What's New in the Nitrogen Cycle?

BY BESS B. WARD, DOUGLAS G. CAPONE, AND JONATHAN P. ZEHR

Of all the biogeochemical cycles, nitrogen is the one most intimately and thoroughly associated with microbes. Essential and unique steps in the nitrogen cycle are performed by a wide array of bacteria, archaea, and eukaryotes, and the broad outlines of the cycle have been understood for over a century. That is why recent discoveries, in both terrestrial and aquatic environments, have surprised and intrigued the biogeochemical community. In this article, we focus on a few of the most exciting, very recent developments in the nitrogen cycle, summarize the changes in our understanding, and point out some questions to guide future research. The main processes of interest are anaerobic ammonium oxidation (anammox), aerobic nitrification by archaea, nitrogen fixation by unicellular marine cyanobacteria, and the issue of the balance and coupling between internal input and removal pathways. The nitrogen cycle in marine environments (Figure 1), in whole and in all of it various parts, is thoroughly reviewed in the recent revision of *Nitrogen in Marine Environments* (Capone et al., in press).

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ANAMMOX

The conversion of fixed nitrogen compounds to nitrogen gas is an essential step in the nitrogen cycle, whereby the total inventory of fixed nitrogen is decreased. This loss has important implications for the nitrogen budget of ecosystems and for the control of primary production and respiration on scales ranging from the microenvironment of sediments to regional and global oceanic systems. While there may be no a priori reason to assume that the nitrogen cycle is or should be balanced, a long-term imbalance is clearly a recipe for global change, so the rate of fixed nitrogen loss is an important question.

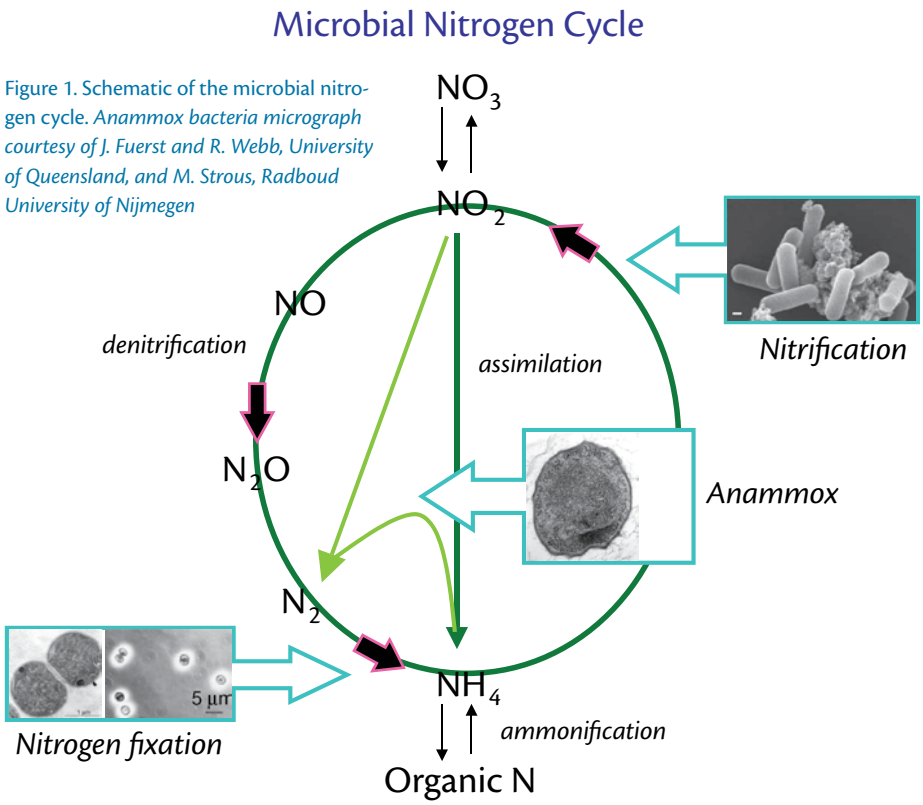

Figure 1. Schematic of the microbial nitrogen cycle. *Anammox bacteria micrograph courtesy of J. Fuerst and R. Webb, University of Queensland, and M. Strous, Radboud University of Nijmegen*

Until quite recently, only one pathway was known for the biological loss of fixed nitrogen, which in the ocean occurs mainly in the oxygen minimum zones (OMZ) and hemipelagic sediments. Conventional denitrification is the anaerobic respiratory pathway by which mostly facultatively anaerobic heterotrophic bacteria reduce nitrate sequentially to nitrite, nitric oxide, nitrous oxide, and N_2 gas. In 1995, a novel biological process (anammox), by which ammonium is anaerobically oxidized by nitrite to N_2 gas, was identified (Mulder et al., 1995; van de Graaf et al., 1995). The organisms that mediate anammox have not been obtained in pure culture, but much has been learned from enrichment cultures and purified cells.

