonium is also the first breakdown product during the decomposition of organic matter. Most dissolved organic matter containing N is not well characterized (McCarthy & Bronk 2008), although there are small compounds such as urea and amino acids that are easily identified, despite their presence at very low concentrations and rapid recycling, because they are so easily assimilated by both autotrophic and heterotrophic organisms (Baker et al. 2009).

BASIC TENETS OF THE "OLD" N CYCLE

The conceptual oceanic N cycle prior to the 1990s can be characterized by a few key principles that set the backdrop for the ensuing recent years of N cycle discoveries.

1. Nitrogen and phosphorus were believed to be the primary nutrients limiting biomass accumulation and productivity in ocean ecosystems, but the geochemical perspective suggested that P is ultimately limiting on millennial timescales, as deficits in N availability could be overcome by biological N_2 fixation.

Table 1 Major forms of nitrogen in the ocean, indicating habitats of importance

Form	Formula	Function	Pathways	Habitats
Nitrate	NO_3^-	Electron acceptor, nitrogen source	Nitrogen assimilation, dissimilatory nitrate reduction and denitrification	Coastal upwelling zones, deep ocean
Ammonium	NH_4^+	Electron donor, energy source, nitrogen source	Nitrogen assimilation, ammonia oxidation (aerobic), and anaerobic ammonia oxidation	Important, rapidly recycled pool in open ocean, intermediate in decomposition of organic matter
Nitrite	NO_2^-	Electron donor and acceptor, energy source, nitrogen source	Nitrification (nitrite oxidation), denitrification	Found at margins of oxic/anoxic regions, intermediate in oxidation and reduction pathways
Dinitrogen	N_2	Nitrogen source	—	High concentration in equilibrium with atmosphere, available to N_2-fixing microorganisms
Nitrous oxide	N_2O	Trace gas, electron acceptor, electron donor	End or by-product of nitrification and denitrification	Intermediate in reductive pathway, also formed in nitrification and at oxic/anoxic interfaces and anoxic or suboxic zones
Dissolved organic nitrogen	Multiple compounds, including, e.g., amino acids	Nitrogen source, nitrogen-containing organic matter, mostly poorly characterized	Remineralization (ammonification)	Complex organic matter found throughout oceans but with varying, not well-known composition
Urea	$(NH_2)_2CO$	Nitrogen source	Nitrogen assimilation	Decomposition product, potentially important nitrogen source in water column

2. The N and P requirements for phytoplankton growth were believed to be fairly constant, resulting in a relatively constant Redfield elemental ratio of C:N:P in marine organic matter (Redfield 1958).
3. It was assumed that all phytoplankton could use the simple, inorganic fixed-nitrogen compounds nitrate, nitrite, and ammonium commonly found in seawater, as well as some organic compounds such as urea and free amino acids.
4. Most algae, or phytoplankton, use nitrate or ammonium as primary nitrogen sources (**Figure 1**). In the open ocean, the primary sources of nitrate are the mixing of deep nitrate-rich water, atmospheric deposition, surface runoff, or N_2 fixation. Ammonium is the first decomposition product from organic matter and, thus, is rapidly recycled in the water column. These concepts were used to formalize a conceptual model that described the link between inputs and outputs of N (along with biologically associated carbon) by calling N assimilated from ammonium "regenerated" (endogenous) and that from nitrate "new" (exogenous), as nitrate had to be derived from outside the surface ocean reservoir (Dugdale & Goering 1967). This concept was developed further to describe the export of associated carbon (from Redfield stoichiometry), or export production (Eppley & Peterson 1979), with contemporary significance for natural and geoengineering solutions for removal of carbon from the atmosphere.

5. It was assumed at the time of the development of the new and regenerated production model (Dugdale & Goering 1967) that N_2 fixation in the open ocean was small. Nitrogen fixation in the open ocean, subsequent to 1961 (Dugdale et al. 1961, Mague et al. 1974, Saino & Hattori 1978) was believed to be primarily due to the filamentous nonheterocyst-forming cyanobacterium *Trichodesmium* but with additional contributions by heterocyst-forming filamentous cyanobacterial symbionts of diatoms (Carpenter 1983).
6. Anaerobic processes, the use of oxidized N compounds as an electron sink for respiration or metabolism, were believed to occur primarily in the sediments but also in the water column of several restricted regions of the ocean basins where there were low oxygen concentrations, called oxygen minimum zones (OMZs) (**Figure 1**). The production and sedimentation of organic material, coupled with oxidized N compounds from deep water, result in depleted oxygen concentrations that allow the use of alternate electron acceptors, such as nitrate and nitrite. These regions may be expanding in the ocean (Stramma et al. 2008), and because they allow important reductive N transformations to occur, are covered in this review. The primary reaction in these OMZs was believed to be denitrification, a respiratory process that uses nitrate as an electron sink for the oxidation of organic matter. This reaction leads to the loss of N from the system as gaseous N_2, ultimately balancing the use of gaseous N_2 by N_2-fixing organisms.
7. The reductive pathways involving oxidized N compounds were limited primarily to denitrification in sediments and OMZs. Nitrate respiration and dissimilatory reduction of nitrate to ammonium were known but were believed to occur in a few specific habitats, primarily sediments, and to have negligible influence on the biogeochemistry of N in the oceans.
8. The use of reduced N compounds as an energy source was limited to several taxa or lineages of the Bacteria (primarily Proteobacteria). These processes, ammonia oxidation and nitrification, were believed to occur primarily in the deep ocean, fueled by decomposing, sedimenting organic N materials (resulting in the nitrate-rich deep water described above). Ammonia oxidation and nitrification also occur in sediments at the interface between oxic and anoxic zones, fueled by diffusion of ammonium from below and oxygen from above.
9. The key microbial players in the N cycle were known primarily from a few cultured isolates, and from amplified rRNA genes using polymerase chain reaction (PCR) primers targeting specific groups of organisms known to be involved in N transformations, such as the nitrifiers and denitrifiers.
10. It was not known whether the oceanic N budget was in balance, or whether the global oceanic denitrification rate exceeded the global N_2-fixation rate. Therefore, it was also unknown, despite speculation, whether the oceans were ultimately N- or P-limited as the N budget was not adequately characterized.

Nitrogen fixation: reduction of N_2 gas (approximately 80% of the atmosphere, and in equilibrium in surface seawater) to ammonium as a source of nitrogen. Biological N_2 fixation is catalyzed by the enzyme nitrogenase, a multicomponent enzyme that requires metals (Fe, and usually molybdenum). The reaction, although exergonic, has a high activation energy and is inactivated by oxygen. The reaction is energetically expensive, requiring approximately 16 ATP and 8 reducing equivalents per N_2 reduced to 2 NH_3

Denitrification: reduction of oxidized compounds resulting in production of N_2 gas that is released to the atmosphere (hence, loss of N from the ecosystem); a primarily respiratory process using nitrate, nitrite, nitric oxide, and nitrous oxide as electron acceptors in respiration. Anammox metabolism also results in production of N_2 gas and is by strict definition a denitrification pathway. The canonical denitrification pathway refers to the respiratory reduction of organic nitrogen compounds coupled to the oxidation of organic matter for energy

OCEANIC N LIMITATION, ELEMENTAL STOICHIOMETRY, AND CARBON FLUX

N and Nutrient Limitation

Climate change can be expected to modify the sources, availability, and speciation of major nutrient pools in the ocean, making it critically important to know the nutrient(s) most limiting to both biomass accumulation and the rate of phytoplankton growth. Nutrients can limit growth, productivity, or ecosystem production (Howarth 1988). Productivity is perhaps the most relevant to evaluate (Howarth 1988). Geochemists have argued that phosphorus (P) is the primary limiting nutrient in the oceans over geological timescales (Redfield 1958, Tyrrell 1999). This argument is

largely based on the existence of biological N_2 fixation, the enzymatic conversion of atmospheric N_2 to ammonium by a variety of microorganisms, which can alleviate ecosystem N limitation (Redfield 1958). The atmosphere is composed of approximately 80% N_2 gas and is an essentially unlimited source of N for N_2-fixing microorganisms. There is no atmospheric source for P, and P must be obtained from recycling organic matter (in shallow or deep water) or terrestrial sources (runoff), with P concentrations ultimately dependent on the balance between terrestrial rock weathering and oceanic burial. Despite the assumption that P ultimately limits ocean ecosystems, marine nutrient addition experiments often result in a growth response when N is added (Elser et al. 2000, Ryther & Dunstan 1971, Thomas 1970) but an even greater response when P is added along with N (Elser et al. 2000, Smith 1984). Although Elser et al. (2000) reviewed the results of over 200 marine experiments, these were largely biased to coastal systems that may be affected by P inputs (Elser et al. 2000). Thus, it is probably true that N limitation is less well proved experimentally in marine systems than in freshwater systems (Hecky & Kilham 1988).

