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THE CONUNDRUM OF MARINE N_2 FIXATION

CLAIRE MAHAFFEY†, ANTHONY F. MICHAELS, and DOUGLAS G. CAPONE*

*Department of Biological Sciences, and Wrigley Institute for Environmental Studies, University of Southern California, Los Angeles, California, 90275

ABSTRACT. Over the last 30 years, immense progress has been made in understanding the global significance of marine nitrogen (N_2) fixation. Development of enzymological, isotopic and molecular techniques for identifying N_2 fixers and quantifying N_2 fixation rates, as well as more frequent and extensive field campaigns have fuelled such progress. Ship-based and laboratory experiments have revealed a large suite of previously unknown physiological characteristics of diazotrophs. More recently, geochemical estimates of N_2 fixation, based upon N and phosphorus (P) stoichiometry, stable N isotope studies and carbon (C) anomalies in nutrient starved regions of the ocean, have provided basin and global- scale estimates of N_2 fixation. While these achievements have revolutionized our understanding of the role N_2 fixers play in global marine N and C cycles, there remain fundamental challenges in the study of N_2 fixation. We here summarize the advances made and highlight the conundrums that remain regarding the basin and global scale quantification of N_2 fixation, as well as the climatological, physical and biological factors that enable or constrain the distribution and growth of diazotrophs.

Presently, direct estimates of N_2 fixation, largely of the planktonic cyanobacterium, *Trichodesmium*, can account for only a quarter to one-half of the geochemically derived rates of N_2 fixation in various ocean basins. This dichotomy may be partially a result of the spatial and temporal scales over which each approach integrates N_2 fixation. However, recently discovered marine N_2 fixers, including coccoid cyanobacteria and heterotrophic bacteria, cyanobacteria symbiotic with eukaryotic algae and the gut flora of zooplankton may account for much of the disparity. While their contribution to the marine N cycle is captured by broad scale geochemical derivations, robust direct estimates of N_2 fixation for these members of the diazotrophic flora have not yet been obtained. Moreover, there is substantial uncertainty among the various geochemically derived estimates of N_2 fixation, each of which is highly sensitive to choices of parameters, such as domain area, used in their derivation. Recent findings from stable isotope studies imply that unraveling the pathway of recently fixed N through the N cycle remains a challenge.

On a basin and global scale, iron (Fe) and P are thought to be primary chemical factors limiting N_2 fixation. The climatological and biological forcings, which individually or cumulatively promote diazotroph growth or trigger blooms, are still poorly understood. Improvements in coupled biological-physical and ecosystem models have allowed for the explicit representation of diazotrophs and, in particular, *Trichodesmium* and thus hold great potential for unraveling the factors that constrain diazotroph distribution and growth.

The role of N_2 fixation in both the global N and C cycle, through both the inter-glacial and glacial cycles as well in the present day ocean, remains an open question. The emerging body of data on global rates of denitrification implies that the oceans are losing nitrate rapidly and, if true, either the rates of N_2 fixation are even higher than we currently estimate or, on some time-scale, the total stock of nitrate in the oceans is more variable than expected. Each of these outcomes has direct implications for the air-sea partitioning of CO_2 on climate time-scales. The question remains: does the importance of marine N_2 fixation relative to denitrification oscillate over various timescales in response to climate forcing or is the N cycle in a homeostatic steady state?

*Corresponding author: capone@usc.edu

†Present address: Department of Oceanography, School of Ocean and Earth Science and Technology, University of Hawaii, Honolulu, Hawaii 96822

INTRODUCTION

More than a century of intensive research has yielded a fairly complete, but un-even understanding of the marine nitrogen (N) cycle. The advent of new tools or the vagaries of scientific fashion have spurred periods of intense focus on one part or another of the overall cycle whilst other aspects were either assumed unimportant or neglected for technical or practical reasons. Recently, a confluence of spatially disparate observations have converged to suggest that dinitrogen (N_2) fixation is much more important than previously thought and that a full understanding of the N cycle and its impact on the carbon (C) cycle will be incomplete without an accurate assessment of the rates and patterns of this process.