Denitrifiers are mostly heterotrophic, capable of and perhaps preferring aerobic respiration, although the thermodynamic yield of growth on nitrate compares very favorably with that on oxygen. They are found in all three domains of life: Bacteria, Archaea, and Eukarya (fungi, foraminifera). In addition to its impact on the nitrogen cycle, a very important role of conventional denitrification is the continued degradation of organic carbon in the absence of oxygen, when obligately aerobic heterotrophs cannot function. Denitrifying bacteria in laboratory culture exhibit generation times on the order of a few hours to a day, with somewhat faster growth under aerobic than under denitrifying conditions. Denitrifying microbes can be isolated from just about any environment, and a vast uncultivated diversity of organisms with the potential to denitrify has been discovered on the basis of their signature genes in both oxic and anoxic waters and sediments.

Anammox bacteria are a much more phylogenetically narrow group, at least as known at present, and they are primarily autotrophic (Jetten et al., 2005). In this sense, they resemble the well-known aerobic autotrophic nitrifiers: their main source of reducing power is ammonium and their only source of carbon is CO_2. Both ammonia-oxidizing bacteria (AOB) and anammox bacteria have been shown to utilize simple organic compounds, but anammox bacteria apparently do not assimilate the carbon. The generation times for anammox bacteria, reported at 11 days or longer, are even longer than those for AOB, despite similar thermodynamic yields from ammonium oxidation. It seems likely that anammox bacteria in the ocean should have faster generation times, but this remains to be determined. When anammox organisms were first reported in wastewater systems, many scientists assumed they could not be important in natural environments because they could not persist under grazing pressure and the turbulence loss terms of the ocean. Both grazing and turbulence, however, are minimized by the same processes that constrain oxygen availability in the water column and sediments, and the anammox process has now been found in many natural environments with less than ~10 micromolar free oxygen.

Reports of anammox rates that are equal to or greater than rates of denitrification in many environments raise the important question of how much of the fixed nitrogen loss can be attributed to denitrification and how much to anammox. Depending on how the estimates of net nitrogen loss are made, the relative importance of the two processes may not change our understanding of the total nitrogen budget, but important questions remain beyond the overall rates of nitrogen cycle balance. First, the basic metabolic differences between the two groups of organisms involved mean that their activities might be differentially regulated and thus they might respond differently to environmental change, such as in oxygen or organic carbon supply. Second, although the enzymatic pathway of anammox is not completely known, it is pretty clear that the powerful greenhouse gas nitrous oxide does not figure as an intermediate, as it does in denitrification. Third, if anammox is established to be the quantitatively most significant pathway for N_2 loss in some extensive marine environments (as currently suggested), it will change our view of how organic matter is remineralized under oxygen-limited conditions. Biogeochemists and modelers assume that respiration switches over from oxy-

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gen to nitrate at some arbitrary oxygen concentration, usually 4–10 µM. If, however, much of the mineralization in the OMZ actually occurs via oxygen-limited aerobic respiration instead of denitrification, then these models and the resulting stoichiometry will need to be revisited.

Two important findings have emerged from field studies on anammox and denitrification: (1) under conditions where conventional denitrification is assumed to occur, especially in the water column, direct measurements often detect only anammox, and (2) in the century or so in which denitrification has been assumed to be the major sink for fixed nitrogen, surprisingly few direct measurements have quantified denitrification specifically in the oceanic environment. In any event, previous methods used to estimate denitrification could not differentiate between conventional denitrification and anammox.

The isotope-pairing method now in use to detect anammox (Thamdrup and Dalsgaard, 2002) can distinguish between these two processes and gives independent estimates of both. Using this method, evidence for only anammox, and not for direct conversion of nitrate to N_2 by denitrifiers, has been reported in shelf waters of the Benguela upwelling region and in the water column off Peru and Chile.