The basic assumption of the argument of N versus P limitation has been challenged because N_2 fixation can be limited by trace elements (Falkowski 1997). It was previously suggested that other nutrients should also be limiting in the open ocean based on analysis of marine N:P ratios (Downing 1997). Building on the work of Martin (Martin & Fitzwater 1988), there is now recognition of the role of iron (Fe) in regulating productivity in wide regions of the oceans (Boyd et al. 2007). Fe has been shown to stimulate general phytoplankton production in high-nutrient, low-chlorophyll regions (Boyd et al. 2007) and may have an even stronger role in limiting N_2-fixing microorganisms because of the need for Fe in the nitrogenase metalloproteins (see Kustka et al. 2003). This importance of Fe for N_2-fixing microorganisms is one of the strongest connections between Fe and the N cycle.

Nutrient regimes, the relative abundance of N, P, and Fe, and their inputs are highly variable across the ocean basins. The most important Fe inputs are believed to be primarily via aeolian deposition from terrestrial sources (Fung et al. 2000), because Fe carried by rivers is rapidly precipitated (Jickells et al. 2005). In some regions, Fe transported horizontally along the pycnocline from shelf regions may be another, less well characterized source (Lam & Bishop 2008). There is also some evidence for the role of deepwater iron enrichment in some regions, including the oligotrophic basins (Boyle et al. 2005), which plays a role in both past and future climate-change scenarios (Blain et al. 2007 and references therein). Wind patterns and the solubility of various forms of Fe-rich compounds create gradients of Fe inputs across and between basins (Jickells et al. 2005, Moore et al. 2006). The patchy distribution of Fe inputs overlays the patchy distribution of N and P. There is an order of magnitude difference in orthophosphate concentrations between the Atlantic and Pacific oceans (Wu et al. 2000), and P appears to limit N_2 fixation in the North Atlantic (Sanudo-Wilhelmy et al. 2001). In contrast, Fe concentrations are low in the ultraoligotrophic South Pacific gyre, but P concentrations are relatively high (Bonnet et al. 2008). Although it has been suggested that Fe limits phytoplankton in this region (Behrenfeld & Kolber 1999), additions of Fe did not stimulate N_2 fixation in this ultraoligotrophic system and few N_2-fixers have been identified in this region (Bonnet et al. 2008).

Whereas Fe enrichment in N-rich areas of the oceans typically demonstrates Fe limitation of productivity (Boyd et al. 2007), nutrient enrichment experiments in oligotrophic regions clearly indicate multiple controls on ecosystem production and N_2 fixation (Arrigo 2005, Elser et al. 2000, Mills et al. 2004). N_2-fixing populations can be limited by different nutrients than the non-N_2-fixing populations, resulting in a complex cross-feeding of N, P, and Fe among populations. Productivity in the tropical Atlantic was shown to respond to N additions, whereas N_2 fixation was enhanced by additions of Fe and P, suggesting that N_2-fixing microorganisms were colimited by Fe and P (Mills et al. 2004).

Nitrification: oxidation of ammonium to nitrate. Strictly defined, nitrification is composed of two distinct processes, ammonia oxidation and nitrite oxidation, catalyzed by different microorganisms. Originally thought to occur primarily in the ocean and sediments but now recognized as occurring throughout the water column, mediated by both Bacteria and Archaea

Polymerase chain reaction (PCR): laboratory procedure that uses repetitive cycles of heating and cooling to enzymatically synthesize a specific region of DNA, using DNA polymerase and oligonucleotides that prime and target the reaction to amplify a specific DNA region (e.g., a gene or part of a gene) for subsequent detection or analysis

The focus on nutrient limitation is complicated by the fact that the oceans are not in steady state (Karl et al. 2001a), and nutrient ratios (N:P) exhibit this imbalance (Pahlow & Riebesell 2000). Annual, decadal, and longer ocean cycles such as glacial-interglacial changes in source waters and aeolian deposition affect the relative rates of the N, P, and Fe biogeochemical cycles (Pahlow & Riebesell 2000). For example, there may have been a microbial community composition shift, over decadal scales, from eukaryotes to prokaryotes (primarily the unicellular cyanobacterium *Prochlorococcus*) at Station ALOHA in the North Pacific driven by a shift from N to P limitation (Karl et al. 2001a,b). The change in N to P limitation is consistent with the Pacific having higher orthophosphate concentrations than the Atlantic and is also consistent with concentrations of soluble reactive phosphorus (SRP) declining for over a decade (Karl et al. 2001b). However, analysis of *Prochlorococcus* populations by comparing NH_4^+ and P incorporation into RNA indicates that current *Prochlorococcus* populations are limited by N but not P availability (Van Mooy & Devol 2008).

Mesoscale physical processes can result in transient increases in nutrients that have large, but episodic, effects on the biota. Perhaps the best characterized of these mesoscale features are the eddy fields associated with much of the open ocean. Whereas cyclonic eddies have long been associated with enhanced upwelling and episodic enrichment of the biota (cf. Ducklow et al. 2009), recent results show that physical/biological interactions in anticyclonic eddies can also have profound impacts on the N cycle. For instance, mode water anticyclonic eddies have promoted diatom blooms in the subtropical Atlantic (McGillicuddy et al. 2007), whereas collapsing anticyclonic eddies stimulated both diatoms and enhanced N_2 fixation within a subtropical Pacific eddy (Fong et al. 2008). In a more comprehensive study, Church et al. (2009) link positive sea surface height anomalies (anticyclonic eddies) in the North Pacific subtropical gyre to enhanced N_2 fixation, suggesting that similar effects may be occurring in the Atlantic basin. Although the exact mechanism(s) for the various shifts in community composition and subsequent adjustments to N biogeochemistry are still being determined, these recent results demonstrate a sensitivity to subtle changes in physical (stratification, temperature, vertical fluxes) perturbations over the relatively short temporal and small spatial scales where mesoscale features dominate (Fong et al. 2008, Woodward & Rees 2001).

There are still relatively few studies that have directly identified nutrient limitation in the open ocean, in part because it is not easy to do. Nutrient addition experiments in bottles, microcosms, or mesocosms require incubation of natural microbial communities, which is fraught with artifacts (Dore & Karl 2001). Nutrient limitation of the individual populations in communities is difficult to ascertain because different species have different stoichiometric requirements for nutrients (Arrigo 2005, Geider & La Roche 2002), whereas algal assemblages also respond to ecological conditions, resulting in wide-ranging N:P ratios (Klausmeier et al. 2004). Some phytoplankton require silica (Si), others can lower their P requirements by replacing P with S in sulfolipids (Van Mooy et al. 2006, 2009), and many organisms can utilize organic P when orthophosphate is limiting (Björkman & Karl 2003, Moore et al. 2006). In addition to N, P, Si, and Fe, interest has rekindled in other potentially limiting substrates such as vitamin B12 (Sanudo-Wilhelmy et al. 2006, Taylor & Sullivan 2008) and other trace metals such as zinc and cobalt (Morel 2008, Morel & Price 2003), further complicating the simple view of a single limiting nutrient in the ocean.

Nutrient addition experiments may perturb the species composition (Arrigo 2005) due to differing uptake and growth kinetics as well as to differing stoichiometric requirements (Marchetti et al. 2010) and, thus, may not really describe how the extant populations were limited by nutrients. Shifts in grazing food chains and the microbial loop (including viruses), which modify the top-down versus bottom-up controls on productivity or production, may further complicate interpretation of the response. To avoid some of these complexities, chlorophyll-fluorescence kinetics have been

used to identify nutrient limitation (Beardall et al. 2001, Behrenfeld & Kolber 1999, Kolber et al. 1994) and have the advantage of being largely noninvasive, circumventing some of the issues with enrichment experiments. An obvious disadvantage is that fluorescence kinetics only track the ecophysiological responses of chlorophyll-containing organisms, precluding its use for many players in the N cycle. Physiological assays, such as examining cellular expression of proteins or RNA, can be good indicators of the nutrient limitation of specific populations (Dyhrman et al. 2002, Graziano et al. 1996). There is great potential for further developing biochemical, biophysical, physiological, or molecular (e.g., RNA or protein) markers to provide information on the physiological state of microorganisms in seawater without requiring incubation (**Table 2**). Such assays exist, although they are in a preliminary state for deployment in routine assays across ecosystems. The most direct way of assessing habitat or ecosystem limitation is by manipulating or fertilizing large enclosures or enriching large patches of the ocean, deliberately (Boyd et al. 2007, Karl & Letelier 2008) or unintentionally (Vitousek et al. 1997).