In the late 1800's, Karl Brandt first speculated on the N cycle of the seas (see Waksman and others, 1933; Mills, 1989) and proposed that the oceans were largely the recipients of the reactive N formed by the then newly discovered process of N_2 fixation in the terrestrial realm. Brandt further suggested that denitrification in the oceans balanced this input and thereby effectively promoted the redistribution of N in the biosphere through the N_2 pool. Systematic studies on the major species of N (Harvey, 1927; Cooper, 1937) and the presence and role of nitrifying, denitrifying and N_2 fixing bacteria in the sea (Waksman and others, 1933) led up to Redfield's fundamental insights on the role of organisms in controlling the stoichiometry of major nutrients in seawater (Redfield, 1934, 1958; Redfield and others, 1963).

In the early 1900s, scholars posited the potential role of N as a major control on marine productivity. Redfield (1934) speculated [after consultation with his colleague G. E. Hutchinson (1944)] that phosphorus (P) was more likely to be the primary limiting nutrient in the sea (*sensu* Liebig), as N_2 fixation should make up any N deficit. Of course, N_2 fixation as an active process in the sea was a purely hypothetical conjecture at this point.

In contrast, experimental (Ryther and Dunstan, 1971) and observational (Thomas, 1966) studies implicated N as the primary nutrient limiting plant productivity and set the stage for the ongoing debates (for example, Cullen, 1999; Tyrrell, 1999) on the relative importance of N, P and, more recently, iron (Fe), in controlling primary productivity in the global ocean. Interestingly, broad scale surveys of marine phytoplankton had already described the distribution of organisms like the planktonic cyanobacteria, *Trichodesmium* and the endosymbiont containing diatom *Hemiaulus* (Wille, 1904; see Carpenter, 1983a) which were later shown to be capable of diazotrophy (= N_2 fixation, see below).

In an important conceptual advance, Dugdale and Goering (1967) and Dugdale (1967) proposed the "New N" model. This mass balance approach considered the distinct sources of N supporting phytoplankton growth within the well-lit surface ocean: "regenerated N," the autochthonous sources of N produced within the euphotic zone by regeneration of organic matter (usually ammonium) and "New N," the allochthonous sources imported into the system from outside its defined bounds, such as nitrate introduced from below the euphotic zone. In steady state, the rate of supply of "New N" can support the export of organic matter from the surface layer of the ocean. This model structured much of marine N cycle research over the next two decades, largely channeling it towards comparing the relative importance among systems of primary production supported by nitrate (new production) versus that supported by ammonium (regenerated production) (Eppley and Peterson, 1979).

The original new production model explicitly recognized that, in addition to nitrate derived from depth, N_2 fixation was also a distinct form of "New N" (Dugdale and Goering, 1967). Dugdale and others (1961) introduced a method to directly quantify the uptake of $^{15}N_2$ and demonstrated its presence in the Sargasso and Arabian Seas, associated with the marine cyanobacteria, *Trichodesmium* (Dugdale and others,

1964). However, at the time, the methods for assaying N_2 fixation were fairly tedious and specialized and not widely adopted, whereas the ^{15}N-based procedures for quantifying the uptake of nitrate and ammonium were widely available and applied. This technological imbalance influenced the development of the research to a large degree. Ironically, the observation that N_2 fixation occurred in the sea was also greeted with some skepticism (for example, Fogg, 1978) as it was not yet appreciated that some cyanobacteria were capable of fixing N_2 without the specialized N_2 fixation structures, termed heterocysts, found in higher cyanobacteria.

