

# Marine nitrogen fixation: what's the fuss?

## Commentary

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Biological nitrogen fixation is a much more important process in the nitrogen cycle of the oceans than previously thought. Further, nitrogen fixation may have an influence on the capacity of the oceans to sequester carbon. A greater diversity of marine nitrogen fixers has also been uncovered but their quantitative significance remains to be determined.

### Addresses

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### Abbreviations

**DON** dissolved organic nitrogen  
**Tg** teragram

### Introduction

A revolution in our understanding of the marine nitrogen cycle and the role of microorganisms in it is currently underway. Nitrogen is generally accepted as the most common nutrient limiting primary production for phytoplankton throughout much of the world's upper ocean [1], although we now recognize large areas of the ocean that are iron limited [2]. The large reserves of nitrogen (in the form of nitrate) in the deep ocean have been considered to be the main external sources supplying the inorganic nitrogen needs of primary production in the surface ocean. Until recently, microbial nitrogen fixation had been thought to be a relatively minor process in the oceans and in the global marine nitrogen balance [1]. Recent observations in biological oceanography, molecular ecology, geochemistry and paleoecology have now prompted us to review and radically revise our view of the quantitative importance of this process in the nitrogen cycle of the present, as well as the past, ocean. Moreover, the simple view that there are only a few key nitrogen fixers in the sea is being challenged as new studies unveil a much broader suite of potential diazotrophs resident in the oceans. Importantly, marine nitrogen fixation has been proposed to be one of the key components in a suite of interactions and feedbacks between the ocean and atmospheric CO<sub>2</sub> [3] and, thereby, the role of the oceans in the dynamics of atmospheric CO<sub>2</sub> [4–6]. Gaining a firmer understanding of the controls on marine nitrogen fixation has taken on immediate importance.

### What is the new evidence?

Diverse studies in oceanography and geochemistry now point towards a much greater role for marine nitrogen fixation. Early nitrogen isotope studies had observed a low

natural abundance of <sup>15</sup>N (relative to <sup>14</sup>N) in the organic matter of particles in the upper layers of warm tropical seas, and lower than that in many other marine ecosystems [7,8]. In organic nitrogen, a ratio of <sup>15</sup>N to <sup>14</sup>N similar to that of nitrogen in air is often taken as evidence for the input of nitrogen by biological fixation. Low <sup>15</sup>N signatures in surface particles have now been observed across a broader suite of tropical and subtropical ecosystems [9–13]. Moreover, nitrate from the upper thermocline of several highly oligotrophic regions (such as the Indian Ocean and the Eastern Tropical North Pacific) has been revealed to be isotopically light in contrast to the enrichment that is often observed in near-surface pools of nitrate as a result of assimilatory nitrate reduction [14]. Again, this has been interpreted to indicate a source of nitrogen relatively depleted in <sup>15</sup>N (a likely result of nitrogen fixation) in the surface layers of these systems.

The observation of relatively high concentrations of dissolved organic nitrogen (DON) in surface waters of regions of the tropical oceans have also been attributed to nitrogen fixation [15–18]. At the Hawaiian Ocean Time (HOT) series station, pools of DON increased during a period in which microbial nitrogen fixation also became more prominent [4,11].

Similarly, patterns in the concentration of nitrate and phosphate in mid-waters of some areas of the ocean point towards nitrogen fixation [19,20]. Organic matter forms in surface planktonic food webs with an average nitrogen to phosphorus (N : P) content of about 16 : 1. Some of this material falls into the deep sea, where it is degraded and its nitrogen and phosphorus content regenerated back to nitrate and phosphate, thereby contributing to the relative abundance of these two nutrients into the deep sea [21]. A linear expression of the relative regeneration of nitrate and phosphate, termed N\*, has been defined [19,20]. Positive values of N\* indicate an excess of nitrate relative to phosphate (and with respect to the nitrogen and phosphorus content of average surface plankton), whereas negative values of N\* identify zones with a relative deficit of nitrate compared to phosphate. Strong positive values have been observed in the tropical and subtropical North Atlantic (Table 1) and are proposed to be a result of the input into these areas of diazotrophic (i.e. nitrogen fixer) biomass with a higher N : P content than that typical of eukaryotic phytoplankton of the upper ocean ([19,20]; C Deutsch, N Gruber, RM Key, JL Sarmiento, in press).