The application of mathematical models to examine how nutrients may limit populations across ocean biomes is a complementary approach to direct experimentation. Moore et al. (2006) showed how Fe inputs can result in limitation of different phytoplankton groups in the world's oceans and that the biogeochemistry of the oceans is also highly sensitive to indirect effects caused by changes in N_2 fixation. These global models have allowed investigators to examine the balance of N_2 fixation, denitrification, and the linkages to aeolian Fe flux (Moore & Doney 2007), supporting the hypothesis that widespread Fe limitation in the modern ocean has decoupled N_2 fixation and denitrification, leading to the apparent deficit of N in the modern ocean. In contrast, more available Fe during the last glacial-interglacial cycle may have led to a P-limited ocean as the N cycle was better balanced, whereas anthropogenic changes in N:Fe deposition in dust may lead to the suppression of N_2 fixation in the near-future ocean, leading once again to a more P-limited ocean (Krishnamurthy et al. 2010). A fundamental issue with these models is that many of the physiological processes, and even the organisms (Zehr et al. 2008), are unknown or poorly described, making it difficult to parameterize the models (Moore & Doney 2007). Development of better methods for detecting nutrient limitation in individual populations would make it possible to both test and improve such models (DeLong 2009, Doney et al. 2004).

N limitation is linked to the biogeochemical cycling of other nutrients and trace elements. Nutrient limitation in the sea varies between basins, is sensitive to a variety of environmental factors, and varies as a function of time, from intervals of seasons and years to decades and eons. These environmental factors are also linked such that minor modifications to the nutrient stoichiometry of the oceans coupled with concomitant physical and chemical changes (mixing, temperature, alkalinity) can cause dramatic shifts in the coupled ocean-atmosphere conditions of our planet (Peacock et al. 2006). Clearly, different nutrients can be the primary limiting nutrient at different times (both short and long timescales) and in different places. The question is not whether the ocean is N limited but how does N limitation interact with the biogeochemical cycles to constrain productivity over long timescales, and how will it respond to global climate change over the ensuing decades?

N Limitation, Elemental Ratios, and Export

Elemental ratios have implications for not only nutrient limitation but carbon flux because photosynthetic C fixation in surface waters is stoichiometrically related to nutrient consumption, followed by sedimentation of particulate matter to the deep ocean. Eppley & Peterson (1979) first proposed the stoichiometric relationship of "new" nitrogen inputs to surface water export because inputs must equal outputs in the surface mixed layer of the ocean. This proposal, however,

Table 2 Major oceanic nitrogen cycling pathways and relevant genes

Reaction name	Chemical reaction	Genes
Nitrogen fixation	$N_2 + 8H^+ + 8e^- + 16\,ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16\,P_i$	*nifH*, *nifD*, *nifK* in alternative nitrogenases (those that use Fe or V in place of Mo in component I); there is also a *nifG* gene (between *nifD* and *nifK*)
Ammonium oxidation	$NH_3 + O_2 + 2\,H^+ + 2e^- \rightarrow NH_2OH + H_2O$ $NH_2OH + H_2O \rightarrow HNO_2 + 4H^+ + 4e^-$ $0.5O_2 + 2H^+ + 2e^- \rightarrow H_2O$	*amoC*, *amoA*, *amoB*, *hao*
Nitrite oxidation	$2NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$ $2H^+ + 2e^- + 0.5O_2 \rightarrow H_2O$	*norA*, *norB*
Heterotrophic nitrification	R-NH2 $\rightarrow NO_2$ R-NH2 $\rightarrow NO_3$	The genes are not well known but may be the nitrate reductase genes involved in heterotrophic denitrification *narH*, *narJ*
Anaerobic ammonia oxidation	$HNO_2 + 4H^+ \rightarrow NH_2OH + H_2O$ $NH_2OH + NH_3 \rightarrow N_2H_4 + H_2O$ $N_2H_4 \rightarrow N_2 + 4H^+$ $>HNO_2 + NH_3 \rightarrow N_2 + 2H_2O$ $>HNO_2 + H_2O + NAD \rightarrow HNO_3 + NADH_2$	Over 200 genes involved in anammox metabolism (Strous et al. 2006), including 9 *hao-like* genes, hydrazine hydrolase (*hzf*), and hydrazine dehydrogenase
Dissimilatory nitrate reduction and denitrification	$5[CH_2O] + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_2O$ $5\,H_2 + 2NO_3^- + 2H^+ \rightarrow N_2 + 6H_2O$ $NO_3 \rightarrow NO_2^-$ $NO_2^- \rightarrow NO + N_2O$ $N_2O \rightarrow N_2$	*narDGHIJ;* *napA,B,D,E;* *nirB,C,K,U,N,O, S;* *norB;* *nosZ*
Assimilatory nitrate and nitrite reduction	$NAD(P)H + H^+ + NO_3^- + 2e^- \rightarrow NO_2^- + NAD(P)^+ + H_2O$ 6 ferredoxin (red) + 8 H^+ + 6 e^- + NO_2^- $\rightarrow NH_4^+$ + 6 ferredoxin (ox) + $2H_2O$	*nasA*, *nasB*, *nasC*, *nasD* (noncyanobacterial Bacteria); *narB* (cyanobacteria); *nrtA*, *nrtB*, *nrtC*, *nrtD* (or *nap*) permeases (cyanobacteria)
Dissimilatory nitrate reduction to ammonia	$NO_3^- + 2H^+ + 4H_2 \rightarrow NH_4^+ + 3H_2O$	*nir*, nar, *nap*, *nrfABCDE*
Ammonification/regeneration/remineralization	$R\text{-}NH_2 \rightarrow NH_4^+$	—
Ammonium assimilation	NH_3 + 2-oxoglutarate + NADPH + H^+ $\rightleftarrows$ glutamate + $NADP^+$ (glutamate dehyrdrogenase) NH_3 + glutamate + ATP $\rightarrow$ glutamine + ADP + Pi glutamine + 2-oxoglutarate + NADPH + H^+ $\rightarrow$ 2 glutamate + $NADP^+$ (glutamine synthetase and NADH-dependent glutamine:2-oxoglutarate amidotransferase)	*gdhA*,*gdhA*, *gltB*

assumed that the ocean was in steady state over short time intervals, and it is now realized that episodic phenomena, such as mesoscale features, including eddies, can result in uncoupling of new production from export (Karl 2002, Karl et al. 2001b).

The downward export (removal from surface waters) of C and N occurs through a number of mechanisms (detritus, fecal pellets, zooplankton, and sinking phytoplankton; Buesseler et al. 2008). Of particular importance in the N cycle is the rapid sinking of blooms of diatoms containing N_2-fixing cyanobacterial symbionts (Scharek et al. 1999), because it is directly coupled with new production (N input via N_2 fixation). These diatom-diazotroph (N_2-fixer) associations may be important in specific areas that select for these organisms (N-limited, Si-rich), such as at the edge of the Amazon River plume (Subramaniam et al. 2008), and perhaps more generally where enrichment with Si, Fe, and/or P can support diatom growth. Microorganisms are also involved in controlling upward fluxes of nutrients between surface and deep water. Diatom mats may transport nitrate upward from the nitracline (Villareal et al. 1993), whereas buoyant N_2-fixing microorganisms have been proposed to mine and transport P into surface waters (Karl et al. 1992, White et al. 2006). Modeling studies suggest that this mechanism could result in ~10% of the upward vertical P flux, depending on development of large *Trichodesmium* aggregates and carbohydrate ballasting (Diaz et al. 2008, White et al. 2006). Ultimately, the significance of vertical downward (or upward) fluxes of microorganisms and organic material is dependent on the elemental composition of the transported materials, because the ratio of elements in sinking particulate material determines the ratios of regenerated nutrients that will be recirculated to the surface water from below the pycnocline. Sedimenting organic material is remineralized in deep water, and the elemental composition of organic matter affects future surface water nutrient availability because deep waters are advected, upwelled, or diffused to surface waters (**Figure 1**). For several possible reasons (Quan & Falkowski 2009), the concentrations of remineralized nutrients with depth are not exactly correlated, resulting in offsets of remineralized N and P.