THOROUGHLY MODERN METHODS

As in many fields, major advances in understanding are often constrained by the availability of appropriate data and tools to formulate and test hypotheses. Evidence that N_2 fixation might be an important component of the N cycle of some areas of the ocean began to first accumulate in the late 1970s with several pioneering N isotope studies examining the natural abundance of the ratio of ^{15}N:^{14}N in the western Pacific Ocean (Wada and Hattori, 1976; Saino, ms, 1977; Saino and Hattori, 1987). Surface particulate and dissolved inorganic N pools in the Kuroshio often had a ^{15}N-depleted isotopic signature, indicative of a N_2 fixation source (Delwiche and others, 1979).

In parallel, major ongoing geochemical and physical oceanographic experiments such as Geochemical Sections (GEOSECS), Transient Tracers in the Ocean (TTO) and World Ocean Circulation Experiment (WOCE) have accumulated a wealth of nutrient data in the major ocean basins. The synthesis and modeling of these data sets have provided new insights into processes occurring in the sea and, in particular, N_2 fixation (see Geochemical Section).

While a field assay for N_2 fixation had been introduced earlier on (Dugdale and others, 1961; see above), field studies of N_2 fixation only reached full stride with the introduction of a convenient enzymological assay, the C_2H_2 reduction method, in the early 1970s (Stewart and others, 1967; Hardy and others, 1968; Capone, 1993; Capone and Montoya, 2001). Major field campaigns largely focused on *Trichodesmium* spp., and attempted to quantify N_2 fixation in the western (Saino and Hattori, 1980) and eastern Pacific (Mague and others, 1974; Mague and others, 1977) and the Sargasso and Caribbean Seas (Carpenter, 1973; Carpenter and McCarthy, 1975; Carpenter and Price, 1977) (see summaries by Carpenter, 1983a; Capone, 2001).

Most of these early studies of N_2 fixation focused on the sub-tropics, or on marginal seas with few observations in the open tropical oceans (Capone and Carpenter, 1999). Attempts to scale these studies to the broader global ocean, using the larger dataset from historical plankton surveys, suggested substantial N inputs in the marginal tropical seas but a relatively small global input (Carpenter, 1983a). In retrospect, many of the very early plankton surveys also likely underestimated the biomass of *Trichodesmium* (Capone and Carpenter, 1999). The net impact of these early papers led to the conclusion that the global rates of N_2 fixation were small, which is consistent with the apparent co-limitation of N and P and the very small surplus of soluble reactive phosphorus (SRP) in most surface oceans.

Instrumentation for mass spectrometry and field methodologies for the direct determination of N_2 fixation by $^{15}N_2$ tracer uptake have now greatly improved (Capone and Montoya, 2001; Montoya and others, 2002). For instance, 99 percent enriched $^{15}N_2$ is commercially available in compressed gas cylinders and this technique is being more broadly applied. In fact, the C_2H_2 reduction method and direct $^{15}N_2$ tracer methods provide distinct estimates of N_2 fixation (Carpenter, 1973; Karl, 2002; Mulholland and others, 2004). Nitrogenase activity assessed with C_2H_2 reduction should detect all activity in a sample for a given set of physiological conditions and may be considered a measure of gross activity. In contrast, using routine protocols (for example, 24 hour incubation time), the $^{15}N_2$ technique measures the incorporation of

the ^{15}N isotope into planktonic particulate organic N, but does not include the exudation of ^{15}N enriched ammonium or dissolved organic nitrogen, and thus approximates net activity (see Bronk and Glibert, 1991; Glibert and Bronk, 1994).

Coupling isotope ratio mass spectrometric approaches with chromatographic separations of complex mixtures has opened a whole field of compound-specific isotope ratio analysis (Macko and others, 1987; Hare and others, 1991; Beaumont and others, 2000; McClelland and Montoya, 2002). This technique has already been profitably exploited in several areas of marine N_2 fixation (see below).