Budgets of the measured inputs and outputs of combined forms of nitrogen into specific areas of the tropical ocean have often discerned an excess of removal, relative to

Table 1

Estimates of pelagic N<sub>2</sub> fixation by mass balance and N\* in different systems.

System	Units	Inputs	Outputs		References
<b>Mass balance</b>				<b>Difference (outputs–inputs)</b>	
N Pacific, HOT	mmol m <sup>-2</sup> y <sup>-1</sup>	53–130*	161–321	31–268	[23]
Coral Sea	10 <sup>9</sup> g y <sup>-1</sup>	4.4–9.3*	18–50	13–42	[24]
Arabian Sea	Tg y <sup>-1</sup>	2*	30	28	[22]
Pacific	Tg y <sup>-1</sup>	4*	63	59	†
<b>N* based estimates</b>				<b>N<sub>2</sub> Fixation</b>	
N Atlantic	Tg y <sup>-1</sup>	n/a	n/a	75–100	[19]
N Atlantic	Tg y <sup>-1</sup>	n/a	n/a	25	[20]
Oceans	Tg y <sup>-1</sup>	n/a	n/a	110	[20]

\*does not include estimate of pelagic N<sub>2</sub> fixation; †C Deutsch, N Gruber, RM Key, JL Sarmiento, in press. HOT, Hawaiian Ocean Time series station.

inflows, and have speculated that biological nitrogen fixation may account for the observed imbalances (Table 1) [22–25]. Attempts to close annual carbon budgets have also implicated nitrogen fixation in some ecosystems. Sharp decreases in concentrations of inorganic carbon in the euphotic zone waters of the Bermuda Time Series Station (BATS) occurs in the absence of combined nitrogen that has been depleted earlier in the year [26].

Over the years, larger scale budgets of the present ocean have generally concluded that, given the large zones of denitrification in the ocean and shelves, removal of combined nitrogen through microbial denitrification probably exceeds inputs of nitrogen, including nitrogen fixation [27,28]. An unbalanced oceanic nitrogen cycle is not unreasonable, given the time scale of circulation of the deep ocean (~2000 years). Indeed, paleoecological evidence indicates that the relative proportions of the complementary

microbial processes of nitrogen fixation and denitrification may vary over a glacial–interglacial timescale (i.e. ~10<sup>5</sup> years), such that marine denitrification exceeds nitrogen fixation during warm interglacial periods whilst nitrogen fixation takes on greater relative importance during glacial periods [3,29–31]. During such periods, this results in a build up of combined nitrogen reserves and a net sequestration of carbon from the atmosphere to the oceans. Interestingly, the paleogeological record also records that glacial–interglacial trends in some areas of the ocean (e.g. the Cariaco Trench) oppose global trends [32].

The role of marine cyanobacteria in the original oxygenation of the atmosphere about 2 x 10<sup>9</sup> billion years ago is generally accepted [33]. It is provocative to consider that they may also be involved with regulation of atmospheric CO<sub>2</sub> and, possibly, with the major state shifts of the earth between glacial states.

