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Dinitrogen fixation in the world's oceans

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Abstract. The surface water of the marine environment has traditionally been viewed as a nitrogen (N) limited habitat, and this has guided the development of conceptual biogeochemical models focusing largely on the reservoir of nitrate as the critical source of N to sustain primary productivity. However, selected groups of *Bacteria*, including cyanobacteria, and *Archaea* can utilize dinitrogen (N_2) as an alternative N source. In the marine environment, these microorganisms can have profound effects on net community production processes and can impact the coupling of C-N-P cycles as well as the net oceanic sequestration of atmospheric carbon dioxide. As one component of an integrated 'Nitrogen Transport and Transformations' project, we have begun to re-assess our understanding of (1) the biotic sources and rates of N_2 fixation in the world's oceans, (2) the major controls on rates of oceanic N_2 fixation, (3) the significance of this N_2 fixation for the global carbon cycle and (4) the role of human activities in the alteration of oceanic N_2 fixation. Preliminary results indicate that rates of N_2 fixation, especially in subtropical and tropical open ocean habitats, have a major role in the global marine N budget. Iron (Fe) bioavailability appears to be an important control and is, therefore, critical in extrapolation to global rates of N_2 fixation. Anthropogenic perturbations may alter N_2 fixation in coastal environments through habitat destruction and eutrophication, and open ocean N_2 fixation may be enhanced by warming and increased stratification of the upper water column. Global anthropogenic and climatic changes may also affect N_2 fixation rates, for example by altering dust inputs (i.e. Fe) or by expansion of subtropical boundaries. Some recent estimates of global ocean N_2 fixation are in the range of 100–200 Tg N ($1–2 \times 10^{14}$ g N) yr^{-1}, but have large uncertainties. These estimates are nearly an order of magnitude greater than historical, pre-1980 estimates, but approach modern estimates of oceanic denitrification.

Introduction

Nitrogen (N) is an essential major element for life, accounting for nearly 10% of the dry weight of most microbial cells in the sea. In organic tissue, N is distributed primarily in proteins and nucleic acids, but is also an important constituent of bacterial cell walls (as muramic acid), energy transfer compounds such as nucleotides, photosynthetic pigments including chlorophylls and phycobilins, nucleic acids, vitamins and selected storage products (e.g. cyanophycin granules).

The N cycle in the sea involves a complex series of primarily microbiological transformations including (Figure 1): (1) nitrate (NO_3^-) and nitrite (NO_2^-) reductions to nitrous oxide (N_2O), dinitrogen (N_2), ammonium (NH_4^+) and organic-N by any one of several independent assimilatory or dissimilatory processes, (2) NH_4^+ production from the decomposition of organic-N (ammonification), (3) NH_4^+ oxidation to NO_2^-, N_2O and NO_3^- (nitrification) and (4) N_2 reduction to NH_4^+ and organic-N (N_2 fixation). Most of these N pool interconversions affect the oxidation state of N, and therefore the free energies of the various molecules and compounds. Consequently, most of these biological interconversions are either energy-yielding (e.g. nitrification) or energy-demanding (e.g. nitrogen fixation) and are fundamental processes in microbial biosynthesis and bioenergetics.

Although the thermodynamically-favorable form of N at the pH and redox state of seawater is NO_3^-, the ocean is far from chemical equilibrium in this regard; N_2 is by far the dominant form (e.g. the N_2:NO_3^- ratio is ≥ 25 in deep ocean waters and is ≥ 100 in most surface ocean waters). The relative stability of the triple bond of N_2 ($N \equiv N$) and the continual production of N_2 by the process of bacterial denitrification both contribute to this chemical disequilibrium. Thus although N, as an element, is present in a nearly inexhaustible supply in the marine environment, N that is combined in chemically 'fixed' or 'reactive' compounds, either as oxidized [NO_2^-/NO_3^-] or reduced [NH_4^+/organic N] forms may limit organic productivity. The low nutrient, fixed N-starved habitats of the near surface waters of the open ocean provide a seemingly ideal niche for N_2-fixing microorganisms. It would appear that this potential niche is largely unoccupied; or is it?

The planktonic, prokaryotic microorganisms that are responsible for N_2 fixation are taxonomically, physiologically and ecologically diverse, including: (1) *Bacteria* (phototrophs, heterotrophs, chemolithotrophs), (2) heterocystous and non-heterocystous cyanobacteria and (3) *Archaea*. Some species such as *Trichodesmium* are conspicuous by their sometimes massive open ocean blooms, other species are more cryptic. Biochemical considerations and accumulating field evidence suggest that Fe bioavailability

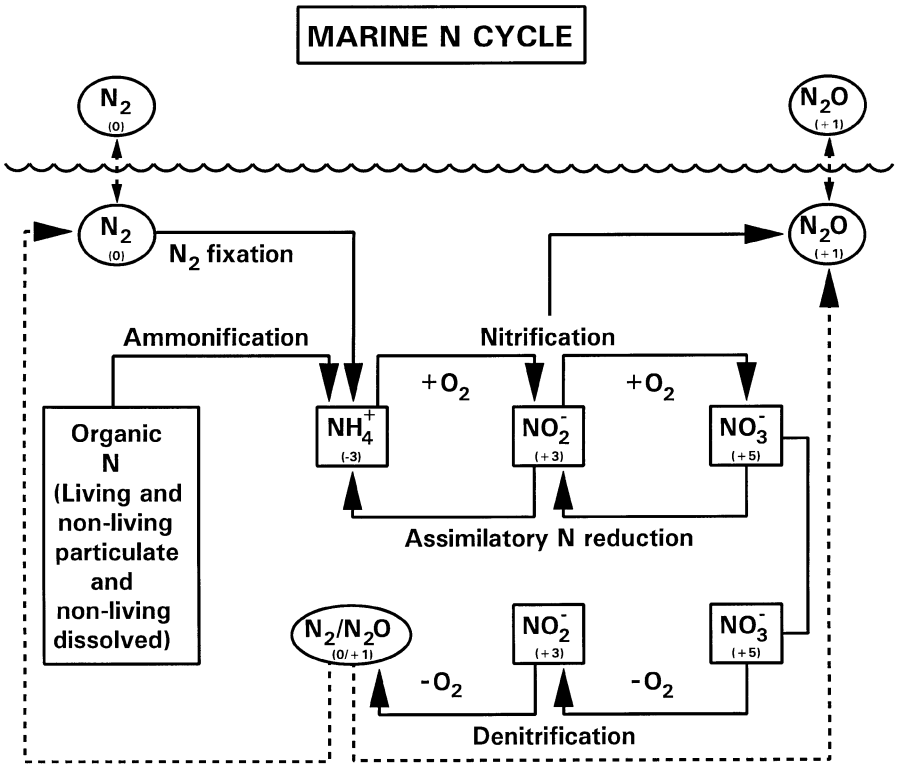


Figure 1. Schematic representation of the marine N cycle showing the major N pools and fluxes. Solid lines indicate transformations that are typically accompanied by direct or coupled energy release to the cell or organism or transformations that require an investment of energy and dotted lines indicate mass redistribution by physical-chemical processes such as gas exchange or water mass movements. Common terms that are assigned to selected pools or fluxes are also included. The small numbers in parentheses refer to the valence of N in each molecule or ion.

may control the distribution and abundance of N_2-fixing microorganisms in the sea. The primary pathway of Fe delivery to the upper oceans is via atmospheric deposition (e.g. dust), with upwelling and cross-shelf transport increasingly important in coastal and high productivity environments.

The global balance between denitrification, or the loss of bioavailable N ($NO_3^-/NO_2^- \rightarrow N_2$) and nitrogen fixation, or gain of bioavailable N ($N_2 \rightarrow NH_4^+$/organic-N), is a key for sustaining life in the sea on time scales of millennia. Furthermore, oceanic N_2 fixation may directly influence the sequestration of atmospheric carbon dioxide (CO_2) by providing a source of 'new' N to sustain the net production and export of organic matter from the

euphotic zone. N_2-fixing microorganisms are involved in global feedbacks with the climate system and these feedbacks will exhibit complex dynamics on varying time-scales. The hypothesized feedback mechanisms will have the following component parts: the rate of N_2 fixation can impact the concentration of the greenhouse gas, carbon dioxide (CO_2), in the atmosphere on time-scales of decades (variability in surface biogeochemistry) to millennia (changes in the total NO_3^- stock from the balance of N_2 fixation and denitrification); CO_2 concentrations in the atmosphere can influence the climate; the climate system, in turn, can influence the rate of N_2 fixation in the oceans by controlling the supply of Fe associated with dust, and by influencing the stratification of the upper ocean. Humans also have a direct role in the current manifestation of this feedback cycle by their influence on dust production, through agriculture at the margins of deserts, and by our collective discharge of CO_2 into the atmosphere. These influences can lead to a cyclic feedback system, particularly on longer time-scales. Consequently, a large challenge in contemporary biogeochemical oceanography is to understand the molecular-to-global scale controls on N_2 fixation in the sea.

This chapter will review data on the physiological ecology of N_2-fixing marine microorganisms in an attempt to (1) re-assess the global ocean rates of biological N_2 fixation, (2) determine the major controls on rates of N_2 fixation, (3) analyze the impact of N_2 fixation on the oceanic carbon cycle, including carbon sequestration and (4) consider how human activity, including eutrophication, habitat alternation and climate change, might affect N_2 fixation in the world's oceans. In preparing this report, we have made use of several excellent and up to date reviews on N_2 fixation (Fay 1992; Gallon 1992; Gallon & Stal 1992; Stal 1995; Zehr 1995; Bergman et al. 1997; Zehr & Paerl 1998; Capone & Carpenter 1999; Paerl 2000; Paerl & Zehr 2000). A recent NATO Advanced Science Institutes Series volume devoted to 'Marine Pelagic Cyanobacteria: *Trichodesmium* and other Diazotrophs' provides a comprehensive summary of the process of N_2 fixation in the sea (Carpenter et al. 1992). More recently a workshop was convened at Catalina Island, USA to examine the conceptual and practical issues concerning the integration of N_2 fixation into global ocean carbon models (Hood et al. 2000). Our report will focus on the open ocean habitat and the eco-physiological controls on environmental N_2 fixation. Various methods for estimating local, regional and global scale rates of N_2 fixation will be presented along with the inherent assumptions and caveats. Finally, a research prospectus for the future will be presented and discussed.

Diversity of N_2-fixing microorganisms

N_2-fixing microorganisms are exclusively prokaryotic (including both *Bacteria* and *Archaea*); however, beyond that single distinguishing characteristic they show a remarkable diversity in form and function. Much of the research in the marine environment, especially that in the open ocean, has focused on the relatively conspicuous, non-heterocystous, filamentous cyanobacterium *Trichodesmium* (Carpenter & Romans 1991; Capone et al. 1997). These planktonic microorganisms are cosmopolitan in the low nutrient tropical and subtropical seas that dominate our planet and often form massive near-surface blooms (Carpenter & Capone 1992). Despite years of research focusing on this organism as the principal oceanic N_2 fixer, rigorous proof that *Trichodesmium*, and not the associated heterotrophic bacteria, actually fixed N_2 did not come until Zehr and McReynolds (1989) examined the associated *nifH* gene sequence and subsequent direct microscopic, immunochemical localization of nitrogenase in *Trichodesmium* cells (Paerl et al. 1989b; Bergman & Carpenter 1991).

Five *Trichodesmium* species have been identified based on cytomorphological (Janson et al. 1995) and 16S rDNA and *hetR* gene sequence analysis (Janson et al. 1999a): *T. thiebautii*, *T. erythraeum*, *T. tenue*, *T. hildebrandtii* and *T. contortum*. Among these groups, three main clades (*T. thiebautii* and *T. hildebrandtii*, *T. contortum* and *T. tenue*, and *T. erythraeum*) are present. These major groups often coexist in nature (Carpenter et al. 1993). At least three laboratory cultures of *Trichodesmium* are now available: strain NIBB1067 isolated from Kuroshio waters by Ohki and Fujita (1982), strain IMS101 isolated from North Atlantic coastal waters by Prufert-Bebout et al. (1993) and strain MACC0993 isolated from coastal waters near Qingdao, China by Haxo et al. (1987). NIBB1067 and IMS101 are most closely aligned to *T. erythraeum*.