Finding of anammox in the absence of denitrification raises serious challenges to our understanding of organic matter cycling. If organic matter degradation is proceeding in the OMZ in the absence of oxygen (i.e., by an anaerobic pathway), it seems that conventional denitrification must be a significant process in the OMZ regions of the world ocean because anammox would be dependent ultimately upon denitrification, or at least nitrate reducers, for the supply of NH_4^+ and NO_2^-. Although anammox bacteria can be shown to reduce nitrate and to oxidize simple organic carbon compounds, their main impact on ocean biogeochemical cycles is the conversion of the nutrients ammonium and NOx to N_2 gas, supporting autotrophic CO_2 fixation. Even if denitrifiers preferentially utilize nitrogen-rich substrates, the net stoichiometry requires that denitrification or nondenitrifying nitrate reduction must be responsible for the production of NH_4^+ and NO_2^-, which is accompanied by the production of N_2. Equations 1 and 2 are the balanced reaction for the complete oxidation of Redfield OM by denitrifiers that allows for the maximum amount of anammox (i.e., all remineralized NH_4^+ is converted to N_2 via anammox):

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 94.4NO_3^- + 94.4H^+ \rightarrow 106CO_2 + 16NH_4^+ + 16NO_2^- + 39.2\,N_2 + 145.2H_20 + H_3PO_4 \quad [1]$$

and

$$16NH_4^+ + 16NO_2^- \rightarrow 16N_2 + 32H_2O \quad [2]$$

In this case, 29% of the N2 is produced from anammox and 71% from canonical denitrification. This is clearly not in agreement with the observations.

How then can the detection of anammox in the absence of denitrification be explained? The open-ocean OMZs are much more problematic than anoxic sediments because unlike in the OMZ, denitrification is usually detected in sediments, even in the presence of anammox, and there are many reports of sites where anammox was investigated and not detected. We describe two possible scenarios for the OMZ, the hypotheses they raise, and the obvious research needs that might help resolve the question.

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Scenario I

As slow-growing autotrophs, anammox bacteria may persist even under unfavorable conditions (e.g., too much oxygen), turn on the anammox metabolism when the conditions are right, and plod along at slow but relatively constant rates. They do not respond strongly to increased supplies of NH_4^+ and NO_2^- because their metabolic rates are thermodynamically constrained, resulting in their intrinsically slow growth rates. When oxygen concentrations are raised by ventilation or mixing, the organisms are not poisoned. Rather, they cannot

grow fast enough to escape aerobic grazers, so they do not persist under typical oceanic oxygen conditions. The organisms are consistently present in OMZs of pelagic waters and in stratified sediments. Denitrifying bacteria, in contrast, grow rapidly when appropriate organic carbon substrates are present. They are limited by carbon, not nitrate, and have the capacity to respond rapidly to the episodic supply of carbon derived from overlying phytoplankton blooms or seasonal inputs to sediments. Most denitrification occurs during pulses of carbon degradation, supplying NH_4^+ and NO_2^- to anammox.

This scenario lends itself to several predictions: we would expect to find that growth rates of anammox organisms are relatively invariant in environments where they occur, but that they are not found at all in chronically aerobic environments. Denitrifiers are present everywhere, including in oxygenated environments, but grow rapidly only occasionally and in response to direct carbon input. Different kinds of denitrifiers might be expected to bloom in response to different kinds of carbon input. We would expect transient ammonium accumulations in the OMZ because anammox bacteria cannot respond fast enough to the episodically enhanced supply of ammonium during such denitrifier "blooms."

Scenario II

An alternative explanation for the apparent absence of denitrification in OMZs is that organic matter mineralization proceeds via oxygen-limited aerobic pathways instead of via denitrification. Equations 3 and 4 show the balanced reaction for complete oxidation of Redfield OM by oxygen-limited aerobic mineralization, again with all the ammonium converted to N_2 via anammox.

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 118O_2 \rightarrow 106CO_2 + 8NO_{2-} + 8NH_4^+ + 114H_2O + H_3PO_4 \quad [3]$$

and

$$8NH_4^+ + 8NO_2^- \rightarrow 8N_2 + 16H_2O \quad [4]$$

In this case, 14.75 moles oxygen are consumed for every mole of NH_4^+ lost as N_2 via anammox. The amount of ammonium needed to fuel anammox is generally less than 100 nmol l^{-1} d^{-1}, assuming anammox rates on the order observed in the OMZs. This would require a maximum of 1.5 µmol O_2 l^{-1} d^{-1}. Oxygen fluxes on this scale are below the detection limit of most conventional oxygen measurements and could be supplied by small lateral inputs.