Table 2

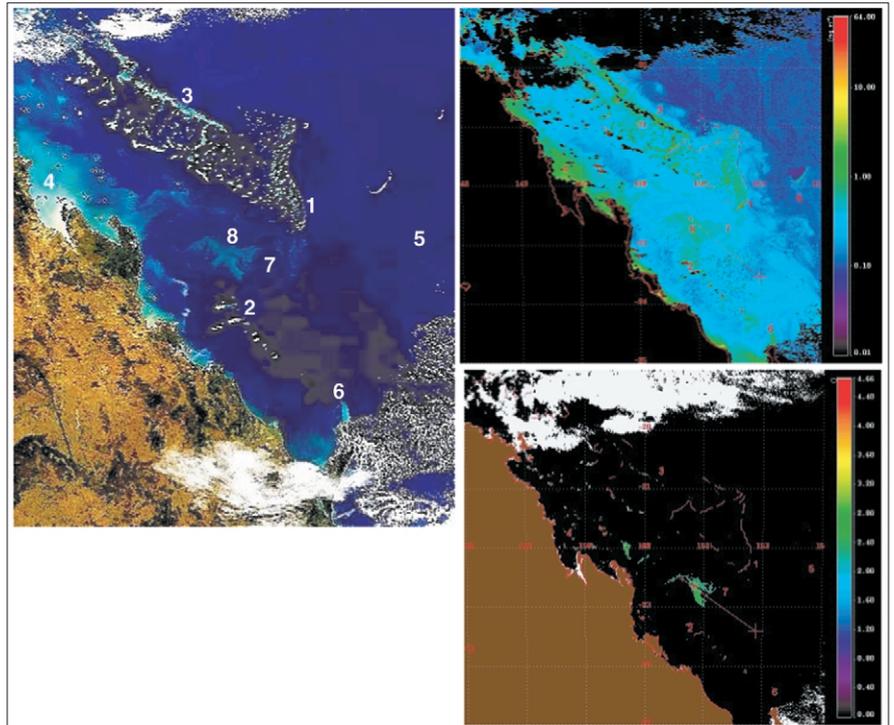
Some recent direct and indirect areal estimates of pelagic N<sub>2</sub> fixation.

Location	Date	Comment	Areal estimates avg se μmol N/m <sup>2</sup> * d		Number of stations or observations	References
<b>DIRECT</b>						
<i>Trichodesmium</i> /tropical regions						
N Pacific: HOT/ALOHA	1990–92	AR, 3:1*	85	49 (?)		[11]
Arabian Sea, 7–10°N	May '95	AR, 3:1	35	7.4	9	[12]
Arabian Sea @ 10°N	May '95	bloom	99†	25	5	
SW N Atlantic, 0°–17°N	Apr '96	AR, 3:1	258	98	15	‡
SW N Atlantic, 7°–27°N	Oct '96	AR, 3:1	206	63	20	‡
<i>Richelia/Hemiaulus</i>						
SW N Atlantic, 7°–27°N	Oct '96	AR, 3:1	3110	1315	14	[13]
<b>INDIRECT/MASS BALANCE</b>						
N Atlantic		extr	2100			[39]
N Pacific: HOT/ALOHA		mass balance/N:P	93			[11]
N Pacific: HOT/ALOHA		mass balance: 15N	137			[11]
N Atlantic		N*	197			[20]
Pacific		mass balance	107			§

\*acetylene reduction method for N<sub>2</sub> fixation using a conversion ratio of 3:1; †for surface blooms, rates extrapolated over top 0.5 m; ‡DG Capone, EJ Carpenter, unpublished data; §C Deutsch, N Gruber, RM Key, JL Sarmiento, in press. AR, acetylene reduction method for nitrogen fixation; avg, average; extr, extrapolation; se, standard error.

**Figure 1**

Example of a bloom of *Trichodesmium* as detected by the SeaWiFS satellite and using an algorithm to specifically resolve *Trichodesmium* chlorophyll [35,42]. Provided by the SeaWiFS Project, NASA/Goddard Space Flight Center and ORBIMAGE. The left-hand image is a SeaWiFS true color image of Capricorn Channel, Great Barrier Reef, Australia, taken on 16 July 1998. The following locations have been numbered: Swain Reefs (1), Capricorn Group islands (2), Southern Great Barrier Reef (3), Broad Sound (4), Coral Sea (5), Frasier Island (6), Capricorn Channel (7). A *Trichodesmium* bloom is numbered (8). The top right-hand image shows OC2-derived chlorophyll (using SeaWiFS data) for the same scene. The bottom right-hand image shows *Trichodesmium*-specific chlorophyll, as determined by the classification scheme presented in [42].