Although the measured composition of marine particulate material roughly averages the Redfield ratio (Arrigo 2005), in recent years the flexibility of the elemental composition of microorganisms has been reevaluated, and observed elemental ratios in biological materials or those estimated by carbon and nutrient drawdown often exceed Redfield ratios (Sarmiento & Gruber 2006). C:N:P ratios do not appear to be strictly biochemically constrained (Arrigo 2005, Geider & La Roche 2002, Sarmiento & Gruber 2006). In fact, the N:P requirements of organisms and microorganisms can vary as a function of the nucleic acid and amino acid composition of organisms, providing some selective advantage under different N:P supplies (Elser et al. 2000). N_2-fixing microorganisms can have high N:P ratios (Mulholland et al. 2004), which has implications for regenerated nutrients. The elemental ratios of resupply also deviate from the Redfield ratio (Dunne et al. 2005, Sarmiento & Gruber 2006). The pattern of regenerated inorganic nutrients in subsurface waters provides a "memory" of the nutrient ratios in surface organic matter that was exported to deep water. As a result, geochemists have used N:P ratios in subsurface waters to infer past surface water N-cycling activities. Specifically, the deviations from the expected Redfield ratio have been used to determine where nitrogen fixation and denitrification occur. By assuming that inorganic nutrients in the deep ocean should approximate Redfield proportions (106:16:1 C:N:P, molar ratio), it is possible to identify imbalances from this expected ratio, which must be caused by either changes in the source (sinking particulate organic material) or sink (metabolic processes mediated by microbes) terms or by existence of alternative sources for these elements. This approach assumes that positive (N-excess) deviations from the canonical Redfield N:P ratio (termed N^*) are sites of N_2 fixation, and negative deviations are sites of N loss through denitrification (including anammox) (Gruber & Sarmiento 1997, Michaels et al. 1996). A related approach examines use of the parameter P^* (calculated in a similar fashion to N^*) to identify regions of the ocean where N:P

Anammox: conversion of equimolar amounts of ammonia (ammonium at the pH of seawater) and nitrite to dinitrogen gas; suggested to be a dominant pathway within the N cycle in regions of reduced oxygen, e.g., sediments and oxygen minimum zones

P^*: amount of phosphorus (μM) excess relative to N in ocean water, assuming P concentrations are in Redfieldian ratios with N concentrations. Regions of elevated P^* are expected to be associated with N_2 fixation

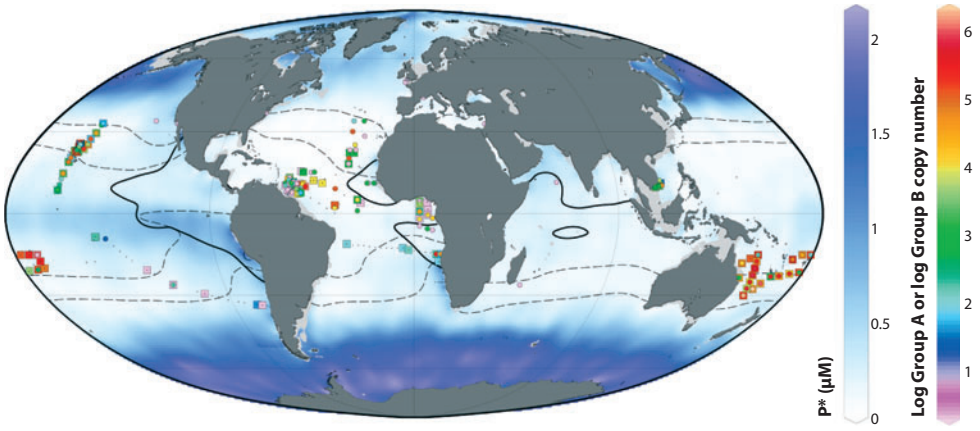

Figure 2

Rates and locations of nitrogen fixation in the global ocean have been both directly measured and inferred based on biogeochemical signatures and the presence/absence of N_2-fixing organisms. The map shows P* (the amount of "extra" P based on Redfieldian proportions relative to N) for the surface ocean, using World Ocean Atlas 2005 data. Dark blue regions indicate excess P, which should promote nitrogen-fixation activity. The symbols indicate locations where Group A (*circles*) and Group B (*squares*) nitrogen fixers were recently identified using molecular techniques (Moisander et al. 2010). Dashed lines denote the 20°C- and 25°C-surface-temperature isotherms, considered the biogeographic boundary for N_2-fixation, particularly by *Trichodesmium*. The solid black line denotes the global oxygen minimum zones (OMZs), here defined as 50% oxygen saturation at 100-m depth. Recent authors have hypothesized that the OMZs and N-processes (consumption and production) in the ocean are closely linked in space and time.

ratios are low and should select for N_2 fixation (Deutsch et al. 2007) (**Figure 2**). Although there are assumptions involved in these methods (Moore & Doney 2007), they nonetheless provide hypotheses and estimates of N_2 fixation over basin scales that are otherwise impossible to determine with current methods.

Karl & Letelier (2008) hypothesized that pumping water from a specific depth to the surface (using floating ocean pumps) could be used to adjust N:P ratios to experimentally induce blooms of non-N_2-fixing species, followed by selection for N_2-fixing cyanobacteria. This approach facilitates experiments to test the effect of different N:P ratios, similar to the various mesoscale Fe-enrichments (Boyd et al. 2007), as water derived from different depths would have different N:P ratios. Fennel (2008) challenged the potential for stimulation of N_2 fixation by this enrichment mechanism because of water column destratification due to mixing cold dense water with surface waters. Since older studies suggested a link between stratification and N_2 fixation (based on *Trichodesmium* abundance and activity), it was hypothesized that the artificial mixing of low N:P water to the surface would not result in enhanced N_2 fixation. This was rebutted by Letelier et al. (2008), who noted that the model presented by Fennel (2008) did not include the effects of solar heating on the stratification of surface waters, highlighing the complexity of the biogeochemical processes being invoked. Also, the distributions of recently discovered N_2-fixing microorganisms are not controlled by the same factors as *Trichodesmium* (Moisander et al. 2010; see below), raising the issue of whether we can adequately predict how changes in nutrient availability, elemental ratios, and stratification will interact under different future-climate-change scenarios.

The basic tenets of nutrient cycling in the ocean, starting with Redfield (1958) and extending to the early 1990s, supported the concept of nitrogen as the proximal limiting nutrient in the modern ocean, with P most likely to be limiting on millennial timescales. We arguably still do not know

what is "ultimately limiting" phytoplankton growth in the oceans, but research in the last two decades demonstrates the need to track much more than just N:P ratios in the ocean, as we now know that biogeochemical cycling of nutrients in the modern ocean is more complex and spatially variable than previously thought. The roles of N, P, and trace-elements such as Fe also change on millennial (glacial-interglacial) scales, whereas the modern age (the so-called Anthropocene) may alter ocean biogeochemical cycling in ways that are difficult to predict based on our classic understanding of the N-cycle and the historical record.

THE NITROGEN BUDGET

The relative rates of N inputs and losses determine the long-term N oceanic inventory, which determines both the total production of the ocean and the proximal limiting nutrients (N, P, Fe, etc.). Although conceptually simple, the oceanic N budget is difficult to balance because of the spatial and temporal scales involved, complexity of circulation, inherent assumption of steady-state dynamics, and chronic undersampling of the fundamental rates and processes associated with the N cycle (Brandes et al. 2007).