This scenario leads to a major difference in the predictions of expected microbiological observations: heterotrophic bacteria with the potential to denitrify are again very widespread, but grow most of the time via oxygen-limited aerobic metabolism rather than denitrification. This scenario, however, does not exclude denitrification altogether. Episodic carbon input may still drive the system temporarily to true anoxia, and that is when denitrifiers grow rapidly. Outside of these anoxic episodes, aerobic mineralization dominates. Hence, the transient increase in ammonium concentrations in the OMZ should be a rare phenomenon.

To distinguish between these hypotheses, we need to determine whether the nonanammox assemblage is growing via aerobic or anaerobic respiration (i.e., using oxygen or nitrate). If the portion of the in situ microbial assemblage that is capable of neither denitrification nor anammox (but relies on oxygen respiration instead) is large, then clearly aerobic metabolism must be important in this environment. If most of the microbes are capable of denitrification, then how fast and when they respire oxygen versus nitrate must be determined. This could be accomplished by careful mass-balance measurements of oxygen and dissolved inorganic nitrogen (DIN) in incubations under "natural environmental" conditions. Technological advances in incubation methods that avoid major perturbations to substrate and oxygen conditions are essential. Perhaps most critical is the rigorous application of existing and newly developed methods for accurate measurement of submicromolar oxygen concentration in the water column and inside incubation devices. Additional information could be derived from gene expression assays for key genes in the alternative metabolic pathways (e.g., terminal oxidases that distinguish between denitrification and oxygen respiration).

NITRIFICATION

Until very recently, organisms known to be capable of nitrification were restricted to two phyla, Proteobacteria and Nitrospira, and appear to have arisen from a photosynthetic ancestor, diverging before the ability to nitrify was developed in the various groups (Teske et al., 1994). The known nitrifiers were all bacteria and comprised two functionally distinct groups: those that

oxidize ammonium to nitrite (ammonia-oxidizing bacteria, AOB) and those that oxidize nitrite to nitrate (nitrite-oxidizing bacteria, NOB). No organism is known to carry out both reactions.

A new group of aerobic, ammonia-oxidizing nitrifiers was recently discovered, first by detection of ammonia-oxidizing genes of apparent archaeal origin in environmental metagenomic libraries (Schleper et al., 2005) and subsequently verified with the enrichment cultivation of a strain of archaea (ammonia-oxidizing archaea, AOA) that oxidizes ammonia to nitrite, apparently using a pathway very much like that known in AOB (Könneke et al., 2005). It now seems likely that the AOA are much more abundant than AOB in marine systems (Wüchter et al., 2006) and are also prevalent in soils (Leininger et al., 2006). How many of the marine Crenarchaea are functional ammonia oxidizers, and the extent of the AOA metabolic repertoire, remain open questions.

The only cultivated AOA, *Nitrosopumilus maritima,* is apparently an obligate chemoautotroph, but uncultivated marine Crenarchaeota may be capable of amino acid assimilation. Facultative nitrifiers that grow heterotrophically most of the time would seem to be an advantageous compromise, but mixotrophy is surprisingly uncommon in the microbial world. Most of the archaeal biomass in the deep ocean appears to be autotrophic in origin (Ingalls et al., 2006), however, suggesting that mixotrophy is not the dominant way of life for AOA.

Nitrosopumilus maritima depends on CO_2 as its only carbon source, and the presence of even low levels of organic carbon is inhibitory to growth. Crenarchaeota generally utilize a 3-hydroxypropionate pathway or a reductive tricarboxylic acid (TCA) cycle (also known as the Krebs cycle) for autotrophic carbon fixation, so it is likely that AOA do not use the Calvin cycle, as do the AOB. *N. maritimus* has a minimal generation time of 21 hours, longer but roughly on the same scale as AOB.

The 16S rRNA sequence of only one AOA is currently available, and that sequence places *Nitrosopumilus maritima* within the low-temperature marine Crenarchaeota, a group that is abundant in seawater and distinct from low-temperature Crenarchaeota in soils. Phylogenetic analysis of several hundred AOA partial *amoA* (gene that encodes ammonia monooxygenase, the first enzyme in the ammonium oxidation pathway) sequences identified major groups that clustered by environment: water-column sequences clustered, whether they originated from the Black Sea, Monterey Bay, or the eastern tropical North Pacific, and marine sediment sequences clustered with other sediment and soil sequences (Francis et al., 2005). It is assumed that these are all derived from Crenarchaeota, but this is a large and more diverse group than previously appreciated, so the phylogeny of AOA remains largely unexplored. The fact that the *amoA* genes from AOB and AOA are homologous raises the question of the ultimate origin of the ammonia-oxidizing phenotype. If the ancestral Crenarchaeota were thermophiles, it is possible that ammonia oxidation originally arose in thermophiles and spread from the Archaea to the Bacteria. Alternatively, the acquisition of *amoA* genes could be a late addition to an otherwise heterotrophic Crenarchaeal background, and thus the original AO metabolism arose in the purple sulfur line shortly after the advent of oxygen in the atmosphere. Determination of the subsequent steps and genes in the AOA pathway will help resolve this issue.