In the field, the filamentous cyanobacterium *Trichodesmium* is polymorphic and can exist as free trichomes (single filaments of cells, about 100–200 cells long), or in one of two characteristic colony morphologies (Figure 2): (1) fusiform colonies composed of trichomes arranged in a generally parallel but often twisted orientation (also called tufts or rafts) or (2) spherical colonies composed of trichomes arranged in a generally radially symmetric pattern (also called puffs). For *Trichodesmium* sampled in the Kuroshio current off Japan, N_2 fixation (more specifically, acetylene reduction – hereafter, AR; see '***Direct field measurements of N_2 fixation***') in free trichomes was only about 10% of the trichome-normalized rate measured in colonies (Saino & Hattori 1979). However, in a recent study conducted in the North Pacific subtropical gyre, the chl *a*-normalized free trichome vs. colony rates differed by only a factor of three (Letelier & Karl 1998). There is no

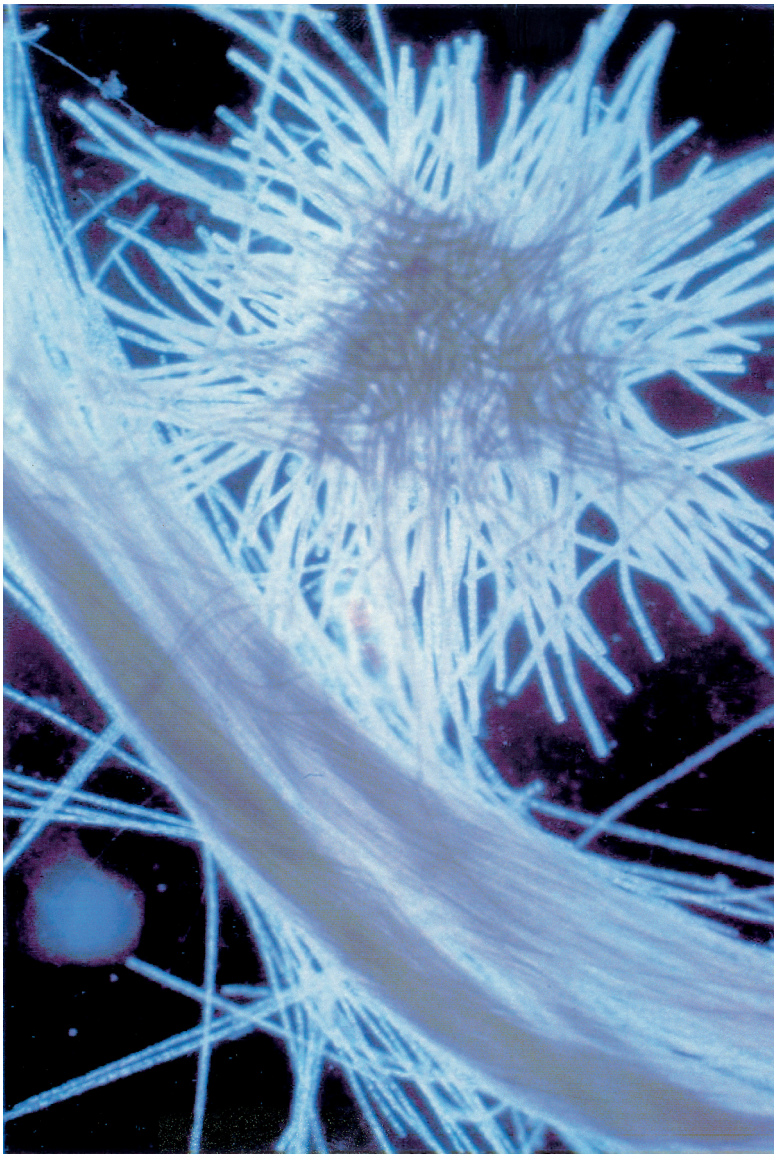


Figure 2. Morphological variability in field-collected samples of *Trichodesmium*. This light micrograph shows both the spherical (puff) colony morphology and fusiform (tuft) colony morphology. *Trichodesmium* can also be present as free trichomes, chains of approximately 100–150 cells (not shown). All three forms of *Trichodesmium* can co-exist in nature. Photo courtesy of Pernilla Lundgren and Birgitta Bergman, Stockholm University.

doubt that colony formation appears to enhance, but it is not a prerequisite for N_2 fixation.

First conducted by Christian Ehrenberg more than a century ago, field research on *Trichodesmium* has been promoted by the occurrence of prominent and extensive near surface ocean accumulations of colonies ('blooms'), especially during conditions of calm wind and sea (Figure 3). Its positive buoyancy (presence of gas vacuoles), high light-adapted photosynthetic apparatus and phosphorus-sparing effect (ability to grow with anomalously high N:P and C:P ratios) coupled with its high capacity for N_2 fixation and buoyancy control are ecologically-relevant adaptations for survival in marine environments that are chronically depleted in fixed N.

While *Trichodesmium* is undoubtedly the most well-studied marine N_2-fixing organism and perhaps one of the most important (Capone et al. 1997), it is not alone in the sea of diazotrophic microbes. Other known or suspected marine N_2 fixers include (see Postgate 1982; Benson 1985; Capone 1988; Sprent & Sprent 1990; Bergman et al. 1997; Paerl & Zehr 2000): (1) free-living (unicellular and filamentous), heterocystous and non-heterocystous, photoauto- and photoheterotrophic cyanobacteria including selected species of the genera *Synechococcus*, *Synechocystis*, *Oscillatoria*, *Aphanizomenen* and *Nodularia*, and two recently described non-heterocystous species, one unicellular (*Erythrosphaera marina*; Waterbury et al. 1988) and one filamentous (*Katagnymene* spp.; Lundgren et al. 2000), (2) anoxygenic photoautotrophic and photoheterotrohic *Bacteria*, (3) free-living, facultatively anaerobic pelagic chemoheterotrophic *Bacteria*, including *Vibrio diazotrophicus* (Guerinot et al. 1982; Guerinot & Colwell 1985; Urdaci et al. 1988), (4) chemoautotrophic *Bacteria*, including selected species of the genera including *Thiobacillus* and *Beggiatoa*, (5) obligately anaerobic *Bacteria* (e.g. *Desulfovibrio desulfuricans*) and *Archaea* (*Methanosarcina* spp.), (6) epiphytic cyanobacteria growing on pelagic Sargassum and other macroalgae (Carpenter 1972; Hanson 1977), (7) chemoheterotrophic bacteria growing as endosymbionts within mat-forming diatoms *Rhizosolenia castracanei* and *R. imbricata* var. *shrubsolei* (Alldredge & Silver 1982; Martinez et al. 1983), (8) the heterocystous photoautotrophic cyanobacterium, *Richelia intracellularis*, growing either as a free-living population or in a more common endosymbiotic association within several diatom genera including *Rhizosolenia* and the more ubiquitous *Hemiaulus*, or epiphytically with *Chaetoceros* (Mague et al. 1974; Venrick 1974; Villareal 1991; Janson et al. 1999b), (9) *Bacteria* living as ecto- and endosymbionts with marine invertebrates (Carpenter & Culliney 1975; Guerinot & Patriquin 1981; Proctor 1997), and (10) oxygenic, photoautotrophic cyanobacteria of the genera *Synechococcus* and *Synechocystis* growing as endosymbionts within several heterotrophic dinoflagellate

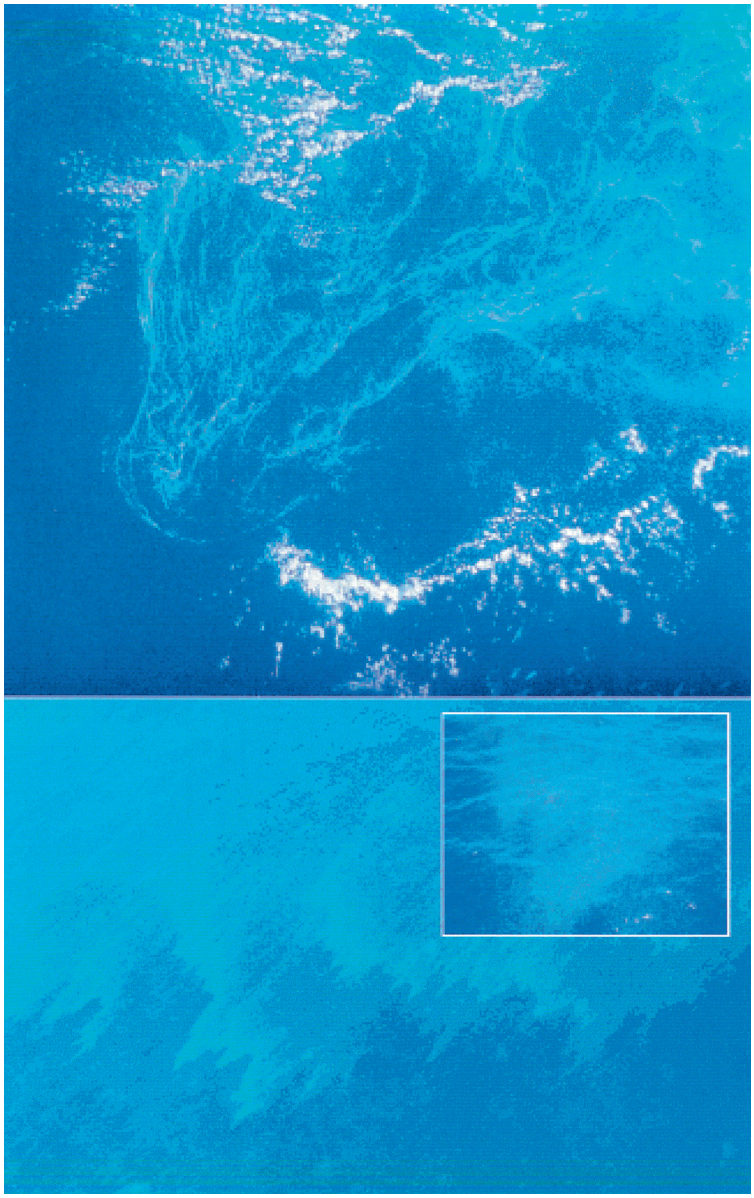


Figure 3. Trichodesmium bloom as viewed from space to the sea surface. [TOP] A massive *Trichodesmium* bloom in the Capricorn Channel of the southern Great Barrier Reef, Australia (near 22°50′S, 152°50′E) as viewed from the U.S. Space Shuttle flight STS-9, November 1983. This photo was taken using a hand-held 70 mm Hasselblad camera from a perspective 300 km above the Earth; the scale is approximately 1:850,000 (Reproduced from Kuchler & Jupp 1988); [BOTTOM] A massive *Trichodesmium* bloom in the North Pacific Subtropical Gyre (near 22°48′N, 158°11′W) as viewed from a U.S. Coast Guard C-130 aircraft (altitude 1.2 km), August 1989. The inset is a shipboard view of this same bloom from the deck of the *SSP Kaimalino* (photo credits: K. Louder and D. Hebel, inset).

genera and presumably supplying both C through photosynthesis and N through N_2 fixation to the host cells (Gordon et al. 1994). The continued use of direct optical and electron microscopic techniques, immunochemical and molecular procedures and novel isolation and culture methods are likely to reveal additional species of N_2-fixing microorganisms. At the present time it is not possible to determine the relative contributions of these various groups to global ocean N_2 fixation.

Field studies of laminated marine microbial mats and other benthic habitats have documented the presence of complex N_2-fixing microbial consortia with interrelated metabolic associations (Paerl & Pinckney 1996). A similar complexity is revealed upon close microscopic and physiological examination of field-collected *Trichodesmium* colonies (Paerl et al. 1989a; Siddiqui et al. 1992) suggesting that photoautotrophic-chemoheterotrophic syntrophy may be the rule rather than the exception for many natural microbial assemblages.

More recently, molecular methods have been used to ascertain the diversity of nitrogenase genes (*nifH*) in natural samples (see '***Nitrogenase Form and Function: Ecological Considerations***' section). Application of these methods to seawater samples collected from the Atlantic and Pacific Oceans has revealed an unexpected variation of *nifH* gene sequences suggesting the presence of previously undescribed N_2-fixing microorganisms (Zehr et al. 1998, 2000; Paerl & Zehr 2000). Picoplankton-sized ($< 2\ \mu$m) organisms with *nifH* gene sequences included those with likely phylogenetic affiliations with α- and γ-proteobaceria, β-proteobacteria and unicellular cyanobacteria clades. Major phylotype differences were observed between ocean basins in waters of similar physical and chemical characteristics (Zehr et al. 1998); a majority of the Pacific Ocean *nifH* sequences aligned with group II genera *Myxosarcina* and *Xenococcus* whereas the Atlantic ocean samples revealed a greater preponderance of group I cyanobacteria. The most remarkable aspect of this study was the extremely high phylogenic diversity of *nifH* genes (Zehr et al. 1998, 2000); it is possible that some marine N_2-fixing microorganisms have, to date, evaded detection. The metabolic activities of these previously undescribed 'virtual' microbes may require a revision of current dogma and, more importantly, may help to balance the marine N cycle in open ocean, low nutrient habitats once systematic ecological studies have been conducted.

Nitrogen fixation by *Trichodesmium* now appears to be much more important that we had previously suspected, and most likely many other presently unknown diazotrophic microorganisms also contribute to the global ocean N budget. However, one thing seems to be clear: heterocystous cyanobacteria are quite rare in the marine environment and in most estuaries,

while such organisms are very common in freshwater environments. They are occasionally common in a few brackish environments such as the Baltic Sea or very shallow estuaries during periods of low salinity (see Howarth et al. 1999). Even when present in marine environments, heterocystous cyanobacteria are usually the symbionts of other algae and, thus, not living in a typical marine environment. There is also no doubt that, considering the non-compatibility of N_2 fixation and oxygenic photosynthesis (see '***Oxygen***' section), heterocystous cyanobacteria are by far superior to non-heterocystous species with respect to diazotrophic growth.

Howarth et al. (1999) suggest that the balance between slow growth rates from trace element limitation (e.g. iron or molybdenum) and grazing losses limits the biomass of heterocystous cyanobacteria in most saline waters of estuaries waters and thus, by extension, the saline open ocean (see also Vitousek et al. this volume). The salinity-dependent process in that model is the hypothesized sulfate inhibition of molybdenum uptake (Howarth et al. 1999), however any balance of nutrient inhibition of growth and high grazing could yield this result. Under this scenario, *Richelia* and the other symbiotic heterocystous cyanobacteria, may persist and occasionally bloom in the open ocean if they experience a lower grazing loss with their host or if the host provides a trace nutrient environment that enhances symbiont growth. The balance of processes that control the abundance of these cyanobacteria is logically some complex mix (Howarth et al. 1999; Paerl & Zehr 2000), and the question of why heterocystous cyanobacteria are not more common in the oceanic environment remains an enigma. Understanding what factor makes these organisms unsuitable to proliferate in the sea, and how non-heterocystous diazotrophs evolved in the marine pelagic environment remain as important scientific challenges.