Balancing the oceanic N budget requires assumptions regarding homeostasis over time, that is, that N_2 fixation and denitrification have to be balanced over relatively short timescales (Codispoti 2007). There are three approaches used to address this question: biological data determined experimentally on oceanographic research cruises, biogeochemical analyses based on measured nutrient ratios over basin scales, and coupled biophysical models incorporating both (see above). The geochemical analyses are based largely on observed ratios of dissolved nutrients (N and P), their deviations from expected N:P ratios, and estimates of deepwater flow, necessary to extrapolate the deep-ocean signature back to the surface-ocean source of the nutrient "fingerprint." Using these approaches, a big discrepancy between basin scale estimates of N_2 fixation and denitrification was inferred (Gruber & Sarmiento 1997, Michaels et al. 1996). These estimates and the geographic boundaries used to make the calculations can be controversial, adding to the already large uncertainties in interpreting the nutrient ratios (Hansell et al. 2004, Moore & Doney 2007).

Although it is possible to estimate N_2 fixation and denitrification rates from experimental measurements (Capone et al. 2005, Mahaffey et al. 2005, Montoya et al. 2007, Ward 2005), these estimates are prone to large error and are not necessarily representative of the larger spatial and temporal patterns. In contrast, geochemical analyses of the relative distributions of dissolved inorganic nutrients in seawater should integrate the effects of remineralization of N_2-fixing and and non-N_2-fixing microorganisms over time and space (Gruber & Sarmiento 1997). Nonetheless, some experimental data suggest that N_2 fixation by *Trichodesmium* may have been underestimated and that the biological and biogeochemical estimates converge to some degree (Capone et al. 2005). Other discoveries in N_2 fixation, such as previously unknown N_2-fixing cyanobacteria, may also help to close the gap (Zehr et al. 2001; see below).

One of the findings of the biogeochemical approach based on nutrient remineralization ratios suggests coupling of denitrification zones (OMZs) with N_2 fixation, because of the effect of denitrification on the N:P ratio (Deutsch et al. 2007). This coupling could take place in the same water mass (N_2 fixation is an anaerobic process and can co-occur with denitrification); if so, rates associated with both processes would be underestimated (Codispoti 2007) because tropical OMZs have been expanding vertically over the past 50 years (Stramma et al. 2008). It is also possible that the effect of the OMZs will be to select for N_2 fixation in water advected from the OMZ rather than in the OMZ itself. This hypothesis can be, and is being, tested by examining the biology of N_2 fixation and denitrification in these regions, and by examination of the decoupling between

N^* and P^* in the Atlantic basin, where enhanced N_2 fixation must be supported by efflux of excess P from distant OMZs (Moore et al. 2009).

The balance of the N budget is still highly controversial (Gruber & Galloway 2008). Over time the estimates of global ocean denitrification have increased (Codispoti 2007) but so have those of N_2 fixation (Capone et al. 2005, Mahaffey et al. 2005). Fundamentally, the N input (N_2 fixation) and output (denitrification) terms are poorly constrained. New discoveries in the N cycle have raised questions about the organisms that catalyze the N_2-fixation and denitrification processes (Kuypers et al. 2005, Ward et al. 2009, Zehr et al. 2001), such as where they occur and what regulates their activity. These new discoveries may help to explain the global metabolic distribution of the N cycle. The feedback loops between atmospheric CO_2 and the N cycle are complex and not well known (Gruber & Galloway 2008). It is critical to understand the balance of N_2 fixation and denitrification, as it may have been key to past Earth atmospheric CO_2 levels, and humans have perturbed the N and the C cycles, which will lead to unknown changes (Galloway et al. 2004).

OPEN OCEAN N NUTRITION: NITROGEN ASSIMILATION AND N_2 FIXATION

Most marine phytoplankton can use ammonium, nitrite, and nitrate as N sources for growth but cannot fix N_2. Many phytoplankton can also use some forms of organic N, including urea (Baker et al. 2009), but less is known about the composition and utilization of marine dissolved organic matter and the direct utilization of these organic compounds by phytoplankton, particularly compared with our understanding of nitrate, nitrite, and ammonium utilization. In the 1980s, *Prochlorococcus* was found to be one of the dominant phytoplankton in the sea (Chisholm et al. 1988). Subsequent work with cultures showed that strains had different light and nutrient requirements, with the variants referred to as ecotypes (Moore et al. 1998), and suggested that some strains could not grow with nitrate as an N source. This discovery suggested that ecological competition among phytoplankton might be based at least partially on different N sources. Entry into the genomic era, with full sequences of a number of strains of *Prochlorococcus* and *Synechococcus* (Dufresne et al. 2003, Palenik et al. 2006, Rocap et al. 2003) available, showed that *Prochlorococcus* did not contain the assimilatory nitrate reductase genes (**Table 2**), providing the explanation for why strains did not grow on nitrate. One of the two strains, a low-light strain (Moore et al. 1998), did contain the gene for nitrite reductase (**Table 2**). It was hypothesized that this low-light strain grew at a depth in the ocean where low light intensities were coupled with higher nitrite availability at the top of the nitricline. These findings provided an ecological selection force involving N substrates; whereas some strains of *Prochlorococcus* were entirely dependent on ammonium, others could use nitrite and ammonium, and *Synechococcus* could use nitrate, nitrite, or ammonium. Some strains of *Prochlorococcus* have also retained the nitrate reductase (*nar*) genes, further complicating the attempt to define the niches of open ocean phytoplankton at the genus level. A feature of most open-ocean marine microbes is reduction in genome size (Dufresne et al. 2003, Giovannoni et al. 2005), and loss of the nitrate utilization genes apparently coincides with the lack of their use in high-light ecotypes that rely primarily on regenerated ammonium. Interestingly, at approximately the same time, it was discovered that not all marine heterotrophic bacteria could use nitrate, because they also lacked the nitrate reductase gene (Allen et al. 2001, 2005). The presence or absence of nitrite reductase genes in *Prochlorococcus* appeared to be correlated with whether the strains or ecotypes were adapted to high light intensity (presumably shallower) or low light intensity (deeper; reviewed in Partensky & Garczarek 2010). This suggested a relationship between genome evolution and selection forces (shallow with high light intensity, low N versus

deep water with low light intensity, nitracline) in the subhabitat. Using molecular techniques that targeted the ribosomal RNA operon that distinguishes these groups of ecotypes, Johnson et al. (2006) showed that the different strains are widely distributed latitudinally and that there is a general trend with the depths in which the high- and low-light ecotypes were found. These trends were supported by results of a novel modeling approach that allowed 78 "species" to compete on the basis of properties that included P use, growth kinetics, and N-source utilization (Follows et al. 2007). The resulting "species" maps corresponded well with distributions reported by Johnson et al. (2006). These results suggested differential roles of ecotypes of *Prochlorococcus* in new and regenerated production processes.

Nitrogen assimilation: assimilation of nitrogen into biomass. Eukaryotic and prokaryotic microorganisms can assimilate simple inorganic N (nitrate, nitrite, ammonium, and urea) into biomass using enzymes that reduce oxidized N into ammonium and, subsequently, amino acids

The *Prochlorococcus* N-utilization story has been confounded by both field measurements of nitrogen assimilation in natural assemblages of *Prochlorococcus*, which show that 5–10% of N is directly assimilated as nitrate in the Sargasso Sea (Casey et al. 2007), and the more recent discovery of assimilatory nitrate reductase (*nar*) genes (**Table 2**) in metagenomic DNA fragments from marine microbial communities (Martiny et al. 2009). The presence or absence of the nitrate reductase genes provides information on the relative roles of these nutrient acquisition systems in nature. With this knowledge in hand, it has been possible to develop molecular biology methods to examine microorganisms in natural populations that have the *nar* or *nas* genes (Ahlgren & Rocap 2006, Allen et al. 2001, Cai & Jiao 2008, Jenkins et al. 2006, Paerl et al. 2008) (**Table 2**). These tools will ultimately provide information on the spatial distribution of nitrate-assimilating populations, including their gene expression, and will provide information on what controls individual strains and ecotypes in space and time.

Nitrate is a stable form of N but is generally not available to most phytoplankton, because it is consumed in surface waters and largely replenished by mixing water from depth or from terrestrial inputs. Surface-ocean microbial growth is largely supported by regenerated production, that is, recycling from both cell death and reduced N in the form of NH_4^+. "New" nitrogen in the form of nitrate or N_2 supports a small percentage of production in the oligotrophic ocean but is important to balance sedimentation losses from the surface ocean. Gaseous N_2 (hundreds of micromoles per liter) is one of the most abundant forms of N (e.g., compared with nanomoles per liter of nitrate or ammonium) in seawater but is biologically available to only N_2-fixing microorganisms (diazotrophs, or nitrogen eaters). N_2 fixation was believed to be absent or minor at the time of conceptualization of the new and regenerated N models (Dugdale & Goering 1967). Even after the discovery of N_2-fixation associated with filamentous cyanobacteria (*Trichodesmium*), N_2 fixation was not appreciated as a source of N in the open ocean until biogeochemical analyses indicated that N_2 fixation may have been underestimated, based primarily on the comparison of ratios of regenerated nutrients to those expected from particulate material in Redfield proportions (Gruber & Sarmiento 1997, Michaels et al. 1996). The interest in N_2 fixation and balancing the N budget resulted in new approaches, new data, and some surprising discoveries.