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The capacity for nitrifier denitrification may be ubiquitous among AOB (Shaw et al., 2006; Casciotti and Ward, 2005), and the distribution of nitrous oxide in the ocean is attributed to production of N_2O by nitrifiers. The AOA may have a different pathway for ammonia oxidation and their potential for N_2O production remains unknown.

There is abundant evidence from culture studies that both AOB and NOB are photosensitive. Although the relative abundance of the marine Crenarchaeota is greatest below the photic zone, their absolute abundances are greater in surface waters, as is observed for the overall distribution of microbes. Thus, light might be a regulating factor for AOA as well as AOB.

The affinity of AOA for ammonium and competitive substrates has not been

investigated. Values derived from incubation of natural samples obviously include contributions from AOA, but very little autecological information is available at present because of the lack of cultivated representatives. The absolute rates and distributions of nitrification measured in marine environments generally do not depend on the kind of organism responsible. The rate of nitrification in the open ocean appears to be linked to the supply of organic matter and the resulting mineralization rate for ammonium supply: particulate organic carbon flux (Berelson, 2001) and nitrification (Ward and Zafiriou, 1988) both show exponential decays with depth, with maximal nitrification rates near the bottom of the photic zone. The discovery of AOA does not change the magnitude or distribution of nitrification rates. Thus, the in situ abundance and activities of AOA, when determined, must be compatible with biogeochemical constraints implied by ammonium supply and turnover.

At this point, it is unclear how extensively the discovery of the AOA will change our understanding of nitrification in the ocean. There are several obvious high-priority questions that should be addressed immediately:

1. What fraction of the ubiquitous Crenarchaeota in the ocean are AOA and what fraction of ammonia oxidation in the ocean is due to AOA versus AOB?
2. Are the marine AOA predominantly autotrophic or do they have a facultative or mixotrophic metabolism that allows them to utilize alternative energy generation modes? What are the pathways of ammonia oxidation in AOA? Are they homologous with those of AOB? Do AOA possess a microaerophilic or anaerobic metabolism similar to the nitrifier denitrification pathway of AOB?
3. How do the physiological characteristics of the AOA compare with those of the AOB in terms of substrate affinity, light sensitivity, growth rates, oxygen requirements, N_2O production, etc.? (For example., are AOA and AOB regulated in the environment by similar factors?)
4. Why have AOA never been cultivated before? The explanation could lie in analogy to *Candidatus Pelagibacter ubique*, identified in 16S rRNA clone libraries as SAR11. Although ubiquitous in clone libraries, *Candidatus P. ubique* was never cultivated until very-low-substrate, clean-culture techniques were applied (Rappé et al., 2002). *Candidatus P. ubique* and its close relatives are estimated to contribute up to 50% of the surface ocean microbial communities, yet they do not grow under standard, rich-media conditions. Perhaps AOB are the weeds of the marine nitrifiers and AOA are the *Candidatus P. ubique* of ammonia oxidizers, the ever-present dominant assemblage that is important in normal—low-substrate, clean—environmental conditions. Alternatively, perhaps most AOA use ammonia oxidation only as a background or support metabolism and therefore don't compete well in the obligately autotrophic conditions usually employed to enrich for nitrifiers.
5. Are nitrite-oxidizing archaea (NOA) waiting to be discovered? Even less is known about the abundance and growth characteristics of important marine NOB than the AOB; it is entirely possible that NOA are also present. Nitrite does not accumulate in most of the world's ocean, specifically in the deep ocean where the Crenarchaeota, suspected to be AOA, comprise up to 40% of the microbial cells. If the Crenarchaeota are mainly AOA, and are abundant and active, the resident nitrite-oxidizing assemblage is clearly capable of keeping up with them. This suggests that bacterial nitrite oxidizers might comprise a similarly large fraction of the total microbial assemblage.