### How much nitrogen fixation occurs in the oceans and who are the important contributors?

Estimating the extent of nitrogen fixation in the world's oceans has moved from being a largely academic exercise to a means of setting a constraint on the capacity of the upper ocean biota to sequester atmospheric CO<sub>2</sub>. To date, the bulk of research on nitrogen fixation in oceanic systems has focused on the nonheterocystous cyanobacteria, *Trichodesmium* spp. [34], which often occur as aggregates (often referred to as colonies) visible to the naked eye ('sea sawdust'). It also occurs as individual filaments. *Trichodesmium* is conspicuous, as it can form extensive surface slicks and is one of the very few microorganism that is directly visible from space (Figure 1) [35].

Two important issues remain immediately at hand. First, have we accurately estimated nitrogen fixation by *Trichodesmium*? Second, are there other, presently unquantified sources of nitrogen fixation in the open ocean? Direct global estimates of oceanic nitrogen have largely considered input by *Trichodesmium* [36]. Earlier attempts to scale rates of nitrogen fixation [37], based on available data of the abundance of the most prominent oceanic diazotroph, may have suffered from systematic underestimation of its biomass by the techniques used for collection [38]. However, direct extrapolation of the more recently collected datasets based on careful quantification of the abundance of *Trichodesmium* and directly determined depth-integrated rates of nitrogen fixation point to much higher areal [11,39] and globally integrated inputs [34,38], consistent with geochemical analyses (see Table 2). Rates observed *in situ* for *Trichodesmium* for

tropical oligotrophic environments are typically more than 100  $\mu\text{mol N m}^{-2}\text{d}^{-1}$  (Table 1), making this process comparable in magnitude to the estimated flux of nitrate across the base of the euphotic zone of these highly stable tropical and subtropical ecosystems [11,40].

One major challenge facing accurate quantification of the input of nitrogen by *Trichodesmium* is factoring in nitrogen input by large-scale surface blooms. These phenomena can occur over very extensive temporal (days to weeks) and spatial (1000 to >100,000 km<sup>2</sup>) [41] (Figure 1) dimensions and amplify nitrogen input, compared to nonbloom conditions [12]. Because of their high reflectance, resulting from the presence of gas vesicles and their possession of phycoerythrin, we may be able to specifically map these events using color remote sensing [35,42] and to incorporate that information into biogeochemical models to predict areal nitrogen fixation by blooms [43].

Intriguing data that are emerging demonstrate that there may be systematic differences among oceanic ecosystems harboring populations of *Trichodesmium*. For example, a larger proportion of the biomass (about 80–90%) is reported to occur as aggregates in the Sargasso Sea and tropical North Atlantic Oceans (EJ Carpenter, DG Capone, unpublished data), whereas studies in the South China Sea [44] and North Pacific [45] have found that free filaments predominate (~90%). Furthermore, the capacity of free filaments to fix nitrogen may not be as great as that for filaments in aggregates [44,46]. These observations bear directly on our ability to accurately quantify *in situ* nitrogen

Table 3

**Hypothetical estimates of required cell specific rates of N<sub>2</sub> fixation to provide a global rate of 100 Tg/y at several assumed cell densities of diazotrophic picocyanobacteria in warm oligotrophic ocean waters.**

	Annual global input Tg N	Ocean surface area >25C* km <sup>2</sup> *10 <sup>6</sup>	N <sub>2</sub> fixation umol/m <sup>2</sup> d	Integration depth m	Hours/d	nmol N/m <sup>3</sup> h	Cells/ml	Cell-specific rate fmol N/cell h
Picocyanobacteria	100	128	153	100	12	127	1000 100 10	0.1 1.3 13
Cocoid cyanobacterial diazotrophs								1.3–4.0 <sup>†</sup>
<i>Trichodesmium</i>								20 <sup>‡</sup>

\*From annual averages of sea surface temperature. <sup>†</sup>Maximum rates reported or derived from [50,76-78]. <sup>‡</sup>From a survey of 28 studies of *Trichodesmium* in tropical waters (DG Capone, unpublished).

fixation by *Trichodesmium* populations, as many studies have focused largely on the macroscopic aggregates that may be selectively isolated.