Nitrogenase form and function: ecological considerations

N_2 fixation is dependent upon the expression of an enzyme system, nitrogenase, which is a complex of highly conserved proteins among the various terrestrial and aquatic N_2-fixing prokaryotes. The most well studied nitrogenase enzyme system requires the activity of two related proteins (Postgate 1982): N_2 reductase, an Fe-protein (*nifH*) and dinitrogenase, an Fe-Mo protein (*nif*DK). Alternative vanadium and tungsten-requiring N_2 fixation systems have also been identified (Bishop et al. 1980; Fallik et al. 1991). Importantly, neither the detection of nitrogenase genes nor the presence of the coded proteins can be used to unambiguously determine rates of catalysis under *in situ* conditions. The synthesis and eventual expression of nitrogenase are ultimately determined by a broad range of physiological and ecological

variables, including presence of fixed N compounds, oxygen concentration, availability of P and enzyme cofactors (notably Fe and Mo), a sufficient supply of energy and, perhaps, temperature. Several comprehensive reviews of nitrogenase enzyme structure and function have appeared (Broughton & Puhler 1986; Smith & Eady 1992; Dean et al. 1993; Kim & Rees 1994), so only a few key control mechanisms that relate to nitrogenase activity in *open ocean* ecosystems will be discussed here.

Oxygen

Molecular oxygen (O_2) is a potent inhibitor of nitrogenase synthesis and activity. A comprehensive discussion of the probable mechanisms of O_2 inactivation of nitrogenase has been presented by Gallon (1981, 1992), which should be read to fully appreciate the complexity of this otherwise straightforward enzyme-catalyzed reaction.

In most surface seawaters of the world's oceans, the O_2 concentration is either at or slightly above equilibrium with O_2 in the atmosphere (250–350 μm, depending upon sea surface temperature and salinity). These relatively high O_2 concentrations would, in theory, preclude nitrogenase activity in these habitats. The fact that *in situ* N_2 fixation does occur suggests at least one of the following: (1) the presence of an efficient O_2 protection or removal mechanism, (2) the presence of an altered, O_2 insensitive, form of nitrogenase or (3) a high rate of nitrogenase enzyme turnover (replacement). While the presence of an O_2 insensitive form of nitrogenase might be expected following more than 3.5 billion years of selection and evolution of marine N_2-fixing prokaryotes, there is presently no evidence for its existence.

In large heterocystous, filamentous cyanobacteria, there is evidence for a spatial separation of O_2-producing (photosynthetically-active) cells from the differentiated heterocysts which do not perform oxygenic photosynthesis. Furthermore, high respiration within the heterocysts and decreased permeability to dissolved O_2 of the cell surface all combine to reduce or eliminate O_2 inhibition of nitrogenase. However in non-heterocystous cyanobacteria, especially small unicells, structural modification is not possible so other behavioral or metabolic strategies of adaptation become important. Ironically, in the non-heterocystous cyanobacterium *Trichodesmium*, N_2 fixation is coupled to O_2 production via photosynthesis (Ohki & Fujita 1988).

It was initially thought that N_2 fixation in *Trichodesmium* was confined to the central portions of colonies, where net O_2 consumption would be favored (Fogg 1974; Carpenter & Price 1976; Bryceson & Fay 1981). It was suggested that these segregated, weakly pigmented and photosynthetically inactive internal cells might be analogous to the differentiated heterocysts (Carpenter & Price 1976). Independent studies using localized tetrazolium

salt reduction confirmed the presence of reduced microzones in *Trichodesmium* colonies (Bryceson & Fay 1981; Paerl & Bland 1982); microelectrode observations of O_2 gradients in *Trichodesmium* aggregates confirmed the presence of O_2-depleted microzones (Paerl & Bebout 1988). Both studies support a model of aggregation control of N_2 fixation.

A re-evaluation of the aggregation hypothesis using *Trichodesmium thiebautii* samples collected from the Caribbean Sea, however, failed to support key ecological predictions of the aggregation control model (Carpenter et al. 1990). Specifically, there were no differences in the distribution of photosystem I and II between central (protected) cells and O_2-unprotected cells near the periphery of the colonies, nor were there any pigmentation gradients. Diffusion model calculations revealed that respiration alone would be unlikely to maintain reduced intracolony O_2 concentrations.

Bergman & Carpenter (1991) first suggested that nitrogenase expression in *Trichodesmium* may be filament specific. More recently, it has been demonstrated that each trichome of *Trichodesmium* may contain one or more consecutively arranged cells containing nitrogenase in what appear to be differentiated cells. This spatial compartmentation of nitrogenase would provide a separation of photosynthetic and N_2-fixing activities that is necessary for optimum growth of the colony (Janson et al. 1994). Ultrastructural characterization of *Trichodesmium* cells, with and without nitrogenase, revealed significant differences that were consistent with the proposed functional interpretation (Fredriksson & Bergman 1997). Finally, whole cell immunochemical localization of nitrogenase (Lin et al. 1999) confirmed the spatial segregation model and it is likely that natural populations of filamentous N_2-fixing microorganisms have adopted a similar strategy for optimum growth and survival.

Additional evidence suggests that N_2 fixation in *Trichodesmium* must be sustained by intracellular adaptations including cellular differentiation, metabolic O_2 consumption, hydrogenase activity or rapid rates of nitrogenase synthesis and, therefore, turnover. Bergman et al. (1993) reported high levels of cytochrome oxidase in *Trichodesmium* and Kana (1993) reported rapid rates of oxygen cycling in field collected samples of *Trichodesmium*. Both of these studies are consistent with respiratory protection of nitrogenase as a possible adaptation. Stal and Krumbein (1985) suggested that a high rate of nitrogenase synthesis may provide a critical pool of enzyme necessary for N_2 fixation in *Oscillatoria*, and Capone et al. (1990) demonstrated a diel cycle in nitrogenase synthesis that was consistent with this hypothesis. The overall process of simultaneous N_2 fixation and photosynthetic oxygen production in *Trichodesmium*, however, remains enigmatic.

N_2 fixation has also been detected in free trichomes of *Trichodesmium* (Paerl 1994; Letelier & Karl 1998) which would not exhibit the hypothesized 'colony O_2 protection mechanism,' and in other small, unicellular prokaryotes exposed to O_2-saturated seawater (Mitsui et al. 1986). At low light levels (< 100 μEinstein m^{-2} sec^{-1}), photosynthesis is subsaturated and oxygen evolution rates are low. This would enable respiration to remove photosynthetically-produced oxygen, minimizing the impact of oxygen on N_2 fixation but still providing reducing agents for N_2 fixation. However, in field populations N_2 fixation occurs even at high light levels. Saino and Hattori (1978) have reported a 200-fold difference between N_2 fixation in the day versus night for field-collected samples of *Trichodesmium* with maximum activity at highest light levels. With regard to O_2 inhibition of nitrogenase, it would seem that N_2-fixing chemoheterotrophic bacteria may have a distinct metabolic advantage over O_2-producing photoautotrophic N_2 fixers, provided these bacteria can find refuge in a low oxygen microzonal habitat (e.g. organic aggregate, biofilm, etc.). Heterotrophic bacteria fix N_2 at reasonably high rates in organic-rich, anoxic sediments even in the presence of large amounts of ammonium (Howarth et al. 1988), implying that much of the ecological cost and relative disadvantage of diazotrophy may be related to the difficulties associated with the oxygenic environment (Vitousek et al. 2002 and ***Energy limitation*** section).

Energy limitation

The microbiological fixation of N_2 demands a significant amount of cellular energy in the form of ATP, and an appropriate electron donor, usually as reduced ferredoxin. This energy is a mixture of the direct costs of N fixation and the energy requirements to maintain an appropriate cellular environment (e.g. low oxygen, trace-element incorporation, resynthesis of enzymes, etc). Sixteen moles of ATP per mole of N_2 are directly required. Most of this energy is required to split the dinitrogen molecule (4 ATP molecules for each pair of electrons), not to reduce it. The thermodynamic investment to reduce NO_3^- to NH_4^+ is actually larger than that needed to reduce N_2 to NH_4^+ The overall reaction, $3H_2 + N_2 \rightarrow 2NH_3$, is actually exothermic (ΔG° = –33.4 $kJmol^{-1}$; Sprent & Sprent 1990). The higher energetic costs associated with nitrate reduction may be one reason that some N_2 fixation can occur even when NO_3^- is present. Organisms that are designed for diazotrophy have few ecological reasons to invest in a switch to NO_3^- uptake when it is added to oligotrophic waters, however, they are highly unlikely to switch to diazotrophy when growing on nitrate. Furthermore, fixed N, in the form of NH_3, may diffuse out of the cell, where it is protonated to NH_4^+ and subsequently taken back into the cell by an energy-dependent uptake mech-

anism. Depending upon the environmental conditions, this futile cycle of NH_3 can account for a significant portion of the cell's energy budget for N_2 fixation.

Other important bioenergetic considerations include the costs of enzyme synthesis and regulation; the former may be especially acute for non-heterocystous species who tackle the oxygen inhibition problem by high nitrogenase turnover. The energetic costs of maintaining a low oxygen environment to minimize nitrogenase turnover may also be considerable (Vitousek et al. this volume). With regard to free-living, microaerophilic and anaerobic chemoheterotrophic *Bacteria* with the potential for N_2 fixation, having access to oxygen-free microzones or participating in net oxygen consuming reactions is a key constraint on plankton N_2 fixation. In such associations, substrate and energy limitation play important regulatory roles.

The ATP required to drive N_2 fixation is supplied through photophosphorylation, substrate level phosphorylation and oxidative phosphorylation, depending upon the species. Both sources of potential energy, light and bioavailable dissolved organic matter, are present in limiting concentrations in most marine environments. The ability to survive in high light environments (Li et al. 1980; Carpenter & Roenneberg 1995; Kana 1993; Subramaniam et al. 1999a, b), and its buoyancy may be important adaptations for N_2 fixation in *Trichodesmium* during periods of low turbulence.

Temperature control

While temperature, *per se*, does not restrict the growth of N_2-fixing microorganisms (e.g. nitrogenase activity has been detected at subzero temperatures in Antarctic soils; Davey & Marchant 1983), the global distribution of *Trichodesmium* appears well constrained by seawater temperature. With rare exception, most reported *Trichodesmium* blooms occur in subtropical and tropical marine habitats with surface water $\geq 25\,^{\circ}C$ (Carpenter & Capone 1992). Nevertheless, individual colonies and free trichomes of *Trichodesmium* actively fix N_2 under *in situ* conditions of light and temperature to depths of at least 75 m in the subtropical North Pacific (Letelier & Karl 1998) corresponding to temperature of 21–23 °C. Because temperature and nitrate are significantly negatively correlated in the marine environment, it is not certain whether the global patterns of N_2 fixation versus surface water temperature derive from an inhibition of nitrogenase by low temperature or selection against N_2-fixing microorganisms under conditions of high ambient nitrate, or both.

N and P nutrient control

Despite its role as the marine 'model' for N_2 fixation, *Trichodesmium*, and probably most other diazotrophs, can grow on fixed N compounds, including both reduced and oxidized inorganic N and some forms of dissolved organic N (Ohki et al. 1986, 1991). In general, nitrogenase synthesis is repressed by NH_4^+ and is induced by depletion of fixed N substrates. However, careful laboratory studies conducted with *Trichodesmium* sp. (NIBB1067) have demonstrated that nitrogenase activity in cells grown on N_2 was not suppressed after 7-hr incubations with 2 mM $NaNO_3$ or 20 μM NH_4Cl, but was repressed by 0.5 mM urea (Ohki et al. 1991). *Trichodesmium* grown on NO_3^-, NH_4^+ or urea as a source of fixed N completely lacked the ability to fix N_2. The authors reported a complex pattern of regulation of the proteins of nitrogenase system, one that involved both transcriptional and post-transcriptional controls (Ohki et al. 1991). An independent laboratory study of N assimilation in *Trichodesmium* sp. (NIBB1067) revealed both a high affinity and a high uptake capacity for NH_4^+, urea and glutamate, and a low capacity for NO_3^-, but the culture was able to grow on NO_3^- as the sole source of fixed N (Mulholland et al. 1999). This latter study emphasized the inextricable links between N_2 fixation and N assimilation, and the complex patterns of intracellular regulation and control. Despite these careful laboratory studies, it is difficult to reproduce the conditions found in nature where the rapid recycling of generally low concentrations of N substrates ($[NO_3^-]$ typically $\leq$ 20 nM, $[NH_4^+]$ typically $\leq$ 40 nM, bioavailable [DON] $\geq$ 1 μM, all with turnover rates of approximately 1 day) may result in different strategies of biochemical adaptation.

It is generally agreed that a low N:P ratio of available nutrients selects for N_2-fixing microorganisms (Niemi 1979). On the other hand, once a bloom of N_2-fixing microorganisms is established, the N:P ratio of the ambient dissolved and particulate matter pools increases dramatically as a result of an overproduction of fixed N and an efficient scavenging of bioavailable P (Karl et al. 1992). Consequently the longer-term signature for regions that support net N_2 fixation is a high ($>$ 16:1), rather than a low, molar N:P ratio.

In contrast to the effects of N substrates on nitrogenase activity, very few studies have been conducted on potential control by P bioavailability. In most marine environments, N and P bioavailability are tightly coupled, so selection for N_2-fixing microorganisms will generally imply P limitation as well. During periods of intense N_2 fixation there must be a specific mechanism for P delivery to sustain net organic matter production. Several possibilities including atmospheric deposition, the passive upward flux of low density, P-enriched organic matter and vertical migration of *Trichodesmium* colonies have been suggested as potential mechanisms (Karl et al. 1992; Karl &

Tien 1997). Two additional physiological adaptations in *Trichodesmium* (and perhaps other N_2-fixing microorganisms) are the use of dissolved organic P (DOP) pools, and the ability to grow with an altered P cell quota. In most oligotrophic environments, DOP concentrations exceed the preferred substrate, orthophosphate, sometimes by 1–2 orders of magnitude. Induction of specific transport and hydrolytic enzymes, such as alkaline phosphatase, may be crucial for survival. A reduction of the P per cell quota, the so-called 'P-sparing effect,' is common for most microorganisms and may involve reduced intracellular pools of nucleotides and a lower nucleic acid content, especially RNA. Furthermore, an intensification of bioavailable P recycling rates under P limitation and a retention of P by an interdependent, remineralization-intensive food web may promote efficient N_2 fixation and microbial growth under P-controlled conditions. These strategies are likely to impact the growth rate of the population and, in the case of *Trichodesmium*, the low growth rates that have been measured under *in situ* conditions may be a manifestation of such a P-sparing strategy.