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NITROGEN FIXATION

Nitrogen fixation (the reduction of N_2 gas to biologically available ammonium) is a common feature in many benthic environments and in marsh sediments, but nitrogen fixation in the water column of the ocean has long been controversial. The paradigm of nitrogen fixation in the open ocean began to change with the discovery that the filamentous nonheterocyst-forming (heterocysts are specialized cyanobacterial cells in filamentous species that fix N_2) *Trichodesmium* fixed nitrogen (Dugdale

et al., 1961). Early attempts to scale rates of N_2 fixation by *Trichodesmium* globally indicated a relatively small input, which was underestimated by: (1) using historical estimates of *Trichodesmium* abundance, which were systematically low because of the unique requirements to accurately quantify this plankter, and (2) ignorance of the quantitative importance of other potential agents of diazotrophy (see below).

In the 1990s, reanalysis of the nitrogen budget of the open ocean indicated that there must be additional unrecognized sources of nitrogen, presumably nitrogen fixation, to the surface waters of the ocean. The discrepancy between measured nitrogen fixation rates and biogeochemical estimates of nitrogen fixation could be satisfied if rates of known N_2 fixers were underestimated, or if additional, previously unknown N_2-fixing microorganisms were active in oceanic nitrogen fixation. Both of these solutions appear to be true.

More recent efforts have confirmed that the abundance of *Trichodesmium* can be much greater than documented in conventional phytoplankton surveys, and its contribution to nitrogen fixation can be of quantitative significance relative to other sources of nitrogen to the open ocean, such as diffusive nitrate flux (Capone et al., 2005). Nonetheless, the newer scaled estimates of *Trichodesmium* based on more robust estimates of their density in the field can still only account for a fraction (25–50%) of some of the recent geochemically based estimates. Other sources are required.

Studies aimed at determining the diversity of microorganisms with the potential for nitrogen fixation, by characterizing the nitrogenase (the enzyme that catalyzes N_2 fixation) gene sequences in ocean waters demonstrate that there were previously unrecognized microorganisms with the ability to fix N_2 (Zehr et al., 1998). These organisms include unicellular cyanobacteria as well as presumed heterotrophic bacteria affiliated with the gamma and alpha-Proteobacteria based on phylogenetic analysis of the *nifH* gene. These organisms have been identified primarily by the presence of gene sequences and most of them have yet to be brought into culture.

One of the unicellular cyanobacterial nitrogenase genes amplified from oceanic DNA samples was very closely related to the gene from an isolate, called *Crocosphaera watsonii* WH8501, previously cultivated from the South Atlantic (Zehr et al., 2001). *nifH* gene sequences that are very similar to the *C. watsonii* gene sequence have now been reported from a wide variety of locations in the tropical and subtropical ocean, including the North Pacific, the Great Barrier Reef, the Arabian Sea, and the North Atlantic.

Interestingly, metagenomic fragments of *C. watsonii* were cloned in a study of picoplankton of the North Pacific Subtropical Gyre (DeLong et al., 2006). Analysis of these metagenomic fragments revealed a surprising level of conservation of genome sequence, with DNA sequences from the Pacific Ocean matching the cultivated isolate with 99% sequence identity. This level of genome conservation is very high compared to the diversification of clades of picoplanktonic bacteria and cyanobacteria.

One of the newly discovered nitrogen-fixing microorganisms, still uncultivated, is a presumed cyanobacterium of a unicellular group similar to that of *C. watsonii*, but more closely related phylogenetically to strains of *Cyanothece*. This organism is very abundant, but may be smaller in size than *C. watsonii*. The geographic range of this organism appears to be greater than that of *Trichodesmium*, and it is even found in lower-temperature water. Intriguingly, the pattern of *nifH* gene expression in this organism is consistent with gene expression and nitrogenase activity during the day, which is unusual for a unicellular cyanobacterium, because cyanobacteria have plantlike oxygenic phototrophic metabolism and nitrogenase is inactivated by oxygen.

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fixing microorganisms than about the unicellular cyanobacteria. Some *nifH* sequences attributed to these organisms have been recovered. None of them have been cultivated, except for *Vibrio diazotrophicus*, which has been detected in a few plankton samples. Bacterial *nifH* genes are apparently expressed even in waters containing fixed inorganic nitrogen and in the mesopelagic. It will be interesting to determine how these bacteria are able to support the energy requirements of N_2 fixation in the oligotrophic ocean. There is a hint of a diel cycle of *nifH* gene expression in the heterotrophic bacteria, which could point to a photoheterotrophic mode of metabolism, or energetic links between photosynthetic and heterotrophic microorganisms.