Other potential diazotrophs have been known to exist in the marine plankton. For instance, a small heterocystous cyanobacterium, *Richelia intracellularis*, is an endosymbiont in diverse marine diatoms [47,48]. However, only recently have these associations been shown to have quantitatively substantial inputs of nitrogen on regional scales [13] (Table 2). There are suggestions of a much broader range of putatively diazotrophic marine cyanobacteria, both free-living [49,50] and associated with eukaryotic algae ([51,52]; EJ Carpenter, personal communication).

Much of the past work in this area has been observational, largely depending on microscopic (including more recent epifluorescent) approaches. The availability of molecular probes to the structural genes of nitrogenase has provided means with which to go beyond direct observation. *NifH* genes, which express the Fe subunit of nitrogenase, have been found in the picoplankton, as well as in heterotrophic bacteria from the guts of copepods [52].

To date, all marine nitrogen fixers, either isolated or identified by gene sequencing, are members of the bacterial domain. Dense populations of archaea also exist in the upper water of the marine environment [53,54] and it is tempting to speculate that, in the low-nutrient upper layers of the ocean, archaea may also contribute to diazotrophy. However, there is no current evidence to substantiate this speculation.

Determining whether picoplanktonic diazotrophs are active in nitrogen fixation and accurately quantifying their input to compare with known sources is a major current challenge in the field. The relatively dense (typically 10<sup>4</sup> cells/ml of seawater) populations of picocyanobacteria (largely *Synechococcus* sp.) in the upper mixed layer of warm tropical seas, the isolation of diazotrophic cocoid cyanobacteria from marine waters [49,52] (please note that the cocoid marine cyanobacterium, *Erythrophaera marina* [49], has been

redesignated *Crocospaera watsonii*; J Waterbury, personal communication), and recent reports of relatively high densities *in situ* of these putatively diazotrophic forms (EJ Carpenter, personal communication; J Waterbury, personal communication) has focussed recent attention on this component of the picoplankton. A simple calculation indicates that it would take only a small portion of these populations to be actively diazotrophic at moderate cell-specific rates, in order to contribute substantially to oceanic nitrogen inputs (Table 3). Furthermore, activity at this level should be detectable with current direct <sup>15</sup>N tracer methods.

Attempts have been made to derive estimates of net nitrogen fixation from N\* distributions (Table 1) — an attractive approach, as it should (under circumstances in which concurrent denitrification does not obscure the signal) provide an integrative measure of all diazotrophic inputs. This analysis, which requires the assumption of an N : P ratio for diazotrophs in excess of the N : P ratio of conventional plankton, resulted in an estimate of about 25 teragrams/year (1 Tg = 10<sup>12</sup> grams) for the North Atlantic and a global nitrogen fixation rate of about 110 Tg/year [20], comparable to recent direct extrapolations (Table 1). The N : P value chosen for this extrapolation, 125 : 1, was taken from Karl *et al.* [23]. However, limited field data suggest that natural populations of *Trichodesmium* have substantially lower N : P ratios (30 to 50) [45,46]. Furthermore, we have observed large changes in the N : P ratios of diazotrophic cultures of *Trichodesmium* over the growth cycle, ranging from low values (~16 : 1) under phosphorus-replete conditions to values exceeding 100 : 1 under severely phosphorus-depleted conditions (J Krauk, DG Capone, unpublished data). Similarly, diazotrophic cocoid cyanobacteria and heterotrophic bacteria may be expected to have relatively low N : P ratios in their biomass. Importantly, as the N : P ratio assumed in this exercise decreases from 125 : 1, derived rates of nitrogen fixation increase exponentially [20].