Fe bioavailability

Iron is a critical metal co-factor for nitrogenase (Howard & Rees 1996), and Fe bioavailability may be the most important overall control on oceanic N_2 fixation. Raven (1988) estimated that photolithoautotrophic growth using N_2 as the sole source of N requires two orders of magnitude more Fe per cell than for growth on NH_4^+. Fe has been shown to limit *Trichodesmium* N_2 fixation under field conditions (Rueter et al. 1992; Paerl et al. 1994). The supply of Fe to support N_2 fixation differs significantly between pelagic and benthic habitats. In the open ocean, Fe is generally depleted in the surface waters (Johnson et al. 1997). There is accumulating evidence to suggest that the delivery of Fe to the oceans in airborne dust may ultimately control the rate of N_2 fixation on the global ocean scale (Michaels et al. 1996; Falkowski 1997).

The processes controlling Fe availability in the upper ocean add several layers of biogeochemical complexity. In open ocean systems, Fe is supplied both by upwelling/mixing from below and from the atmosphere above. When Fe-enriched waters from beneath the euphotic zone are mixed into the surface ocean, they deliver a suite of other required major (C, N, P, Si) and trace elements in approximately the proper stoichiometry to sustain plankton production and coupled export. Under these conditions, there would be little selection for N_2 fixation due to the relatively high NO_3^-:Fe ratio in the upwelled waters. Alternatively, the dust-associated Fe flux is N-depleted, so it would select for N_2-fixing microorganisms and would ultimately serve to decouple the otherwise linked C-N-P-Si cycles in the sea. Consequently,

atmospheric dust inputs to oligotrophic, open ocean ecosystems could alter community structure, bioelemental stoichiometry and the net sequestration of atmospheric carbon dioxide. These and other potential biogeochemical consequences of N_2 fixation are discussed later.

Most of the Fe associated with atmospheric dust is locked into inaccessible aluminosilicate lattices and only a small amount is released as bioavailable Fe after dust contacts seawater. Aqueous dissolution studies on Atlantic (Zhu et al. 1997) and Indian Ocean (Siefert et al. 1999) aerosols have found that only about 1% of the total Fe is released as Fe(II). The mechanisms controlling the release of Fe from dust are not well understood and several otherwise unrelated processes are potentially important: (1) partial dissolution of Fe (III) oxides by acidic aerosols (Keene & Savoie 1998), (2) photochemical reduction to Fe (II), especially in the presence of organic matter (Zhuang et al. 1992; Siefert et al. 1996; Zhu et al. 1997) and (3) organic ligand complexation (Gledhill & Berg 1994; Rue & Bruland 1995; Wu & Luther 1995). Rueter et al. (1992) have suggested that *Trichodesmium* colonies may intercept dust particles, facilitate dissolution and, hence, enhance Fe(II) flux. Little is known about the bioavailability of the various forms of Fe in seawater, but recent reports indicate that even some forms of colloidal and particulate Fe might be taken up by plankton assemblages. Algal phagotrophy of Fe-rich chemoheterotrophic bacteria is another possible physiological adaptation to life in the 'Fe-free' zone.

Molybdenum-sulfate antagonism

Molybdenum (Mo) is a another required cofactor of nitrogenase and, like Fe, has been proposed to limit N_2 fixation (Howarth & Cole 1985; Cole et al. 1993). Even though the concentration of Mo in seawater exceeds that in freshwater systems replete with diazotrophy, Howarth and Cole (1985) have proposed that the relatively high concentrations of sulfate (SO_4^{2-}) in seawater ($\sim$28 mM), a structural analogue of molybdate (MoO_4^{2-}), could compete with Mo uptake and inhibit N_2 fixation. Competitive inhibition of MoO_4^{2-} uptake by high SO_4^{2-} concentrations was demonstrated by Cole et al. (1993). However, the inhibition is not complete and, in their experiments, only a partial inhibition of the uptake of Mo would be predicted at sulfate and Mo concentrations comparable to seawater. In the brackish Baltic, increased concentrations of Mo did not stimulate increases in N_2 fixation, but decreases in sulfate did (Stal et al. 1999). Thus, there appears to be a very complex interplay between Mo, sulfate, N_2 fixation and other cellular processes in the diazotrophs that characterize fresh and brackish waters.

The sulfate-Mo competition hypothesis has been the basis of an elegant model (Howarth et al. 1999) to explain the strong gradient in N_2 fixation

from freshwater lakes (high N_2 fixation rates) into estuaries (low rates and rare occurrence of heterocystous cyanobacteria). This model implicates a complex mix of processes, the balance of which control the amount of diazotrophy. Since a salinity gradient characterizes the differences among these ecosystems, the model fits the observations quite well. Sulfate concentrations vary with salinity and the increasing competitive reduction in Mo uptake as the salinity increases slows the growth rate of the cyanobacteria. At the salinity where grazing losses exceed growth rates, the net losses of biomass prevent blooms by the diazotrophs. Iron limitation is not concurrently explored in the model, in part, due to a lack of data on which to parameterize this process in estuaries. The authors recognize that any nutrient-like process that slows diazotroph growth rates in saline compared to fresh waters will yield a similar pattern (Howarth et al. 1999). This model also uses the relationship between heterocysts and filament length (influenced by grazing) to further impact growth rates. The apparent dominance of a non-heterocystous cyanobacterium in the open ocean may make this species less sensitive to the grazing-growth imbalance (Howarth et al. 1999) or provide an alternative explanation for the relative lack of heterocystous forms in the sea.

There are few direct measurements of Mo uptake in the open ocean. N_2-fixing potentials of some marine diazotrophs in the Fe-rich coastal Atlantic waters appear unaffected by this competition (Paerl et al. 1987; Paulsen et al. 1991). Oceanic diazotrophs undergo large changes in biomass under relatively similar Mo and sulfate concentrations suggesting that many other processes must have important ecosystem level effects, even if the sulfate-Mo competition still plays a role in inhibiting growth. Most likely, the small cellular Mo requirements for N_2 fixation are met through reduced, but sufficient, uptake and storage at the low growth rates that are implied by many field observations. In addition, recent research has shown the presence of alternative non Mo-requiring nitrogenases in bacterial and cyanobacterial diazotrophs (Bishop & Premakumar 1992). If such microbes are broadly distributed in nature (but there are no data to evaluate this at present), it would represent a selective mechanism by which Mo limitation could be circumvented. However, the final resolution of this debate will probably require detailed experimentation on the oceanic organisms that are currently adapted to life in this chronically Fe-depleted environment.

Substrate specificity and reaction by-products

All known nitrogenase systems studied to date exhibit a very low substrate specificity (Burris 1991). Although the physiological substrate is N_2, nitrogenase can also catalyze the reduction of many compounds that are

structurally-related to N_2, including acetylene (C_2H_2), cyanide (CN) and N_2O. A shared characteristic of these alternate substrates is the presence of a N-N, N-O, N-C or C-C double or triple bond. The kinetic properties and reaction mechanisms vary considerably among these different substrates. Some investigators believe that the original function of nitrogenase in pre-Cambrian microorganisms was for detoxification rather than N_2 fixation; presumably there was ample fixed N (especially NH_4^+) available under early Earth conditions.

In addition to the above-mentioned substrates, nitrogenase also reduces protons to form hydrogen (H_2) during N_2 fixation. This is an obligate reaction and at least 25% (Simpson & Burris 1984) and generally a larger share of the flow of electrons through nitrogenase is used for H_2 production. There are several potentially important ecophysiological consequences of H_2 formation. First, H_2 is a specific, competitive inhibitor of nitrogenase (Burris 1991) so elevated intracellular concentrations are unfavorable for N_2 fixation. The H_2 produced is either recycled intracellularly (i.e. oxidized and coupled to ATP formation via hydrogenase) or excreted into the surrounding environment. Scranton (1983) has reported both hydrogenase activity and elevated ambient concentrations of H_2 following short-term incubation with field-collected *Trichodesmium* colonies. This coupled N_2 reduction/H_2 formation may have important ecological consequences especially considering the fact that most bacteria can oxidize H_2. In this way, the population of N_2-fixing microbes directly influences the larger microbial community.

More recently, it has been shown that *Azotobacter vinelandii* nitrogenase (and presumably other nitrogenases as well) can reduce both C-S and C-O bonds, including the conversion of carbonyl sulfide (COS) to carbon monoxide (CO) and hydrogen sulfide (H_2S) and the conversion of carbon dioxide (CO_2) to CO and water (Seefeldt et al. 1995). Further investigation demonstrated that several COS analogues, including thiocyanate (SCN^-) can also be reduced leading to the formation of methane (CH_4) and H_2S (Rasche & Seefeldt 1997). The role of N_2-fixing microorganisms has not yet been evaluated as a potential source for the trace levels (typically nM) of CH_4, H_2S or CO that are ubiquitous in most tropical and subtropical marine environments.

Estimation of global ocean N_2 fixation

Chronic undersampling is a fact of life in oceanography (Platt et al. 1989) and still constrains the interpretation of most field data. In the case of N_2 fixation, neither spatial nor temporal uniformity can be assumed; much of the total N_2 fixation in the sea probably occurs during stochastic, heterogeneous

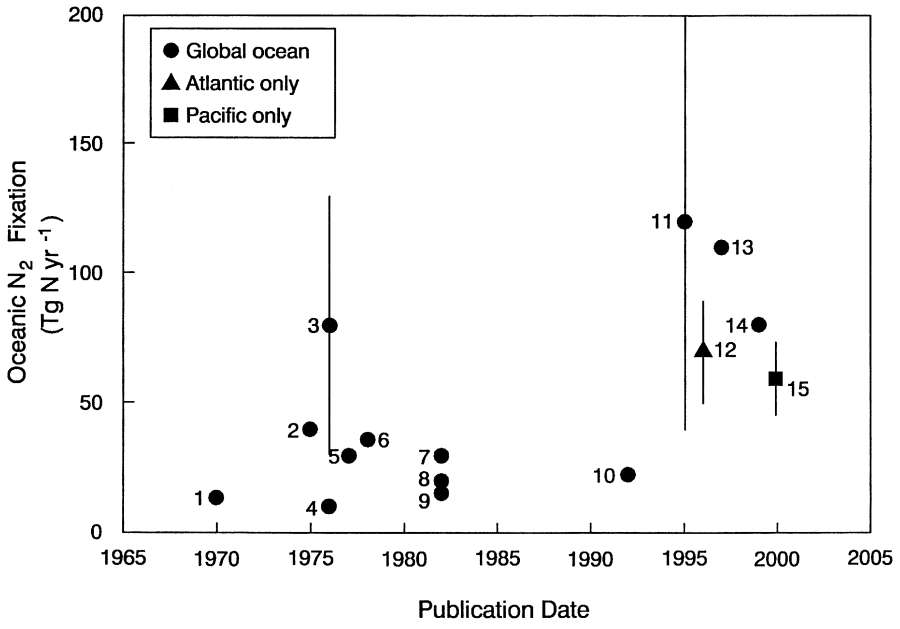


Figure 4. Basin scale and global ocean rates of N_2 fixation estimated by a variety of different methods plotted versus year of publication. These data were extracted from a recent review by Capone and Carpenter (1999) with more recent updates as indicated. The primary literature sources are: (1) Delwiche 1970, (2) Burns & Hardy 1975, (3) Soderlund & Svennson 1976, (4) McElroy 1976, (5) Delwiche & Likens 1977, (6) Paul 1978, (7) Soderlund & Rosswall 1982, (8) Capone & Carpenter 1982, (9) Fogg 1982, (10) Carpenter & Capone 1992, (11) Galloway et al. 1995, (12) Michaels et al. 1996, (13) Gruber & Sarmiento 1997, (14) Capone & Carpenter 1999 and (15) Deutsch et al. 2000. Although the individual estimates may have different integration time scales (see text), the full suite of estimates documents a potentially greater role for N_2 fixation than had been considered only a decade ago.

blooms that are not easily predicted or resolved by marine expeditionary field work. Furthermore, scientists who seek fundamental data on N_2 fixation in the sea usually target geographical regions and seasons when N_2 fixation is most likely to occur, and rarely produce data sets that can be statistically scaled to an unbiased average value for a particular biome. No existing N_2 fixation biogeochemical model captures the complexity of open ocean ecosystems and, as stated previously, we still have not even identified, much less isolated, all of the marine microorganisms with the genetic potential for N_2 fixation. Despite this sobering state of affairs, there exist several complementary approaches that can be used to assess rates of N_2 fixation in the global ocean (Figure 4). Much of the recent research, summarized below, has concluded that previous rates of global ocean N_2 fixation have been significantly underestimated.

Direct field measurements of N_2 fixation

Apart from the isolation of diazotrophs (e.g. Moore et al. 1921), N_2 fixation activity in the sea was initially discovered by Dugdale et al. (1961) in association with *Trichodesmium* colonies collected from the Sargasso Sea. Over the past 40 years numerous field studies have been conducted, many including direct estimation of N_2 fixation rates (see Capone et al. 1997 and Capone & Carpenter 1999). In our view, there is no substitute for direct field measurements if the stated objective is to estimate rates of N_2 fixation in the sea. This approach has provided many of the recent data sets that were necessary to question the existing dogma and to hypothesize an increased role for N_2 fixation in the world's oceans. They have also provided opportunities to sample plankton assemblages and to discover the previously unknown N_2-fixing species biodiversity (Zehr et al. 1998, 2000). More recently, the use of the polymerase chain reaction (PCR) and oligonucleotide probes designed to target the *nifH* gene have been used to detect N_2-fixing microorganisms and to assess the 'genetic potential' for N_2 fixation, whether or not N_2 fixation is actually occurring at the time of sampling (Kirshtein et al. 1993). Further development of these novel molecular methods may provide explicit links between the genetic potential and *in situ* rates of N_2 fixation in the sea (Zehr & Capone 1996; Zehr et al. 1996).