Quantifying the relative contribution of the more recently discovered diazotrophs is important and yet challenging. Measurements of nitrogen-fixation rates in bulk water are needed to determine rates in the nonaggregating, small, unicellular cyanobacteria. Nitrogen-fixation rates by the small size fraction can be significant, but the contribution of these small cells to the global nitrogen budget is unknown. A modeling study based on the growth dynamics of *Trichodesmium*, *C. watsonii*, and the uncultivated Group A unicellular cyanobacteria showed that *Trichodesmium* can dominate annual nitrogen fixation rates at station ALOHA in the North Pacific, but that the relative contribution is very sensitive to a number of parameters of which little is yet known.

Nonetheless, it is also clear that unicellular cyanobacteria are fixing nitrogen, and may do so on different temporal and spatial scales than *Trichodesmium*. If rates of *Trichodesmium* can account for the biogeochemically estimated nitrogen-fixation rates, then the new quandary will be, where does the nitrogen fixed by unicellular cyanobacteria and heterotrophic bacteria go? This leads to perhaps one of the most important implications of multiple nitrogen-fixing microorganisms in the ocean: they undoubtedly have very different limiting factors, different seasonality, but also different fates in the food web and export from the mixed layer.

With regard to differing limiting factors, it has been suggested that diazotrophs in the North Atlantic are more likely to be phosphorus stressed, while those in the North Pacific are more likely to suffer iron limitation. Phosphorus appears more depleted in the North Atlantic, while iron concentrations are higher in the North Atlantic compared to the North Pacific. Nitrogen fixation by natural populations of *Trichodesmium* was positively correlated with cellular P content but not with dissolved or cellular iron, which tends to support the phosphorus limitation hypotheses.

Some current estimates of global oceanic nitrogen removal through denitrification (including here the conventional pathway and anammox) greatly exceed the current geochemical input estimates, implying an unbalanced marine nitrogen cycle (Codispoti, 2006). Denitrification has generally has been associated with major oceanic OMZs (e.g., the eastern tropical North Pacific and eastern tropical South Pacific) and with shelf sediments, whereas the oligotrophic open ocean, and particularly the tropical North Atlantic, has received much of the recent focus of nitrogen-fixation research. Thus, primary sites of nitrogen fixation and denitrification appeared to be spatially uncoupled (at the temporal scales of ocean mixing of a few thousand years), perhaps allowing transient imbalances in input and output terms.

Several modeling efforts have produced results, however, that argue strongly that the oceanic nitrogen cycle must be more closely coupled on shorter time scales. A new analysis of oceanic nutrient distributions provides a mechanism for this coupling. Deutsch et al. (2007) noted that excesses of phosphorus in waters upwelled through OMZs were lost as the water was advected offshore. They attributed this drawdown of phosphorus in the absence of nitrogen fixation with surface waters downstream of major OMZs being major sites of nitrogen fixation. Hence, a mechanism for close coupling between denitrification and nitrogen fixation is provided.

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drop of the discovery of novel organisms participating in known pathways and even of novel pathways, there is the overarching issue of how the marine nitrogen cycle may respond to upper-ocean warming and acidification. Modeling and experimental efforts are now ramping up to discern how the nitrogen cycle may respond to predicted trends. Modeling studies suggest that oceanic nitrogen fixation may diminish through either a reduction of dust inputs or directly through warming, and experimental data show that elevated CO_2 concentrations stimulate growth and nitrogen fixation by *Trichodesmium*. The future likely holds more surprises.

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REFERENCES

Berelson, W.M. 2001. The flux of particulate organic carbon into the ocean interior: A comparison of four U.S. JGOFS regional studies. *Oceanography* 14(4):59–67.

Capone, D.G., J.A. Burns, J.P. Montoya, A. Subramaniam, C. Mahaffey, T. Gunderson, A.F. Michaels, and E.J. Carpenter. 2005. Nitrogen fixation by *Trichodesmium* spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean. *Global Biogeochemical Cycles* 19:doi:10.1029/2004GB002331.

Capone, D.G., E. Carpenter, D.A. Bronk, and M.R. Mulholland. In press. Nitrogen in the Marine Environment. Academic Press/Elsevier.

Casciotti, K.L., and B.B. Ward. 2005. Phylogenetic analysis of nitric oxide reductase gene homologues from aerobic ammonia-oxidizing bacteria. *FEMS Microbiology Ecology* 52:197–205.