Since N is in short supply in oligotrophic oceans, it should be ecologically advantageous to fix N_2 (**Table 2**) from the essentially unlimited N_2 reservoir in the atmosphere. Surprisingly few oceanic microorganisms appear to be capable of N_2 fixation. *Trichodesmium* is a filamentous N_2-fixing cyanobacterium that has been studied for decades (Dugdale et al. 1961), largely because it frequently forms large aggregates that can be seen on the ocean's surface with the unaided eye. It is a filamentous organism that can exist in aggregates (colonies) or as individual filaments and is peculiar because it fixes N_2 during the day, while it is evolving oxygen through photosynthesis. The enzymes involved in N_2 fixation, and therefore N_2 fixation activity, are sensitive to oxygen inactivation. Since *Trichodesmium* can easily be collected and concentrated by plankton nets, studies on N_2 fixation focused on *Trichodesmium* for four decades (1961–1998). A few other N_2-fixing cyanobacteria had been observed anecdotally, including cyanobacterial symbionts of diatoms (Carpenter 1983,

Mague et al. 1974, Villareal 1987, Villareal 1991). A unicellular cyanobacterium, *Crocosphaera*, was isolated from tropical waters in the mid-1980s (Waterbury & Rippka 1989), but its presence elsewhere was unknown.

Based on historical estimates, N_2-fixing microorganisms, although key as a source of N to resupply losses from sinking or denitrification, are present in relatively low abundance. Although *Trichodesmium* is easily observed because of its macroscopic aggregate morphology, it was not clear in the early 1990s whether there were other microorganisms that might be equivalent in abundance and biogeochemical significance but less easily detected because of being small and distributed as individual cells rather than in large aggregates easily collected by net tows. Molecular biology approaches made it possible to answer this question by looking for the genes that encode the enzymes that catalyze N_2 fixation, the *nif* genes (**Table 2**). By sequencing *nif* genes collected from bulk seawater samples, Zehr et al. (1998) found the first evidence for the presence of several groups of cyanobacteria (and possibly heterotrophic bacteria) that were widely distributed in tropical and subtropical waters. Two important discoveries were that (*a*) *Crocosphaera*, previously cultivated from the Atlantic Ocean, was present in the Pacific Ocean in relatively high abundances, and (*b*) there was an uncultivated group of cyanobacteria, called Group A (or UCYN-A), that was generally present in high abundances as well (**Figure 2**). The discovery of the *nif* gene associated with *Crocosphaera* provided a context for previous flow cytometer–based studies that showed the episodic presence of these "large" unicellular cyanobacteria (these are nanoplankton, being 3–5 μm in diameter, but are large in comparison to the <1-μm diameter, non-N_2-fixing oceanic cyanobacteria *Prochlorococcus* and *Synechococcus*) (Campbell et al. 2005, Neveux et al. 1999). These unicellular cyanobacteria, discovered from the presence of the nitrogenase genes, would not have been easily detected as N_2-fixing microorganisms by conventional techniques as they are small, dispersed cells.

Intriguingly, the unicellular N_2-fixing cyanobacteria were found to be substantially different in biology and evolution than their non-N_2-fixing, oceanic microbial counterparts. Comparison of sequences of genomic fragments collected in metagenomic studies were found to be essentially identical to the sequence of *Crocosphaera* sp. WH8501 isolated from the Atlantic Ocean over 20 years before (Zehr et al. 2007). This high similarity of genomic sequences within a marine planktonic cyanobacterial genus is very different from the diversity of gene sequences and genomes of sympatric non-N_2-fixing microorganisms (e.g., *Prochlorococcus* and *Pelagibacter ubique*) in the oceans (Giovannoni & Stingl 2005, Rusch et al. 2007). The Group A unicellular cyanobacteria (UCYN-A), because of the *nif* gene sequence similarity to *Crocosphaera* and related cyanobacterial groups, was expected to be similar to these organisms in biology and physiology as well. However, using flow cytometry, Goebel et al. (2008) found it was smaller, on the order of the size of the non-N_2-fixing cyanobacteria (ca. 1 μm). Fluorescence in situ hybridization (FISH) studies using a 16S rRNA, fluorescently labeled probe targeted to the 16S rRNA of the unicellular N_2-fixing cyanobacteria has also shown that there are submicrometer-diameter, cyanobacteria-like cells in the South Pacific and the Mediterranean Sea (Biegala & Raimbault 2008, Le Moal & Biegala 2009). Ultimately, genomic sequences obtained from flow cytometric–sorting and high-throughput sequencing (Tripp et al. 2010, Zehr et al. 2008) showed that Group A was a highly unusual organism that lacked metabolic pathways typical for cyanobacteria and phototrophs in general, including the tricarboxylic acid cycle. Based on the lack of such key metabolic pathways, Group A would appear to be a likely symbiont, but at present no host has been identified (Tripp et al. 2010).

The molecular approach also made it possible to identify and characterize cyanobacterial symbionts and their hosts (Carpenter & Janson 2000, Foster & Zehr 2006). Symbioses, particularly between N_2-fixing cyanobacteria and eukaryotic, single-celled algae, appear to be an important

Group A (or UCYN-A) picoplankton: nitrogen-fixing (diazotrophic) picoplankton found in the open ocean in waters >20°C that have been characterized based on their genomic and metabolic signatures but have not yet been cultivated in the laboratory

FISH: type of molecular biology approach that uses fluorescently labeled oligonucleotides to target a specific DNA or RNA sequence. These fluorescent probes can be hybridized to whole cells to identify cells with a specific DNA or RNA sequence of interest. A common application is to identify specific microorganisms based on the presence of a sequence in ribosomal RNA (rRNA)

but not well understood component of the oceanic N_2-fixing community (Foster & O'Mullan 2008). Heterocystous cyanobacteria associate with the external surfaces of some diatoms, and between the diatom cell wall (frustule) and cell membrane (plasmalemma) (Janson et al. 1995, Villareal 1992). Some unicellular cyanobacteria also appear to be symbionts of centric diatoms (Carpenter 2002, Villareal 1992). These symbioses present a number of interesting evolutionary and ecological questions; for example, it is not known whether there are free-living stages in the symbioses, or how the symbioses are transferred. This intriguing topic is beyond the scope of this review but will likely emerge as another key aspect of the oceanic N cycle that we are just beginning to grasp (Carpenter & Foster 2002, Foster & O'Mullan 2008, Giovannoni et al. 2007).

Another large unknown regarding the metabolic balance of the global N cycle is the potential role of heterotrophic bacteria in oceanic N_2 fixation. *nif* genes and *nif* gene transcripts from presumably heterotrophic bacterial populations have been reported in a number of studies (Church et al. 2005a; Falcón et al. 2004; Moisander et al. 2008; Zehr et al. 1998, 2001). There are technical difficulties in confirming bacterial *nif* sequences from dilute natural samples because diverse nitrogenase sequences have been reported from most laboratory quantitative polymerase chain reaction (Q-PCR) and reverse-transcription polymerase chain reaction (RT-PCR) reagents (Zehr et al. 2003). Some bacterial *nif* gene expression has been confirmed by quantitative reverse-transcription polymerase chain reaction (QRT-PCR), but abundances are low. Nitrogen fixation genes are present in many aquatic habitats (Bostrom et al. 2007, Zehr et al. 2001), even when N_2 fixation is not detectable (Jenkins et al. 2004, Moisander et al. 2006, Steward et al. 2004). The abundances of these bacteria are low relative to the non-N_2-fixing populations, and it seems unlikely that, at typical in situ growth rates of oligotrophic ocean bacteria, the N fixed could be important from a budget standpoint. This is a difficult research question to address but could be important in specific habitats such as the deep sea (Dekas et al. 2009, Mehta & Baross 2006) and OMZs.