The first credible attempts to estimate rates of global ocean N_2 fixation resulted in a flux of 10–20 Tg N yr^{-1} (1 Tg = 10^{12} g) based primarily on the extrapolation of limited field measurements of N_2 fixation by *Trichodesmium* in the tropical Atlantic Ocean and Caribbean Sea (Capone & Carpenter 1982; Carpenter 1983) scaled globally using a historical data set for *Trichodesmium* abundance over larger portions of the world's oceans (Figure 4). A subsequent re-analysis of *Trichodesmium* abundance data for the tropical North Atlantic and Caribbean Sea increased this flux to 40–200 Tg N yr^{-1}, depending upon the regional boundaries that were selected (Carpenter & Romans 1991). Lipschultz and Owens (1996) provided a critical assessment of North Atlantic, basin-scale N_2 fixation rates. They concluded that the role of N_2 fixation, based on direct measurements of *Trichodesmium*, may have been overestimated and favor an estimate of approximately 15 Tg N yr^{-1}. Most recently, Capone and Carpenter (1999) have extrapolated average rates derived from field studies, which directly determined rates of N_2 fixation at diverse sites in the tropical oceans, across latitudinal bands adjusted for areas of upwelling and monsoonal periods. They derived a global estimate of about 80 Tg N per year, not accounting for input during bloom events. The primary limitation with the direct measurement approach is the spatial and temporal variability in N_2 fixation, relative to the measurement frequency. This makes the extrapolation of measured shipboard rates to regional and

basin scale estimates uncertain. Then there are also the usual sampling and incubation problems that have plagued biological oceanographers for more than a century. By comparison, indirect geochemical estimates suggest that rates of N_2 fixation in the North Atlantic Ocean are near the high end of the range of direct estimates (see section on '***The N* parameter***').

Most field studies of N_2 fixation have relied upon the acetylene reduction (AR) technique to estimate rates of N_2 reduction. Though indirect, the AR method is much more sensitive and much simpler than the alternative $^{15}N_2$ isotopic tracer method as traditionally applied (c.f. Montoya et al. 1996). Convenience and cost have generally dictated the choice of the AR method for most field studies. However, the advent of continuous flow isotope ratio mass spectrometry using multiple collectors has greatly improved the sensitivity of the $^{15}N_2$ uptake method, making it comparable to AR in some systems (Montoya et al. 1996). Zuckermann et al. (1997) have recently described a continuous on-line system for the measurement of AR using a laser-based photoacoustical detection of ethylene. This method is three orders of magnitude more sensitive than the standard gas chromatography-based AR method, but to our knowledge it has not yet been applied to field studies.

Two major assumptions must be made in the extrapolation of measured AR to rates of N_2 fixation: (1) selection of an appropriate C_2H_2 reduction to N_2 fixation stoichiometry and (2) extrapolation of nitrogenase enzyme activity (C_2H_2 reduction) to enzyme product (NH_4^+/amino acid) accumulation. Theoretically, three molecules of C_2H_2 are reduced for each N_2 molecule fixed (Stewart et al. 1967). However, empirical observations reveal significant deviations from theory, with C_2H_2:$^{15}N_2$ ratios ranging from 3.3:1 to 56:1 for samples collected from the North Pacific gyre (Mague et al. 1974). Furthermore, nitrogenase can produce H_2 from H_2O; this reaction accompanies N_2 reduction but not AR (Robson & Postgate 1980), so formation of H_2 can also affect the C_2H_2:N_2 reduction stoichiometry. Variation in the C_2H_2:N_2 stoichiometry may also be caused by failure of the second field assumption relating potential activity to actual *in vivo* product formation. If N_2-fixing microbial assemblages are nutrient (P, Fe, vitamin) limited then the potential rate for N_2 fixation, measured using the AR technique, may never be fully realized. Mague et al. (1974) demonstrated that the anomalously high C_2H_2:N_2 ratios ($>$ 10–20:1) returned to the theoretical value of 3:1 following the addition of excess phosphate. Only under balanced growth conditions, they reasoned, would the AR and $^{15}N_2$ methods be expected to yield comparable estimates of N_2 fixation in field samples. It might, therefore, be possible to use simultaneous measurements of AR and $^{15}N_2$ reduction to assess the degree of nutrient limitation in natural populations, although to our knowledge this approach has not yet been attempted.

^{15}N isotopic abundance as an indicator of N_2 fixation

Nitrogen exists naturally as two stable isotopes, ^{14}N (99.634% by atoms) and ^{15}N (0.366% by atoms). Because the $^{15}N/^{14}N$ ratios of natural materials vary only slightly, they are expressed in δ-notation, where $\delta^{15}N$ (‰) = $(((^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{standard} - 1) * 1000$; the universal reference standard is atmospheric N_2. The nitrogen isotopes can be used to study the marine N cycle by examination of natural variations in the $^{15}N/^{14}N$ ratio, or by addition of tracers that are artificially enriched in ^{15}N. We focus in this section on natural isotopic variations. This subject was reviewed comprehensively by Owens (1987), although the field has evolved significantly since that time.

Two factors control the $\delta^{15}N$ of a given N pool: (1) the $\delta^{15}N$ of its source and (2) isotopic fractionation associated with its production and loss (with enzymatic reactions typically favoring the conversion of ^{14}N-bearing substrates). Dissolved N_2, the substrate for marine microbial N_2 fixation typically has a $\delta^{15}N$ of ~0.6‰, close to the value expected from equilibrium with atmospheric N_2. Microbial N_2 fixation has a small isotopic fractionation, so that the organic-N produced by N_2 fixation is only slightly depleted in ^{15}N compared to its substrate, with a $\delta^{15}N$ of 0 to –1‰ relative to atmospheric N_2 (Wada 1980; Wada & Hattori 1991; Carpenter et al. 1997). Mean oceanic nitrate, the largest reservoir of fixed N, has a $\delta^{15}N$ of ~5‰ (Sigman et al. 1997, 1999, 2000), so N_2 fixation should be discernible in marine systems as a source of ^{15}N-depleted N, providing a potential constraint on the relative importance of N_2 fixation in open ocean environments. Using the low $\delta^{15}N$ of newly fixed N_2, one could potentially trace its path through each of the important N pools, from its origin in the particulate (and dissolved) organic-N of the surface ocean, to its export from the euphotic zone as sinking particulate N, finally to its oxidation to nitrate in the underlying thermocline.

The $\delta^{15}N$ of particulate N in surface waters of oligotrophic basins tends to be low, consistent with a significant contribution of newly fixed N_2 to this pool (Saino & Hattori 1980, 1987). However, the isotopic effect of N recycling represents a competing alternative explanation for this low $\delta^{15}N$ (Checkley & Miller 1989; Altabet 1988). Zooplankton appear to release ammonium which has a lower $\delta^{15}N$ than their food source, making their tissues and solid wastes ~3‰ higher in $\delta^{15}N$ than their food source. The low-$\delta^{15}N$ ammonium is consumed by phytoplankton and thus retained in the surface ocean N pool, while the ^{15}N-enriched particulate N is preferentially exported, potentially leading to a lower $\delta^{15}N$ of surface particulate N in environments where recycled N is an important component of the gross N supply to phytoplankton. The low $\delta^{15}N$ values observed in suspended particulate N from Antarctica and other high latitude regions probably cannot be attributed to N_2 fixation, and thus are most likely due to N recycling. However, in the low-latitude, low-

nutrient ocean surface, such as the Sargasso Sea and western tropical Pacific Ocean, the relative importance of N_2 fixation and N recycling in producing ^{15}N-depleted surface particles is uncertain.

One potential approach to discern the N isotopic effects of N_2 fixation and recycling on suspended particles is the coincident measurement of C isotopic composition. *Trichodesmium* has the highest $\delta^{13}C$ content of any phytoplankton species (–12.9‰, compared to –20 to –22‰ for most others) and the lowest $\delta^{15}N$, as described above. Thus, Carpenter et al. (1997) suggest that dual isotopic tracer measurements of marine particulate organic matter may provide an unique tracer for *Trichodesmium* and of heterotrophs that incorporate their organic matter. Of course, the carbon isotopes will not track the newly fixed N of *Trichodesmium* through its subsequent reincorporation by other autotrophic plankton.

While N recycling can result in a decrease in $\delta^{15}N$ of the suspended particulate N pool due to the preferential export of ^{15}N-enriched material, only N_2 fixation represents a true source of ^{15}N-depleted fixed N. For this reason, the development of an annually integrated N isotope budget for a region of the ocean surface (that is, the study of fluxes rather than pools) should reveal whether N_2 fixation is an important component of the N budget, regardless of N recycling. This approach has been taken for the BATS region in the North Atlantic (Altabet 1988) and the HOT station in the North Pacific (Karl et al. 1997) using $\delta^{15}N$ data for the N sinking flux and assuming that: (1) fixed N input to the surface ocean is either as upwelled nitrate or via N_2 fixation and (2) the total export is well represented by the sinking particulate N collected in sediment traps. Altabet (1988) found no need for a N_2 fixation term to close the isotope budget at BATS, although subsequent changes in our understanding of the Sargasso Sea and in sediment trap processing methods may require a re-analysis of that budget.

By contrast, Karl et al. (1997) required a 25–50% contribution of newly fixed N_2 at Sta. ALOHA (22°45′N, 158°W), in agreement with other measures of N_2 fixation at that North Pacific subtropical gyre site. Karl et al. (1997) also observed a seasonal change in the $\delta^{15}N$ of sedimenting organic matter indicative of a systematic seasonal alternation between predominantly NO_3^- supported export production in winter, and predominantly N_2 supported export production during the more stratified summer period, although N_2 fixation was detected throughout the year. These sediment trap-based studies have, by necessity, ignored certain aspects of the N budget that might also be significant, including: (1) DON and its associated horizontal and vertical N fluxes, (2) atmospheric fixed N inputs and (3) N fluxes associated with zooplankton migration. As these terms are added, N isotope budgets will

provide an increasingly rigorous constraint on the role of N_2 fixation in the N nutrition of the oligotrophic surface ocean.

Much of the sinking flux that exports fixed N from the surface ocean is oxidized to nitrate at thermocline depths. Thus, in regions where significant N_2 fixation occurs, newly fixed N is exported from the euphotic zone and should appear as ^{15}N-depleted nitrate in shallow subsurface waters. In the absence of N_2 fixation, since nitrate consumption is complete in oligotrophic surface waters, the δ^{15}N of the N export will converge on the δ^{15}N of the nitrate supply from the subsurface, so that the 'normal' pathway of N assimilation and dissimilation should not cause the δ^{15}N of nitrate to change from its 'preformed' value of $\sim$5‰. Thus, the δ^{15}N of nitrate in the thermocline should provide a measure of the addition of nitrate to the thermocline from the oxidation of newly fixed N_2. Combining such data with transient geochemical tracers should allow for the calculation of integrated (in time and in space) rates of N_2 fixation.

Liu et al. (1996) measured the N isotopic composition of nitrate in thermocline waters of the Kuroshio near Taiwan. Compared to the δ^{15}N measured for nitrate in deeper waters (+5.5 to 6.1‰ at 500–780 m), the δ^{15}N of nitrate in the overlying thermocline water was 1–3‰, indicating the input of ^{15}N-depleted N, probably from the oxidation of newly fixed N_2. The authors conclude that 40 $\pm$ 15% of the subeuphotic zone nitrate pool was derived from local N_2 fixation (Liu et al. 1996).

Similarly, Brandes et al. (1998) documented a pool of isotopically light nitrate in the thermocline waters of the central Arabian Sea. Based on a simple vertical mixing model, the authors concluded that 40% of the nitrate at 80 m was derived from local N_2 fixation. In the Arabian Sea, the N_2 fixation signal is complicated by local denitrification (Brandes et al. 1998). However, these two processes are theoretically separable if the isotope data are combined with $[NO_3^-]/[PO_4^{3-}]$ ratio data (see following section).

Finally, the δ^{15}N of nitrate also shows an upward decrease across the thermocline of Sargasso Sea in the oligotrophic North Atlantic, with δ^{15}N values decreasing from 5‰ at 800 m to 2.3‰ at 300 m (D. Sigman, unpublished data; Figure 5). As in the studies described above, this pattern can be interpreted as indicating the thermocline-depth nitrification of newly fixed N_2. Thermocline ventilation in the North Atlantic subtropical gyre is conducive to the calculation of a rate for processes that alter the chemistry of the thermocline. This approach has been used for estimation of N_2 fixation rates on the basis of $[NO_3^-]/[PO_4^{3-}]$ ratio data (Gruber & Sarmiento 1997; see following section), and the isotope data (Figure 5) imply that nitrate δ^{15}N data could be used for the same purpose.