### What controls oceanic nitrogen fixation? Iron versus phosphorus

Perhaps one of the most active areas of marine nitrogen fixation research are current efforts to identify major

factors controlling this process in the oceans. Understanding the controls on nitrogen fixation will further our ability to estimate the capacity of specific oceanic ecosystems to produce and export carbon [55]. An improved knowledge of key controls can also help us infer how nitrogen fixation and the primary productivity associated with it may change with climatically induced and/or human-population-associated changes in nutrient fluxes to the ocean (e.g. increase in atmospheric nitrogen deposition, or by changes in patterns of dust transport).

Diazotrophs, by their nature, should not be 'limited' by the availability of combined nitrogen in the environment (although they may be excluded by excesses of nitrogen). Availability of other nutrients, such as phosphorus, trace metals (e.g. molybdenum and iron) or, in the case of heterotrophic diazotrophs, organic carbon, may constrain the extent of nitrogen fixation in a system [56].

Molybdenum occurs in the ocean in trace quantities as  $\text{MoO}_4$ . The molecular similarity of  $\text{MoO}_4$  to sulphate, which occurs at mM concentrations in seawater, prompted the suggestion that sulphate may inhibit the uptake of molybdenum by oceanic diazotrophs [57]. However, the evidence available does not seem to support severe limitation of nitrogen fixation by molybdenum in oceanic ecosystems [56].

For autotrophic diazotrophs in the open ocean marine realm, the hunt is now focusing on iron and phosphorus. The cell requirement for iron should be greater for a diazotroph compared to a nondiazotroph, given the requirements of the nitrogenase enzyme complex [58]. Data for *Trichodesmium* do indicate a higher cell quota [59]. Through much of the upper ocean, iron is generally thought to be delivered by atmospheric deposition [60]. There is some experimental evidence for stimulation of growth and nitrogen fixation in natural populations of *Trichodesmium* [61], as well as indications of chronic iron limitation of phytoplankton populations in some highly oligotrophic regions [62]. Intriguingly, areas of the North Atlantic subject to the largest seasonal inputs of atmospheric iron [63] are also areas of high  $\text{N}^*$  [20] and presumed diazotrophy. However, convincing evidence of chronic iron limitation of diazotrophic populations *in situ* remains to be provided [5,64].

Phosphate concentrations are also extremely low in many environments in which *Trichodesmium* is found. Mechanisms for phosphorus acquisition by *Trichodesmium*, such as the use of the dissolved organic phosphate pool [65] and periodic migration to deep phosphate reserves [23], have been suggested.

There may be a substantial interaction between iron supply and the relative extent of nitrogen versus phosphorus limitation of primary production through nitrogen fixation. Wu *et al.* [64] reported that inorganic phosphorus is

relatively more depleted in the Sargasso Sea than in the subtropical North Pacific, and hypothesized that this may result from the control of nitrogen fixation by iron supply, which is greater in the Sargasso Sea. For samples of *Trichodesmium* from the tropical Atlantic, nitrogenase activity was directly correlated with cell phosphorus content, but not with cell iron [66]. Interestingly (as noted above) Karl *et al.* [11] observed over the last decade a relative increase in diazotrophic populations at the HOT subtropical time series station, as well as a decrease in soluble reactive phosphate and an apparent shift from chronic nitrogen limitation to phosphorus limitation.

With regard to the trends among these ocean basins in iron, phosphorus and nitrogen limitation, it may be relevant that the predominant species of *Trichodesmium* appears to vary systematically across ecosystems. *Trichodesmium thiebautii* is the most common species encountered in the Sargasso Sea, tropical North Atlantic and Caribbean Sea ([67]; EJ Carpenter, personal communication). In contrast, *Trichodesmium erythraeum* appears to predominate in the Indian [68,12] and South West Pacific Oceans ([69]; EJ Carpenter, personal communication). Are these trends real, and do they reflect fundamental differences among the Sargasso Sea, the tropical North Atlantic, the Caribbean Sea and the South West Pacific Ocean?

### Where goes the nitrogen??

Other sustaining mysteries in marine nitrogen fixation by *Trichodesmium* spp. are the routes of transfer of recently fixed nitrogen to higher trophic levels. *Trichodesmium* does not appear to be directly grazed by the conventional calanoid and cyclopoid zooplankton of the upper ocean [70]. However, some specialized harpacticoid copepods (typically benthic forms) that are uniquely adapted to live on *Trichodesmium* [71] have been found.