From the oceanic N cycle perspective, the most important aspect is the relative abundance and activity of different N_2-fixing microorganisms. With different sizes and morphologies and different lifestyles (free-living versus symbiotic), the N fixed by different taxa can have very different fates in the surface water, with differing implications for C and N export. Determining the relative contributions presents technical hurdles as many of these organisms are small and the populations dilute, and even low N_2-fixation rates (near the limit of detection) are significant relative to in situ N turnover in oligotrophic oceans, particularly when integrated over the vast expanse of open ocean. *Trichodesmium* abundances have been quantified using a variety of techniques, including microscopy and video plankton recorders (Davis & McGillicuddy 2006, Taboada et al. 2010, Tyrrell et al. 2003). Nitrogenase (*nif*) gene sequences provide a means to quantify the relative abundance and even gene expression of different groups of N_2-fixing microorganisms in the sea (Church et al. 2005a,b; Langlois et al. 2008; Rees et al. 2009). However, the variability in space and time and the scales of ocean basins present significant hurdles for determining the relative contribution to the nitrogen cycle of N_2-fixing microorganisms. It is well known that *Trichodesmium* distribution is highly variable, but they form blooms that can be detected by remote sensing (Dupouy et al. 1988, Subramaniam et al. 2002, Westberry & Siegel 2006). However, the less easily detected unicellular cyanobacteria also form "blooms" that can occur in both the surface and subsurface (Hewson et al. 2009). Diatom symbioses are also highly dynamic (Fong et al. 2008, Foster et al. 2007). Although molecular methods provide a means of comparing the abundances of different organisms, limitations in sampling over the large time and space scales is problematic in that the populations are very dynamic (Church et al. 2009, Fong et al. 2008). Church et al. (2009), for example, show that the N_2-fixing populations are highly variable over seasonal cycles but that

the Group A cyanobacteria can be the most abundant N_2-fixer over much of the year at Station ALOHA in the North Pacific.

Trichodesmium is assumed to be the dominant N_2-fixing microorganism, and biogeography studies suggest this may be the case (Capone et al. 2005, Goebel et al. 2010, Mahaffey et al. 2005). However, information on the other, less easily observed microorganisms is scant in comparison. Group A cyanobacteria are found over a larger geographic range and in cooler waters than is *Trichodesmium* (Langlois et al. 2008, Moisander et al. 2010, Needoba et al. 2007, Rees et al. 2009). Because of the difficulties in sampling adequately, mathematical modeling has proved useful for determining the relative importance of the known N_2-fixing organisms (Goebel et al. 2007, Monteiro & Follows 2009). Most models that include N_2 fixation have focused on *Trichodesmium*, for which there is the most data, but recent development of modeling approaches have helped to investigate the factors that control the global distribution of other groups (Monteiro & Follows 2009) and to determine their relative contribution to N_2 fixation (Goebel et al. 2007, 2010). So far, modeling suggests that the small Group A cyanobacteria may be less important than *Trichodesmium*, except in specific locations and at certain times, but there are large uncertainties. Determining the factors that control the temporal and spatial distributions of different N_2-fixing microorganisms continues to be an exciting but challenging problem that is central to understanding the oceanic N cycle.

AEROBIC OXIDATION OF REDUCED NITROGEN

The oxidation of ammonium results in formation of nitrite and nitrate. The significance of this process is that it converts ammonium into a less biologically preferable form, as nitrite and nitrate are more energetically expensive N forms to use (**Table 2**). It is a central pathway in the oceans in that particulate nitrogen (PN) that is remineralized to ammonium in the deep ocean is oxidized to nitrate through the nitrification pathway (Ward 2000). This nitrate slowly mixes into the surface water or is upwelled, primarily at ocean boundaries and equatorial regions. Oxidation of reduced N compounds is performed by mainly chemolithotrophic microorganisms who glean energy and electrons from the oxidation (**Tables 1**, **2**). Prior to 2004, this reaction was believed to be catalyzed by mainly Proteobacteria.

Early studies of nitrification in the oceanic water columns used stable isotopes and immunochemistry to measure rates of transformations and enumerate nitrifying bacteria (Ward & Carlucci 1985). Nitrification in surface waters was generally found deeper in the water column but sometimes within the euphotic zone (Ward 2005, Ward et al. 1989). Nitrate formed by oxidation within the euphotic zone and recycled to phytoplankton confuses the concept of new and regenerated production (Clark et al. 2008, Dore & Karl 1996, Wankel et al. 2007, Ward et al. 1989). The use of molecular techniques in marine microbiology made it possible to detect specific microorganisms responsible for oceanic ammonia oxidation, such as *Nitrosomonas* and *Nitrosospira* and others in the beta-Proteobacteria group (Ward 1996) that were found to be widespread (Ward et al. 2007), even in polar oceans (Hollibaugh et al. 2002). The concept of proteobacterial nitrification as the dominant nitrogen oxidation changed upon the discovery of putative crenarchaeal ammonia monooxygenase (*amo*) genes during a metagenomic survey of ocean waters (Venter et al. 2004) and elsewhere (reviewed in Junier et al. 2010). In the early 1990s, it was discovered that the Crenarchaea clade of the Archaea were abundant in the oceans (DeLong 1992, Fuhrman et al. 1992), but they had not been cultivated and little was known regarding their ecological function. Sequences of *amo* genes were linked to archaeal genomic DNA in DNA fragments cloned from the Sargasso Sea (Venter et al. 2004) and from soil (Treusch et al. 2005). In parallel with the metagenomic discovery, an ammonia-oxidizing crenarchaeote that had *amoA* genes was cultivated from a saltwater

aquarium (Könneke et al. 2005) (**Table 2**). Using the polymerase chain reaction (PCR), Francis et al. (2005) showed that these genes were very common in a number of marine environments. It became clear that archaeal nitrification (by ammonia oxidizing Archaea, or AOA) was likely to be an important pathway for ammonia oxidation in the open oceans because Archaea were much more abundant than the ammonia-oxidizing Bacteria (AOB; Mincer et al. 2007, Wuchter et al. 2006). Because these Crenarchaea are ammonia oxidizers, nitrite formed must still be oxidized by the bacterial nitrite oxidizers (e.g., *Nitrospina*). A correlation between the abundance of Crenarchaeal *amo* (including a new novel group not previously believed to be abundant in the marine environment) and in-depth profiles of *Nitrospina* in the North Pacific Ocean suggested that these organisms together result in nitrification in subeuphotic mesopelagic waters (Mincer et al. 2007). Santoro et al. (2010) found a similar correlation between AOA and *Nitrospina* in the central California Current (surface to 500 m), where active nitrification was occurring, further suggesting metabolic coupling between these two N-cycling groups. Intriguingly, Church et al. (2010) found that the archaeal *amoA* transcript abundance per gene copy (interpreted as transcription per cell) was highest in surface waters, suggesting that ammonia oxidation catalyzed by Crenarchaea may occur in even well-lit surface waters. While Crenarchaeota are also abundant in the deep ocean, recent evidence suggests that *amoA* is not being utilized at depth and that these organisms are living heterotrophically, in contrast to the surface populations (Agogue et al. 2008). Examination of nitrification rates in the Gulf of California using the stable isotope ^{15}N in relation to the AOB abundance argued strongly for the role of Crenarchaea in marine ammonia oxidation (Beman et al. 2008), whereas AOB were found to be relatively important in the San Francisco Bay (Mosier & Francis 2008) and to vary in both abundance and community composition along a salinity gradient in a New England estuary (Bernhard et al. 2007).

Since the AOA essentially catalyze the same reaction (ammonia to nitrite), and in the same habitats in the oceans as the previously known AOB, it is unclear how this phylogenetic shift in paradigm changes our view of how the ocean N cycle functions (Ward et al. 2007). Major unknowns are how metabolic differences between AOA and AOB may have implications for the energetics of the N transformation. For example, it has recently been demonstrated that the cultivated ammonia-oxidizing crenarchaeote *Nitrosopumilus maritimus* SCM1 has an NH_3/NH_4^+ affinity more than 200 times greater than that of AOB (Martens-Habbena et al. 2009). How the nitrite formed from the AOA is oxidized, whether by previously known nitrifiers or yet another group of microorganisms, is yet to be resolved (Ward et al. 2007).