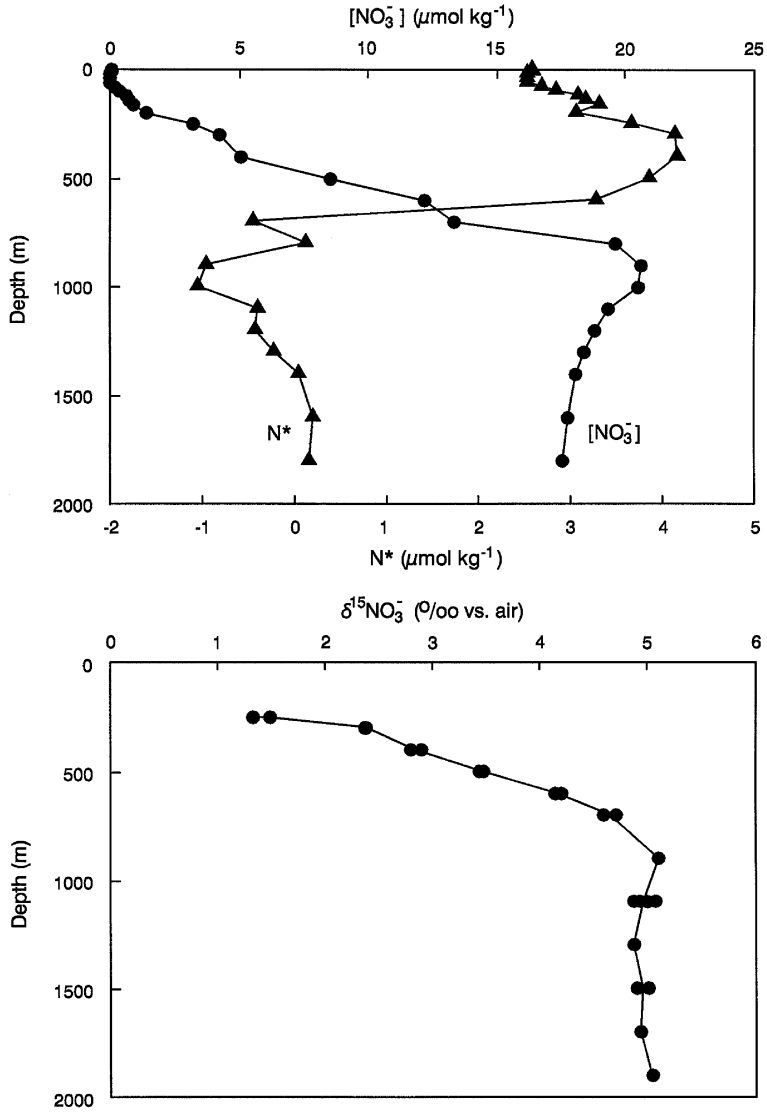


Figure 5. Depth profiles at the Bermuda Atlantic Time-series Study (BATS) station of (top) nitrate concentration and N* (see text), and (bottom) the δ^{15}N of nitrate. The data in (top) were collected as part of the BATS program and the data in (bottom) are from D. Sigman (unpublished results). Individual nitrogen isotope analyses are plotted as filled circles, and the trend line passes the mean value for replicate samples at each depth. The nitrate δ^{15}N below 800 m is close to the oceanic mean value of 5‰. The ^{15}N/^{14}N decrease toward the surface requires the addition of ^{14}N-rich nitrate to the upper water column, such as would result from the nitrification of newly fixed N. The nitrate δ^{15}N is lowest in the shallow thermocline, where the N* data suggest that the addition of newly fixed N is greatest in proportion to the nitrate concentration.

The N parameter*

In a recent report, Michaels et al. (1996) concluded that rates of N_2 fixation between 10–40°N latitude in the North Atlantic Ocean are on the order of 50–90 Tg N yr^{-1}. This value, for one of the Earth's smallest ocean basins, exceeds most previous estimates of *global* ocean N_2 fixation (Figure 4). Their estimate was based on an assessment of the patterns in the ratio of N to P in the upper thermocline, and on nutrient fluxes along isopycnal surfaces in the North Atlantic. Data were also presented for seasonal and interannual variability in ecosystem dynamics; the reported temporal variability makes a mass balance approach like this much more difficult to achieve. Despite these limitations, the authors clearly documented elevated ratios of nitrate-to-phosphate in the main thermocline of the central North Atlantic gyre, the Sargasso Sea, compared to surrounding waters. This had been noted previously by Fanning (1989, 1992), but to date, not fully explained. Michaels et al. (1996) went on to derive an anomaly parameter, which they called N*, as the concentration of N, in excess or in deficit of P, relative to the Redfield stoichiometry of 16N:1P (i.e. $N^* = [NO_3^-] - 16[PO_4^{3-}] + 2.72$). The constant, 2.72, was chosen to set the global ocean mean N* to zero, and implies that in the contemporary ocean, denitrification exceeds N_2 fixation. For samples collected in the upper 800 m of the Sargasso Sea, both near Bermuda and to the southeast in the core of the North Atlantic subtropical gyre, N* exceeds 2.72 implying net N_2 fixation. From independent information on the ventilation time-scale of each isopycnal surface, the N* inventory was extrapolated to an average rate of N_2 fixation, the integral of which was equivalent to 3.4–6.1 $\times$ 10^{12} moles excess nitrate yr^{-1}. They equated this nitrate excess to the net rate of N_2 fixation (i.e. gross N_2 fixation less any denitrification in the same water masses). Thus, gross N_2 fixation rates, as might be extrapolated from direct field measurements, could be even higher.

These larger than anticipated rates of N_2 fixation for the Sargasso Sea suggested that N_2 may be a major source of new N in this low nutrient habitat, accounting for $> 50\%$ of the annual particulate N export measured using free-drifting sediment traps. This mostly summertime input of fixed N in the absence of physical mixing processes is consistent, both in process and in amount, with the enigmatic summertime drawdown (net removal) of dissolved inorganic carbon that occurs in these waters in the apparent absence of new nutrients (Michaels et al. 1994). Consequently, this geochemical anomaly-based estimate of the rate of N_2 fixation appears robust and consistent with certain other complementary field observations.

Gruber and Sarmiento (1997) have taken this nutrient anomaly approach much further. In their more comprehensive treatment of the N* parameter, they redefined N* as a linear combination of NO_3^- and PO_4^{3-} ($N^* = 0.87$

$[NO_3]$ – 16 $[PO_4^{3-}]$ + 2.90 μmol kg^{-1}). As in the report by Michaels et al. (1996), positive N* values, they reasoned, would indicate regions where excess N, relative to P, had been regenerated. Their analyses concluded that the tropical and subtropical North Atlantic Ocean and the Mediterranean Sea were major sources of N, via N_2 fixation, whereas net N* in the subtropical gyre of the North Pacific Ocean was more often than not zero (Gruber & Sarmiento 1997). Gruber and Sarmiento (1997) calculated that the rate of N_2 fixation in the North Atlantic Ocean between 0°N and 50°N was 28 Tg N yr^{-1}, a value that is 2–3 times lower than that estimated by Michaels et al. (1996). However, 28 Tg N yr^{-1} is still larger than earlier estimates for the global ocean (Figure 4). They use a variety of assumptions to extrapolate N* to the global ocean, and derive an estimate of 110 Tg N yr^{-1}.

Deutsch et al. (2001) present a fixed N budget for the Pacific Ocean based on the recently completed World Ocean Circulation Experiment (WOCE) hydrographic data set. Using water mass age tracers, estimates of atmospheric deposition and riverine fluxes and N* calculations, they estimate rates of both denitrification and N_2 fixation assuming steady-state conditions. To achieve mass balance, they conclude that the N_2 fixation in the Pacific, north of 32°S is 59 $\pm$ 14 Tg N yr^{-1}. Based on the N* signals, N_2 fixation was enhanced in the western portion of the subtropical gyres (Figure 6), a result that is consistent with the hypothesis that iron supplied to the ocean via atmospheric dust deposition may be an important control.

Like all other indirect methods for estimating rates of N_2 fixation, the N* method has its unique limitations. First, it assesses net N_2 fixation not gross N_2 fixation. If a given habitat supports simultaneous N_2 fixation and denitrification of comparable rates, then N_2 fixation by this nutrient anomaly method would not be detected. The large areas of denitrification in the eastern tropical Pacific may be balanced by high rates of N_2 fixation elsewhere in the Pacific Ocean basin. There are strong gradients in N* across the Pacific, and the maintenance of these gradients implies a source of new nitrate to balance the denitrification in the east. This is an important limitation of the N* approach because net N_2 fixation 'neutral' ecosystems may function differently from those where N_2 fixation is truly absent.

The rates of N_2 fixation derived from N* are also dependent upon the assumption that diazotrophs produce biomass with an N:P ratio greater than the Redfield ratio (see '***N and P nutrient control***' section), thus accounting for the N excess regeneration anomaly. Furthermore, the rate of N_2 fixation derived from N* is highly sensitive to the reference organic matter N:P ratio that is selected. For instance, Gruber and Sarmiento (1997) used a molar value of 125N:1P derived from Karl et al. (1992). This is a relatively extreme bloom value, compared to more modest estimates for the N:P of *Trichodesmium*

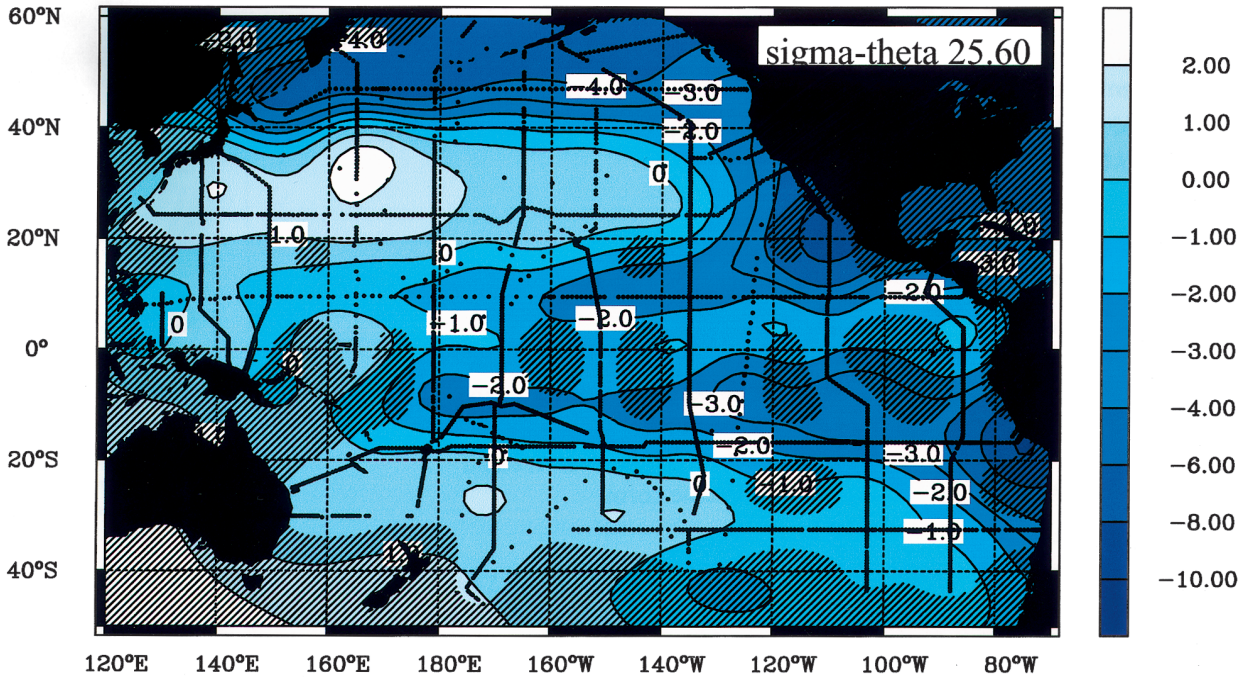


Figure 6. Map of N* (μmol kg^{-1}) in the thermocline on the sigma-theta = 25.6 isopycnal surface (located at approximately 200–300 m in central gyres shoaling to about 100 m in the east). This map was prepared using the objective mapping technique described by LeTraon (1990). Dashed areas indicate regions where the interpolation error in N* from this objective mapping procedure is > 20%. From: Deutsch et al. (2001).

which are in the range of 40 to 50 (Letelier & Karl 1996, 1998). As the assumed N:P of diazotroph biomass decreases to the canonical Redfield ratio of 16N:1P, the N_2 fixation rate derived from N^* increases to infinity (see Figure 18 in Gruber & Sarmiento 1997). Therefore the N:P ratio assumption alone could more than account for much of the difference between the estimates of Michaels et al. (1996) and Gruber and Sarmiento (1997). Finally, by ignoring the dissolved organic matter (DON and DOP) pools one cannot accurately determine the true N:P stoichiometry of the dissolved nutrient pools. Despite these well founded criticisms, the N^* parameter appears to be a robust qualitative, if not quantitative, indicator of the contribution of net N_2 fixation to the regional scale oceanic N cycle. It certainly has opened up a broader range of possibilities for the global scale of this process and its pattern across basins and with depth.

N:P stoichiometry of the suspended and exported particulate matter pools

There remains a major misconception about the stoichiometry of dissolved and particulate matter pools in the sea; more often that not, ambient pools have a N:P molar stoichiometry that deviates significantly from the 'expected' Redfield ratio of 16N:1P (Duarte 1992; Hecky et al. 1993). N_2 fixation is one of two major microbiological processes (the other being denitrification) that can influence oceanic N:P stoichiometry on global scales. In contrast to N_2 fixation, there is no comparable gas-phase to the phosphorus (P) cycle. Thus, N_2 fixation will either lead to variations in N:P stoichiometry or P supply will limit biological activity, or both.