Codispoti, L.A. 2006. An oceanic fixed nitrogen sink exceeding 400 $TgNa^{-1}$ vs the concept of homeostasis in the fixed-nitrogen inventory. *Biogeosciences Discussions* 3:1,203–1,246.

DeLong, E., S. Hallam, T. Mincer, C. Schleper, C. Preston, K. Roberts, and P. Richardson. 2006. Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine crenarchaeota. *PLoS Biology* 4:2,412–2,412.

Deutsch, C., J.L. Sarmiento, D.M. Sigman, N. Gruber, and J.P. Dunne. 2007. Spatial coupling of nitrogen inputs and losses in the ocean. *Nature* 445:163–167.

Dugdale, R.C., D.W. Menzel, and J.H. Ryther, 1961. Nitrogen Fixation In The Sargasso Sea. *Deep-Sea Research* 7: 297–300.

Francis, C.A., K.J. Roberts, M.J. Beman, A.E. Santoro, and B.B. Oakley. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences of the United States of America* 102:14,683–14,688.

Ingalls, A.E., S.R. Shah, R.L. Hansman, L.I. Aluwihare, G.M. Santos, E.R.M. Druffel, and A. Pearson. 2006. Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. *Proceedings of the National Academy of Sciences of the United States of America* 103:6,442–6,447.

Jetten, M.S.M., M. Schmid, K. van de Pas-Schoonen, J.S.S. Damste, and M. Strous. 2005. The anammox organisms: Enrichment, cultivation, and environmental analysis. *Environmental Microbiology Methods in Enzymology* 397:34–57.

Konnecke, M., A.E. Berhnard, J.R. de la Torre, C.B. Walker, J.B. Waterbury, and D.A. Stahl. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437:543–546.

Leininger, S., T. Urich, M. Schloter, L. Schwark, J. Qi, G.W. Nicol, J.I Prosser, S.C. Schuster, and C. Schleper. 2006 Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442:806–809.

Mulder, A., A.A. van de Graaf, L.A. Robertson, and J.G. Kuenen. 1995. Anaerobic ammonium oxidation discovered in a denitrifying fluidized-bed reactor. *FEMS Microbiology Ecology* 16:177–183.

Rappé, M.S., S.A. Connon, K.L. Vergin, and S.J. Giovannoni. 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* 418:630–633.

Schleper, C., G. Jurgens, and M. Jonuscheit. 2005. Genomic studies of uncultivated Archaea. *Nature Reviews Microbiology* 3:479–488.

Shaw, L.J., G.W. Nicol, Z. Smith, J. Fear, J.I. Prosser. and E.M. Baggs. 2006. *Nitrosospira* spp. can produce nitrous oxide via a nitrifier denitrification pathway. *Environmental Microbiology* 8:214–222.

Teske, A., E. Alm, J.M. Regan, S. Toze, B.E. Rittmann, and D.A. Stahl. 1994. Evolutionary relationships among ammonia- and nitrite-oxidizing bacteria. *Journal of Bacteriology* 176:6,623–6,630.

Thamdrup, B., and T. Dalsgaard. 2002. Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Applied and Environmental Microbiology* 68:1,312–1,318.

van de Graaf, A.A., A. Mulder, P. Debruijn, M.S.M. Jetten, L.A. Robertson, and J.G. Kuenen. 1995. Anaerobic oxidation of ammonium is a biologically mediated process. *Applied and Environmental Microbiology* 61:1,246–1,251.

Ward, B.B., and O.C. Zafiriou. 1988. Nitrification and nitric oxide in the oxygen minimum of the eastern tropical North Pacific. *Deep-Sea Research* 35:1,127–1,142.

Wüchter, C., B. Abbas, M.J.L. Coolen, L. Herfort, J. van Bleijswijk, P. Timmers, M. Strous, E. Teira, G.J. Herndl, J.J. Middleburg, and others. 2006. Archaeal nitrification in the ocean. *Proceedings of the National Academy of Sciences of the United States of America* 103:12,317–12,322.

Zehr, J.P., M.T. Mellon, and S. Zani. 1998. New nitrogen-fixing microorganisms detected in oligotrophic oceans by amplification of nitrogenase (*nifH*) genes. *Applied and Environmental Microbiology* 6:3,444–3,450.

Zehr, J.P., J.B. Waterbury, P.J. Turner, J.P. Montoya, E. Omoregie, G.F. Steward, A. Hansen, and D.M. Karl. 2001. Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean. *Nature* 412:635–638.