AOA: ammonia oxidizing Archaea; a group of organisms that derives energy from the oxidation of ammonia to nitrite, using the gene *amoA*; see also AOB

AOB: ammonia oxidizing Bacteria; a group of organisms that derives energy from the oxidation of ammonia to nitrite, using the gene *amoA*; see also AOA

Anaerobic ammonia oxidation: oxidation of ammonia to N_2 gas from coupled nitrite reduction and ammonia oxidation. The reaction is catalyzed by specific lineages of bacteria (*Kuenenia* and *Scalindula*) of the Planctomycetes and is an anaerobic process that occurs in anoxic or suboxic conditions

ANAEROBIC OXIDATION OF AMMONIA AND DENITRIFICATION

Oxidized N compounds, nitrate, and nitrite are good electron acceptors for biological reactions, as they are very close to O_2 in oxidation-reduction potential, and oxidation reactions coupled to the NO_3^- or NO_2^- reduction half-reactions result in generation of almost as much free energy as aerobic respiration. Aside from the newly described anammox pathway (see below), reduction products include nitrite (dissimilatory nitrate reduction), N_2 (denitrification, including N_2O as an end product), or ammonia (dissimilatory nitrate reduction to ammonia, or DNRA; Gardner et al. 2006) (**Table 1**). Denitrification occurs where nitrate is present but there are reduced oxygen concentrations. This occurs in specific habitats, such as the oxic-anoxic interface of benthic sediments, and in the water column at the edge of suboxic or anoxic water masses in OMZs (Naqvi et al. 2008).

In the mid-1990s, a new pathway of ammonia oxidation was discovered in wastewater treatment plants (Jetten et al. 2009, Mulder et al. 1995, van de Graaf et al. 1997) that was then found to occur in marine sediments (Rich et al. 2008, Thamdrup & Dalsgaard 2002) and OMZs

(Hamersley et al. 2009, Kuypers et al. 2005, Lam et al. 2009). This process, called anaerobic ammonia oxidation (**Table 2**), is performed by a few specific lineages of bacteria (including the *Candidatus* genera *Kuenenia*, *Scalindua*, *Brocadia*, *Jettenia*, and *Anammoxoglobus* in the Planctomycetes) with very specific substrate and environmental requirements. Several genera of bacteria were enriched, described, and used as targets for fluorescence in situ hybridization probes, enabling enumeration in the marine environment (Kuypers et al. 2003, 2006; Schmid et al. 2005). The genome of *Kuenenia* was subsequently sequenced (Strous et al. 2006).

Anammox bacteria obtain energy and electrons from ammonia oxidation but liberate N_2 as an oxidation product, rather than nitrite as in canonical nitrification (**Table 2**). Thus, this seemingly peculiar metabolism is by definition a denitrification pathway in that it results in loss of N from the system but does not occur through the same intermediates as canonical denitrification. Because both canonical denitrification and anammox have N_2 as an end product, the two processes have to be differentiated by tracing the N from nitrite, nitrate, or ammonium using the stable isotope ^{15}N. Ammonium and nitrite are not usually found together (in high concentrations) in aquatic habitats, and thus sources of flux of these compounds have to be inferred to support measured anammox activity. In one study, dissimilatory nitrate reduction to ammonium was suggested as the source of ammonium for anammox (Lam et al. 2009). It is possible that previously ignored pathways such as DNRA could help explain the anammox paradox (Lam et al. 2009, Zehr 2009).

Denitrification (**Table 2**) was believed to be the major N pathway in OMZs until the discovery of the anammox pathway. The anammox reaction has now been suggested as the most significant pathway in an increasing number of suboxic environments, including OMZs (Kuypers et al. 2003, 2005; Thamdrup & Dalsgaard 2002). Habitats where these reductive processes occur (OMZs, benthic sediment environments, and even hydrothermal vents; Byrne et al. 2009, Galan et al. 2009) exist because of the input of a large amount of organic matter, which in turn leads to O_2 utilization. Organic matter is also the source of ammonium for anammox. The oxidation of organic matter is stoichiometrically linked to the consumption of O_2 and the release of ammonium from organic matter. Denitrifying organisms can be responsible in part for suboxic conditions as many are facultative aerobic respirers. Denitrification requires an input of electron acceptors (oxidized N) from advection (presumably deep, nitrate-rich water) in suboxic waters. Similarly, anammox requires both reduced and oxidized N, requiring advection of electron donors or acceptors into suboxic waters. Recent studies suggest that anammox and canonical denitrification can dominate at different times and places (Ward et al. 2009). The issue of which process is the dominant pathway and why there are conflicting results remains to be resolved. It is possible that the two processes are linked and that denitrification can provide substrates (nitrite and ammonium) for anammox. The suboxic or anaerobic pathways are difficult to disentagle, particularly given the need to also account for physical advection of the substrates and by-products, and yet they are critical to understanding the global ocean N budget.

CONCLUDING REMARKS

The magnitude of the oceans continues to be one of the greatest challenges facing oceanographers. Its spatial and temporal scales exceed our ability to sample statistically to adequately represent oceanic processes, even with the development of new methods such as genomics. Ironically, processes at the opposite end of the scale spectrum, such as cubic micrometers surrounding microbial cells compared with cubic kilometers of the ocean ecosystems, present equally challenging problems with uncertainties of equivalent magnitude affecting the global N cycle. Although much is known about the players, rates, and controlling mechanisms, the wealth of new organisms

discovered in the last decade demonstrates that understanding and predicting the global N cycle remains a formidable but exciting, and indeed "deliciously complex," task.

The N cycle is unique within our planet's biogeochemical cycles because only microbes and human activities can control the amount of biologically available N in the biosphere through N_2 fixation; humans chemically synthesize N for fertilizer and modify the environments where microbial activities occur, impacting the rates and locations of denitrification and nitrogen fixation and the exchange of N among biomes and habitats. Thus, in future-climate scenarios, the effects of ocean circulation, trace element availability, and P availability may all have independent anthropogenic and natural feedbacks on the relative rates of the components of the oceanic N cycle, in particular N_2 fixation and denitrification.

SUMMARY POINTS

1. Nutrient limitation is a dynamic property, controlling individual species and community composition as well as ecosystem productivity and the balance of recycling versus export of that productivity. P and Fe limitation in particular interact with N limitation to control total productivity, and the relative availability of P and Fe are in turn controlled by terrestrial processes and aeolian transport operating at temporal scales ranging from nearly instantaneous to millennia.
2. The distinction between new and regenerated production has become blurred as we discover new organisms and new places (e.g., the surface ocean) where N cycling is occurring. We now know that marine organisms have adapted to utilize virtually every type of N compound in the oceans, whereas some organisms (e.g., some strains of *Prochlorococcus*) have eliminated some metabolic pathways, such as assimilative nitrate reduction.
3. Estimates of nitrogen fixation have changed dramatically in the last few decades because of both better detection tools for known organisms such as *Trichodesmium* and the newly recognized existence and potential importance of new groups of diazotrophs. Whereas there are many questions about how abundant and active these organisms are, there is no question that N_2 fixation is widespread, and of profound biogeochemical significance, in the oceans.
4. There has been a resurgent interest in OMZs with the discovery of processes such as anammox. Furthermore, the potential for suboxic or microaerophilic conditions to occur in microhabitats is not well known and is also likely to become a focus of research on anaerobic oceanic processes. The relative roles of anaerobic processes are not well understood and are currently the subject of controversy.
5. The diversity of organisms performing ammonia oxidation and nitrification is now known to span both the Bacterial and Archaeal domains of life. In contrast to the perception that this occurs primarily in the deep ocean, we now know that nitrification occurs at all depths in the ocean, potentially at rates significantly faster (days rather than months) than previously thought.
6. The conceptual N cycle is in a state of flux, with new organisms and processes having recently been discovered and their global implications poorly understood. New information regarding the who, when, and where of processes such as nitrification, anammox, and nitrogen fixation are at the center of new discoveries and controversies with wide-ranging global implications.

7. Although there are hypotheses about the global N balance, and how the N cycle may be affected by global climate change, the unconstrained uncertainties suggest that we still cannot balance the global N cycle, we probably cannot properly parameterize all of the various rates and processes (many of which are just now being characterized or inferred from a handful of direct observations), and we therefore can predict only the most obvious responses to past or future climate change, be it natural or anthropogenic.

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Errata

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