At Sta. ALOHA in the oligotrophic North Pacific Ocean, the deviations from the nominal 16:1 N:P stoichiometry (Redfield ratio) are particularly intriguing (Figure 7). During the first two years of the HOT program, the mean N:P ratio for suspended particulate matter in the upper (0–100 m) water column was 15.3 (standard deviation [s.d.] = 3.1, n = 14), a value that was not significantly different from the Redfield prediction of 16.0 (Figure 7). Since 1991, however, there has been an increase in the molar N:P ratio of suspended particulate matter to a value greater than the expected Redfield stoichiometry (Figure 7). There is also much greater temporal variability and a greater overall range. Karl et al. (1997) suggested that the ecosystem N:P stoichiometry drifts out of a Redfield balance en route to phosphorus limitation as the supply of new N shifts from a limiting flux of nitrate from below the euphotic zone to the nearly inexhaustible pool of N_2 that is dissolved in the surface waters of the ocean. This shift to N_2 supported new and export production has significant consequences for biogeochemical cycling pathways and rates. The coherent temporal pattern observed for suspended N:P, with maxima in the summer periods, is consistent with relatively enhanced

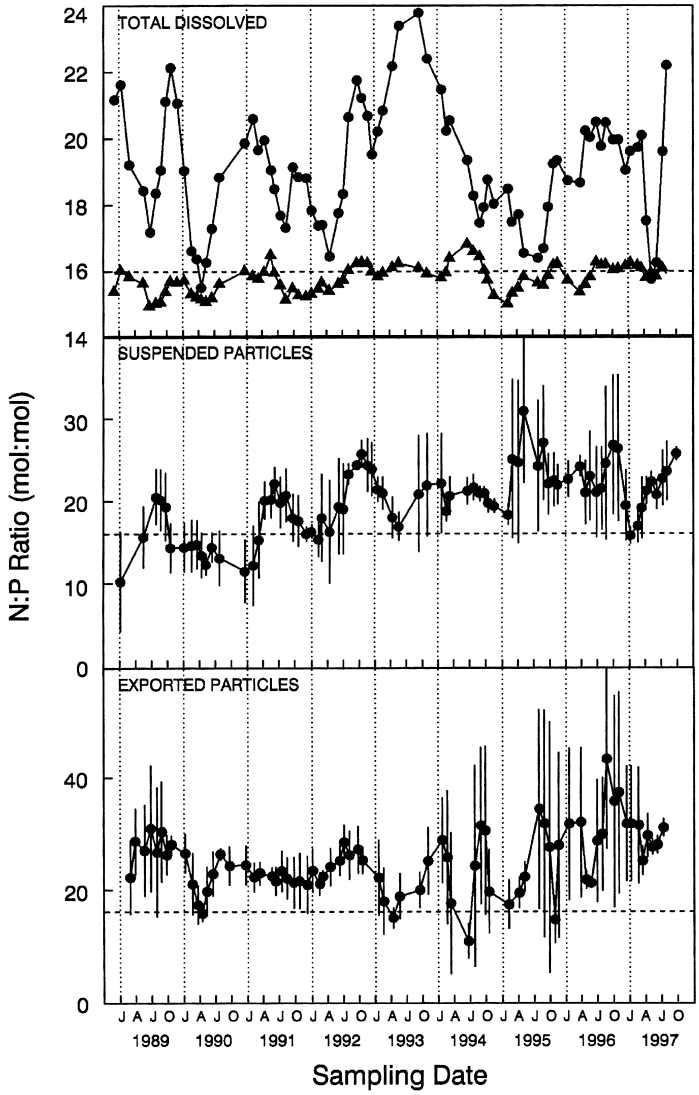


Figure 7. Time series of N and P analyses of dissolved and particulate matter, presented as N:P (mol:mol) ratios, for [TOP] dissolved matter, [CENTER] suspended particulate matter, and [BOTTOM] exported particulate matter. The top panel shows the 3-point running mean N:P ratios for 0–100 m (●) and 200–500 m (▲) portions of the water column. The center panel shows the 3-point running mean (±1 SD) for the average suspended particulate N:P ratio measured in the upper portion (0–100 m) of the water column on each cruise (depth-integrated particulate N ÷ depth-integrated particulate P). The bottom panel shows the 3-point running mean (± 1 SD) for the average N:P ratio of the sediment trap-collected particulate matter at the 150-m reference depth. The Redfield ratio (N:P = 16) is represented by a dashed line in all three panels. From Karl et al. (1997).

bioavailability of N. This most likely results from increased rates of N_2 fixation during periods of maximum water-column stratification when N_2 fixation is also likely to be greater.

The anomalously high N:P stoichiometry (> 16:1) of exported particulate matter at Sta. ALOHA (Figure 7) confirms an important prediction of the N_2 supported new production hypothesis model. The temporal variability of the exported matter N:P ratio, with lower, near Redfield ratio values in late winter and elevated (> 20:1) ratios throughout the remainder of the year is consistent with the previously mentioned seasonal model for an alternation between NO_3^- supported and N_2 supported new production (see '***^{15}N isotope abundance as an indicator of N_2 fixation***' section). The generally increasing trend in both the suspended particulate matter and the exported particulate matter N:P ratios with time, corresponding with a generally decreasing trend in bioavailable P (Karl et al. 1997), are strong independent lines of evidence for the role of N_2 fixation at this site. Based on a simple mass balance model, N_2 fixation at Sta. ALOHA supplied at least 32% of the new N for the period 1989–1995, with significant seasonal and interannual variability (Karl et al. 1997).

Karl et al. (1997, 2001) have also emphasized that the contemporary role of N_2 fixation in the marine N cycle at Sta. ALOHA must be greater than the recent past. The subeuphotic zone waters where the relatively high N:P exported matter is regenerated has not yet achieved the equilibrium N:P value that would be expected under steady-state export (Figure 7; Karl et al. 2001). A major implication of these data trends is that the N^* parameter is a time-variable quantity that may have increased significantly over the past several decades. These potentially dramatic changes in microbial community structure (selection for N_2-fixing prokaryotes) and rates and mechanisms of nutrient cycling may be related to large scale ocean-atmosphere interactions including, but not limited to, a change beginning in 1976 towards more frequent El Niño and fewer La Niña events (Trenberth & Hoar 1997; Karl 1999). In the Atlantic, significant variations in the total iron supply to the Sargasso Sea, caused by changes in the size of the Saharan desert, also imply a dramatic increase in N_2 fixation rates over the past four decades (Michaels et al. 1996). Clearly if the ocean is variable in time, or is exhibiting secular changes in response to climate forcing, it may be misleading to use historical data sets to map present-day conditions or to predict future trends. The dynamic, non-steady state behavior of the Pacific and, probably, Atlantic and Indian Oceans provides an ideal testing ground for the time-dependent behaviors of the hypothesized effects of climate variability on oceanic N_2 fixation (see '***The N_2 fixation-climate feedback hypothesis***' section).

Remote sensing of N_2 fixation

During the past two decades the development of novel ocean observation platforms, including instrumented ocean buoys and drifters and Earth-orbiting satellites, has improved our ability for continuous, large spatial scale surveillance of the world's oceans (Dickey 1991). These data, and in particular satellite remote sensing of ocean color, have revealed the presence of fairly coherent biogeochemical provinces characterized by relatively small horizontal gradients and well-defined boundaries (Longhurst 1998; Platt & Sathyendranath 1999). The presence of these biogeochemical biomes will undoubtedly facilitate regional and global-scale extrapolation of key ecological processes once the interprovince properties are reasonably well understood. At the very least, algorithms based on sea surface temperature, pigment content, wind and dynamic topography – all currently measured from space – could be used to help constrain rates of global ocean N_2 fixation.

It has previously been suggested that periods of calm seas (e.g. low wind, low turbulent mixing rates, low surface wave activity) favor *Trichodesmium* bloom formation (Carpenter & Price 1976). Karl et al. (1992) showed that when the North Pacific gyre sea state is low, there is a significant (> 1 °C) diurnal warming and cooling of the sea surface temperature. It is now possible to monitor sea surface temperature changes of this magnitude using satellite-based Advanced Very High Resolution Radiometer (AVHRR) sensors and these data could, in principle, reveal 'N_2 fixation-probable' regions of the ocean, especially the central gyres. Deployment of satellite-linked moored or free-drifting ocean buoys with thermistor chains, light and fluorescence probes and nutrient sensors could serve to ground-truth the basin-scale synoptic satellite view at key locations within each biome. Over time, an empirical predictive model of N_2 fixation rates might evolve.

When *Trichodesmium* colonies accumulate at the sea surface under calm water conditions, they are clearly visible from space. Dupouy et al. (1988) were the first to present data on this phenomenon based on a Nimbus-7 Coastal Zone Color Scanner (CZCS) image of a 90,000 km^2 *Trichodesmium* bloom near New Caledonia in the southwest Pacific Ocean. Although there was no ground-truth of this image, the CZCS spectral signature was presented as supporting evidence. They estimated that this single bloom could fix 7.2×10^9 g N in 10 days.

Borstad et al. (1992) and Dupouy (1992) later developed spectral-reflectance models applicable for surface ocean *Trichodesmium* blooms observed by the CZCS imager. At moderate colony densities, *Trichodesmium* and other cyanobacteria should be distinguishable from diatoms and dinoflagellates, provided high resolution spectral data are available (Borstad et al. 1992). Continued development of a *Trichodesmium*-specific remote

sensing algorithm relied upon two unique physiological characteristics: (1) the presence of gas vacuoles and (2) the presence of the accessory pigment phycoerythrin (Subramaniam & Carpenter 1994). The former results in high reflectivity and the latter in a specific absorption of light at 550 nm. These two independent parameters can be used to distinguish *Trichodesmium* from most other marine phytoplankton. Further refinements in the reflectance model based on field measurements of the inherent optical properties of *Trichodesmium* colonies collected from the Caribbean Sea (Subramaniam et al. 1999a, b) currently provide a sophisticated empirical expression of surface ocean *Trichodesmium* blooms. Application of this optical model using visible and near-infrared sensors of the NOAA-12 AVHRR satellite mapped the near surface *Trichodesmium* distributions in the central Arabian Sea off the Somali coast in 1995; a major bloom that was observed was also intercepted by the R/V *Malcolm Baldridge* so ground truth data were available (Subramaniam et al. 1999a, b). In addition to the AVHRR imager, Tassan (1995) suggested that the Sea-viewing Wide Field-of-view Sensor (SeaWIFS) ocean color satellite might also be useful for detecting *Trichodesmium* at low, sub-bloom concentrations in open ocean habitats, but to our knowledge these model predictions have not yet been verified with field data. The utility of SeaWIFS, however, was verified during a recent series of *Trichodesmium* blooms in the Melanesian Archipelago. These blooms were both mapped by SeaWIFS imagery and sampled as part of the NSF-NASA Sensor Intercomparison and Merger for Biological and Interdisciplinary Ocean Studies (SIMBIOS) expedition in April 1998 (Dupouy et al. 2000).

Despite these successes, a major limitation with any remote ocean sensing application is the uncertainty in relating the surface ocean conditions of phytoplankton assemblage pigmentation or reflectance to surface ocean biomass and total euphotic zone-integrated population inventories. This would be a much more difficult task for relating the presence of a target N_2-fixing microorganism (i.e. *Trichodesmium*) to the *in situ* rate of N_2 fixation. Furthermore, ocean color imagery will not detect chemoautotrophic or chemoheterotrophic N_2-fixing *Bacteria* or *Archaea*; to the extent that they are important to the N budget, global N_2 fixation rates will be underestimated by the use of these remote sensing methods. Finally, N_2 fixation probably occurs in mid-ocean gyres throughout the year (Karl et al. 1997), so methods based simply on interrogation of sea surface blooms will have a built-in alias that is difficult to quantify. Regardless of their potential, it is simply impossible to conduct microbial ecology from space; however, remote sensing methods are likely to prove invaluable as a complementary approach to traditional ship-based investigations.

Human perturbations and climate variability: effects on oceanic N_2 fixation

Our current estimate of global ocean N_2 fixation (100–200 Tg N yr^{-1}) is similar to the rate of terrestrial N_2 fixation (estimated to be 90–130 Tg N yr^{-1}; Galloway et al. 1995), in the absence of human activities. However, the contemporary rate of terrestrial N_2 fixation is more than double this pre-industrial rate as a result of legume cultivation, energy demands and fertilizer production. As anthropogenic mobilization of N intensifies, fixed N fluxes to coastal and open oceans will likely increase, especially relative to P mobilization. This could impact contemporaneous rates of oceanic microbiological N_2 fixation, and could exacerbate N and P decoupling in open ocean habitats.

Oceanic areas of enhanced N_2 fixation are localized in the subtropical gyres and tropical seas, especially the tropical Atlantic, western Pacific and tropical Indian Oceans. Each of these regions is downwind of a major area of dust production, the Saharan Desert/Sahel, the Gobi Desert and the deserts bounding the Arabian Sea, respectively. For the Atlantic Ocean, the flux of atmospheric dust-derived Fe is comparable to that required to sustain the recent estimates of N_2 fixation in that ocean basin given our present understanding of the Fe requirements of *Trichodesmium* (Michaels et al. 1996). However, the current dust load is nearly four-fold higher than before the expansion of the Saharan desert in the early 1970s (Prospero & Nees 1986; Prospero et al. 1996). This fact alone suggests that contemporary rates of N_2 fixation in the North Atlantic Ocean may have been recently enhanced. On the other hand, human activity is presently causing a reduction in the dust plume from the Gobi desert as a result of an aggressive reforestation effort. However, we have no direct evidence that this has yet impacted N_2 fixation in the North Pacific Ocean.

In addition to anthropogenic influences on the fluxes of desert dust to the world's oceans, the natural climate system also causes large temporal variations. For example, marine sediment and ice core data both suggest that dust deposition was 2–20 times higher during the last glacial maximum than it is currently (Rea 1994; Cragin et al. 1977; compilation in Mahowald et al. 1999). These changes in dust deposition appear to be caused by changes in total global desert source area and atmospheric transport patterns (Joussaume 1993). During the current climate, the desert dust source areas lie mostly in subtropical regions (Husar et al. 1997). In the last glacial maximum, pollen and loess studies suggest that desert regions in mid- and high latitude Asia, North America and South America were significantly larger in extent (Liu et al. 1985; Beget 1996; Prentice & Webb 1998).