Current evidence suggests that a major flux of recently fixed nitrogen to higher trophic levels may be through extracellular release [72–74]. This is consistent with the observations of enhanced DON levels reported in environments with diazotrophs (see above). Analysis of the  $^{15}\text{N}$  signature of DON (and ammonium) would provide more evidence for a direct connection between nitrogen fixation and these key pools in surface waters. Interestingly, zooplankton from the tropical Atlantic are isotopically 'light' (i.e. depleted in  $^{15}\text{N}$ ), relative to zooplankton from more temperate zones, indicating that recently fixed nitrogen does make it to higher trophic levels in these systems (JP Montoya, EJ Carpenter, DG Capone, unpublished data).

One key area that remains largely unexplored is the role of viruses in the life cycle of *Trichodesmium*. Ohki [75] has provided some preliminary evidence for infection and a lytic cycle in cultures and natural populations of *Trichodesmium*. Given the lack of major predators, cyanophages could play an important role in the turnover of *Trichodesmium* biomass.

## Where are we headed?

Research on marine nitrogen fixation is accelerating rapidly. Gene-based studies complemented by high sensitivity isotopic tracer efforts are expanding and helping to better define the roles of picoplanktonic and symbiotic diazotrophs in our current understanding of marine nitrogen fixation.

Studies attempting to better quantify this process in the oceans are underway worldwide, including efforts from field laboratories and at time series stations, ship-based efforts, and efforts using satellite resources to determine the spatial and temporal scales of *Trichodesmium* distributions (e.g. see SeaWiFS Level-3 Standard Mapped Images URL <http://seawifs.gsfc.nasa.gov/cgi/level3.pl?DAY=&PER=&TYP=trif>; Blooms of the cyanobacteria *Trichodesmium* spp. URL [http://orbit-net.nesdis.noaa.gov/orad2/doc/tricho\\_www.html](http://orbit-net.nesdis.noaa.gov/orad2/doc/tricho_www.html)).

For the first time, nitrogen fixation is being included as an explicit component of ocean biogeochemical models aimed at defining ocean-atmosphere interactions and feedback to the carbon cycle. Lastly, the possibility of stimulating oceanic nitrogen fixers with artificial iron additions has also been raised (e.g. Ocean Sequestration, Carbon Sequestration Research and Development URL [http://www.ornl.gov/carbon\\_sequestration/chap3.pdf](http://www.ornl.gov/carbon_sequestration/chap3.pdf); Iron and Marine N<sub>2</sub> Fixation, SOLAS Science Plan URL [http://www.ifm.uni-kiel.de/ch/solas/SP\\_Focus1.htm#activity1.2b](http://www.ifm.uni-kiel.de/ch/solas/SP_Focus1.htm#activity1.2b)), and may seriously be considered as a strategy to reduce atmospheric CO<sub>2</sub> levels.

## Conclusions

Oceanic nitrogen fixation is of far greater importance than realized a decade ago and may have direct bearing on the capacity for the upper ocean to sequester atmospheric CO<sub>2</sub>. Iron and phosphorus are the likely factors controlling oceanic nitrogen fixation, and their relative influence may vary among ocean basins. The most conspicuous oceanic nitrogen fixer, the cyanobacterium *Trichodesmium*, can provide substantial inputs of nitrogen in tropical ecosystems. However, the role of 'blooms' and the fate of *Trichodesmium* biomass in the marine food web needs to be resolved. Other sources of nitrogen fixation are now evident in the open ocean realm, although their input remains unquantified. It is speculated that the large populations of archaea recently recognized in the oligotrophic oceans could also contribute to marine nitrogen fixation. Although <sup>15</sup>N natural isotope abundance studies demonstrate that recently fixed nitrogen can be detected in soluble and particulate pools in the upper layers of the oligotrophic ocean, the pathways of transfer of this nitrogen remain to be elucidated.

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