The historical imbalance between global oceanic N_2 fixation and denitrification is potentially sustained by anthropogenically-fixed N that is delivered to coastal and open ocean environments (currently estimated to be ~59 Tg N yr^{-1}; Galloway et al. 1995). If N_2 fixation rates in the sea have been historically underestimated, as now appears to be the case (Figure 4), then there may well be a pool of 'missing N' or an additional sink for fixed N in the global ocean. Not unrelated to these considerations is the increasing burden of N_2O in the global atmosphere, and the role of the open ocean as a previously unrecognized source of N_2O. Dore et al. (1998) have recently suggested, based on dual ^{15}N and ^{18}O measurements of N_2O in the North Pacific Ocean, that bacterial nitrification rather than denitrification may be a major source for atmospheric N_2O. Nitrification is stimulated by N_2 fixation and the intensified flux of NH_4^+ to NO_3^- in the surface ocean. It now appears that both N_2 fixation and N_2O production may be linked to similar climate variables, such as dust deposition.

N_2 fixation and atmospheric CO_2

The oceans are both a source and a sink for atmospheric CO_2 and, on average, they are thought to absorb about 1–2 Gt C yr^{-1} (Tans et al. 1990; Siegenthaler & Sarmiento 1993; Takahashi et al. 1997). This uptake is a result of a combination of physical and biological processes. The physical processes (the solubility pump), involve the interaction of ocean circulation, the direct thermal effects on pCO_2 and the steady increase in atmospheric CO_2 over the past two centuries. Most of the global C models focus on the solubility pump because it is the one process where there is a clear mechanism leading to oceanic uptake of CO_2 in response to fossil fuel emissions. The biological pump (Longhurst & Harrison 1989) is less well understood and generally less well defined in global models. In its simplest form, surface organisms consume available nutrients and transport them to midwater depths via sinking particles or the mixing of dissolved organic matter. This surface drawdown of nutrients causes a depletion of total C in the surface waters and a concomitant decrease in pCO_2. Subsequent mixing re-introduces nutrients and C to the surface waters and, with simple stoichiometric assumptions, this balance results in little subsequent uptake of CO_2 as long as the mean surface nutrient concentrations remain the same.

N_2 fixation brings a new dimension to the ocean uptake of CO_2. On short time-scales, it adds a gaseous component to the N cycle. The creation of new reactive N in the euphotic zone and its potential to support a downward flux of C will be in excess of the upward fluxes of C by mixing. This should lower pCO_2 locally and sequester C on the time-scale of the ventilation of those waters. This mechanism is further accentuated by the relatively high ratios of

C:P and N:P in marine diazotrophs as evidenced by the anomalous dissolved nutrient ratios in areas of high N_2 fixation (see '***The N* parameter***' section). At an estimated rate of global ocean N_2 fixation of 100–200 Tg N yr^{-1} and a median C:N ratio of 11:1 for remineralization (as estimated from Sargasso Sea data sets), the annual amount of C transport could be about 1–2 Gt C yr^{-1}. Interannual fluctuations in N_2 fixation, or trends due to changing global climate, could be large enough to complicate interpretation of the record of changing atmospheric CO_2. As long as this nitrate remains in the ocean and the surface oceans remain depleted in nitrate, it will continue to sequester carbon in the deep sea. When denitrification removes the nitrate from the water, the subsequent ventilation of that water will result in an outgassing of the, now excess, CO_2 to the atmosphere.

The N_2 fixation-climate feedback hypothesis

On millennial time-scales, any imbalance between N_2 fixation and denitrification will change the total NO_3^- stock of the oceans (McElroy 1983; Codispoti 1989; Falkowski 1997). Increases in total oceanic NO_3^- should sequester C in the deep sea, provided bioavailable P is present and that N:P ratios of organisms can vary within narrow bounds. Decreases in oceanic NO_3^- should cause a gradual release of C to the atmosphere. If climate variations affect both N_2 fixation and denitrification on these time-scales, one might expect an increased dynamic amplitude in these coupled processes and the potential for both positive and negative feedback loops (Michaels et al. 2001).

The hypothesized feedback mechanism will have the following component parts (Michaels et al. 2001; Figure 8): the rate of N_2 fixation in the world's oceans, balanced against the denitrification rate, can have an impact on the concentration of the greenhouse gas, CO_2, in the atmosphere on time-scales of decades (variability in surface biogeochemistry) to millennia (changes in the total NO_3^- stock from the balance of N_2 fixation and denitrification); CO_2 concentrations in the atmosphere will influence the climate on the longer time-scales; and the climate system, in turn, can influence the rate of N_2 fixation in the oceans by controlling the supply of Fe on dust, and by influencing stratification of the upper ocean which also promotes N_2 fixation. Humans have a direct role in the feedback cycle by their influence on dust production, through agriculture at the margins of deserts, and by our own production of CO_2 into the atmosphere. Because of the interaction of the various parts of this system, keyed around the unique behavior and biogeochemistry of the prokaryotic microorganisms that can fix N_2, this feedback loop should exhibit complex behaviors on a variety of time-scales.

From a modeling perspective, the coupled N_2 fixation-climate hypothesis can be segregated by timescale. On interannual to decadal scales, the interac-

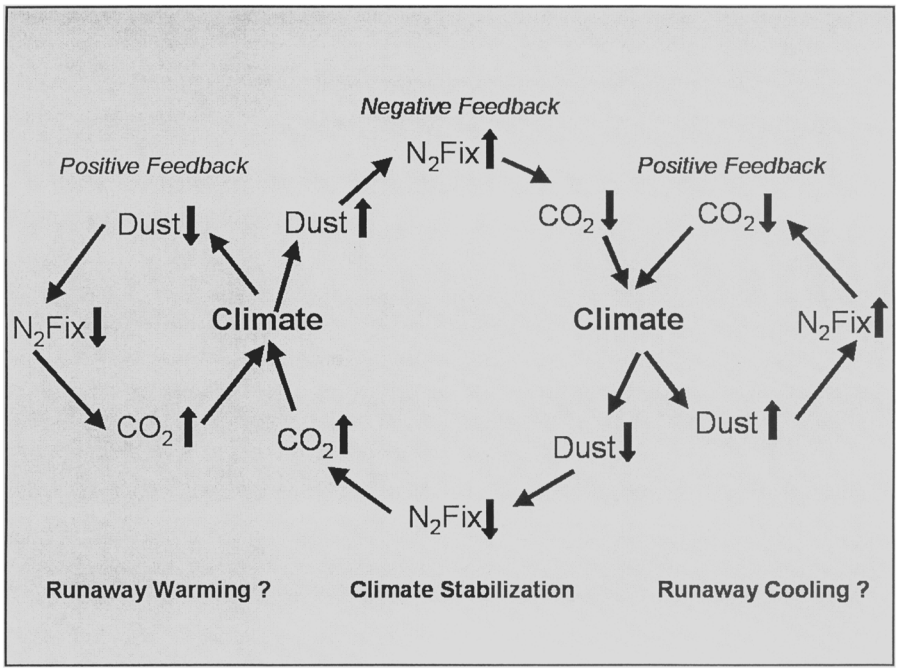


Figure 8. Schematic representation of potential global scale feedbacks between climate and N_2 fixation. Shown are key hypothesized roles of dust deposition as a positive effector for N_2 fixation and the crucial role of N_2 fixation in the potential for global ocean carbon sequestration (redrawn from Michaels et al. 2001).

tions among Fe deposition, climate (mostly ocean surface stratification) and N_2 fixation will be expressed as changes in the rates and community structure of marine ecosystems and will be reflected in the regional and global net air-sea exchange of CO_2. The relatively small resultant changes in oceanic N inventory and atmospheric CO_2 over these time periods do not have strong direct feedbacks on climate, simplifying the problem considerably. Nonlinearities in the dynamics of dust supply, bioavailable Fe release, diazotroph growth rates, bloom dynamics and export/remineralization processes will provide complex model outputs. However, these should still fall within some simpler bounds, namely: more Fe leads to more N_2 fixation leads to more C sequestration, and the inverse. Although denitrification rates may also vary on these time scales, the majority of that process occurs at depth. Thus, the resulting outgassing of CO_2 will be averaged over a longer time scale. The variability in the net impact will be dominated by the variability in N_2 fixation.

On millennial time scales, the changes in the total nitrate stock of the ocean are controlled by the balance of N_2 fixation and denitrification. Here

the climate feedbacks will reach the full range of possible outcomes. If the relationship between high CO_2 and dust is positive, then a negative, stabilizing feedback will result (Michaels et al. 2001; Figure 8). If the converse relationship exists, then a positive feedback will drive the system towards either very low or very high CO_2 levels. In this case, some other process would have to temper the feedback, perhaps an interaction with the total availability of nitrate.

These processes can be studied in the existing framework of uncoupled and coupled ocean general circulation models (GCMs) and atmosphere-land surface models by incorporating the required dust and marine biogeochemical dynamics. The models, with full feedback dynamics, will undoubtedly reveal a variety of complex dynamics (in the mathematical sense of the term), but they may also be able to determine the role of this hypothesized feedback system in our global climate.

Summary and future prospectus

For nearly one hundred years oceanographers have studied the interactions between the photosynthetic production of organic matter and nutrient dynamics in the sea. Classical research efforts by H.W. Harvey, L.H.N. Cooper, A.C. Redfield and others established robust quantitative relationships between the nitrogen and phosphorus contents of phytoplankton cells in relationship to ambient nutrient levels. However, one unique feature of the coupled N-P cycles that has never been fully appreciated or quantified is the role of diazotrophy; the ability of certain microorganisms to use N_2 for cell metabolism and growth. N_2 fixation should 'force' marine ecosystems toward P-limitation.

Trichodesmium blooms are ubiquitous phenomena in tropical and subtropical oceanic waters and they are known to fix N_2 under *in situ* conditions. To date it has been difficult to quantify the importance of diazotrophy because of the stochastic nature of the blooms and, until recently, a lack of pure cultures for physiological studies. Recent budget estimates based upon seasonally- and interannually-averaged N imports to and exports from the epipelagic zone of the subtropical gyres of the North Atlantic and North Pacific Oceans suggest that diazotrophic production of fixed N may be an important source of new nitrogen for these open ocean biomes.

The revised estimates for the North Pacific subtropical gyre suggest that 30–50% of the N required to sustain particulate and dissolved matter export from the euphotic zone (the so-called 'new' N) is derived from N_2 fixation; the remainder is supplied by the vertical flux of NO_3^- from sub-euphotic zone waters. If these data extrapolations are verified by subsequent measurements,

then our present conceptual models of ocean ecosystems will need to be revised. In this regard, we need to fully document both the phylogenetic diversity of N_2-fixing marine microorganisms and understand the breadth of their metabolic strategies for survival in the sea.

Regardless of the apparent importance of N_2 fixation to the global ocean N cycle, it is essential to emphasize that the field observations currently available were not designed to derive global estimates of N_2 fixation. For this reason, and also because there is a lack of physiological research on marine diazotrophs made under controlled environmental conditions, it is still difficult to constrain global ocean N_2 fixation at the present time. With additional field observations on N_2 fixation we may be able to characterize statistically the temporal and spatial distribution of N_2 fixation in the world's ocean. Combining this characterization with the study of the biogeochemical signature of N_2 fixation (elemental stoichiometry, N^*, $\delta^{15}N$) will improve our current estimates and refine our predictions regarding the coupling of climate variability and oceanic N_2 fixation. However, the mechanistic understanding to predict the effect of global change in N_2 fixation will probably require experimental manipulation at different biological levels.

Both conceptually and ecologically, N_2 supported new production is fundamentally different from NO_3^- supported new production even though the two processes were considered together in the original new versus regenerated N model of Dugdale and Goering (1967). For open ocean ecosystems it now appears that N_2 fuels both organic matter production and 'excess' NH_4^+ (or dissolved organic N; Karl et al. 1992; Capone et al. 1994; Glibert & Bronk 1994) production; the latter is mostly regenerated to NO_3^- in the euphotic zone. Consequently the previous paradigm of NO_3^- uptake being equivalent to new production, and NH_4^+ being equivalent to regenerated production, must be replaced by the realization that N_2 supports the production of new organic matter and 'new' NH_4^+; surface ocean NO_3^- pools, on the other hand, are mostly locally 'regenerated' in oligotrophic oceanic habitats.

This antithetical conceptualization has significant implication both for the design and interpretation of field experiments, and for the survival strategies of the resident microbial populations. When NO_3^- enters the euphotic zone from below by vertical advection and diffusion, it is delivered with a suite of other required major (e.g. C, P and Si) and trace (e.g. Fe) elements in the proper stoichiometry to sustain biological activity (Karl 1999; Cullen et al. 2001). However the process of N_2 fixation serves to decouple export from new nutrient import, which can lead to changes in the elemental stoichiometry of surface-ocean particulate and dissolved organic matter and selection for or against certain groups of microorganisms (see Karl 1999). Significant rates of N_2-based new production would eventually result in severe P and,

perhaps, Si limitation because these vital nutrients are supplied from below. Furthermore, selective separation of the otherwise coupled N-P-Si cycles by vertically migrating microbial assemblages (Karl et al. 1992; Villareal et al. 1993, 1999) or positively buoyant particulate matter may further complicate these mass-balance considerations. These observations suggest that it may be inappropriate to assume that biogeochemical processes in open ocean ecosystems conform to the current new vs. regenerated dichotomy; a revised paradigm may be required (Karl 2000).

From research that has been conducted over the past several decades, N has emerged as the master variable for productivity and export modeling due to the perception that it was the production rate limiting nutrient. If current estimates of N_2 fixation are valid, then a re-assessment of this fundamental assertion must be made. In all likelihood, emphasis will shift to the role of P which has a much less complex cycle due to the absence of variable oxidation state chemistry and the lack of a significant biogenic gas phase, or to Fe. Although the debate on whether N or P ultimately limits marine productivity (see Codispoti 1989) will likely continue (e.g. Toggweiler 1999; Tyrrell 1999), it now appears almost certain that N_2 fixation must be considered as an ecologically relevant source of new N in the sea. Finally, the inextricable link between N_2 fixation in the world's oceans to climate variability and certain anthropogenic processes, suggests that predictable changes may occur in rates of N_2 fixation in regions such as those ranging from severely human-impacted to natural landscapes, seascapes and the pre-industrial bioelemental cycles.

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Note added in proof

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