Most recently, the infusion of methods from molecular biology (Zehr and others, 2000) is revolutionizing the study of marine microbes. These molecular ecological approaches have added whole new dimensions to the study of marine N_2 fixation (Zehr and Capone, 1996, 2000; Zehr and others, 2000; Zehr and Ward, 2002) allowing for identification of new sites of active N_2 fixation in the environment (Zehr and others, 2001), and providing sensitive means to study the environmental regulation of this system. *In situ* visualization and identification of diazotrophs (Amann and others, 1990, 1995), as well as the potential to be able to determine the number of copies of the nitrogenase (*nif*) genes (Short and others, 2004; Church and others, 2005) and possibly transcript (mRNA) numbers in natural samples by quantitative PCR (qPCR), may provide future advances we cannot yet even predict or anticipate.

Remote sensing (Subramaniam and others, 2002; Westberry and others, 2005) that for the first time provides yet another modern tool that may be usefully exploited to study marine diazotrophs at broad spatial and temporal scales. *Trichodesmium,* for instance, can be specifically detected at relatively high concentrations using ocean color remote sensing (for example, SeaWiFS) because of its unique optical signal resulting from its possession of phycoerythrin and gas vesicles with high reflectance properties (Subramaniam and others, 1999), and its position in the upper water column. In tandem with the emerging importance of remote sensing in studies of marine N_2 fixation, there has also been a surge in the development of global ecosystem (Moore and others, 2002b), biological and coupled biological-physical models (Hood and others, 2001; Fennel and others, 2002; Coles and others, 2004a; Hood and others, 2004) and algorithms using climatological satellite data (Hood and others, 2002; Subramaniam and others, 2002) that, the first time, explicitly represent N_2 fixation. These models are greatly improving our understanding of the spatial and temporal variability, and constraints of diazotrophs, in particular, *Trichodesmium,* and their impact on the global C cycle (Fennel and others, 2002; Moore and others, 2002b; see below).

Indirect Geochemical Approaches to N_2 Fixation

Setting the Stage: Geochemical Studies of N_2 Fixation

Over the last decade or so, several lines of indirect geochemical evidence have strongly pointed to a greater role for N_2 fixation in the current and past oceans. Inferences and rate estimates of the basin scale and global importance of N_2 fixation have been founded upon anomalies from the ratio of N to P (Michaels and others, 1996; Gruber and Sarmiento, 1997), stable N isotopes studies in the water column (Altabet, 1988; Karl and others, 1997) and sediments (Altabet and others, 2002; Ganeshram and others, 2002), and more recently, through models of inorganic carbon budgets (Gruber, 1998; Gruber and others, 1998; Lee and others, 2002). Despite concerted efforts to compare biogeochemical approaches, some large differences exist among independent geochemical estimates of N_2 fixation, and moreover, they can differ greatly from the direct biological estimates (Capone and others, 2005).

Several recent comprehensive publications discussing the indirect geochemical approaches used to study N_2 fixation in the current (Capone, 2001; Karl and others,

2002) and past ocean's (Gruber, 2004) and comparing the geochemical derivations with direct estimates of N_2 fixation (Capone and others, 2005a) have been used as a springboard for this section.

N_2 Fixation Perturbs Redfield Stoichiometry

In the oceans, the relatively robust stoichiometric balance between dissolved and particulate C, N and P in proportions of 106:16:1 (Redfield, 1958; Anderson and Sarmiento, 1994) is classically referred to as the "Redfield ratio." The remarkable constancy between N and P in the ocean has encouraged a plethora of studies examining which nutrient ultimately controls marine primary production (Falkowski, 1997; Tyrrell, 1999), as well as the putative mechanisms that maintain this apparent perfect balance between two elements (Codispoti, 1989; Gruber, 2004).

The marine N inventory is maintained by inputs of fixed N from the combination of N_2 fixation and other exogenous sources (for example, riverine input, runoff, atmospheric deposition) and losses through water column and sedimentary denitrification and sedimentation. Reactive nitrogen is removed through denitrification, the bacterially-mediated respiratory conversion of nitrate to N_2 (Hattori, 1983; Codispoti, 1995) and through anaerobic nitrification by certain planktomycete bacteria in the Anammox reaction that oxidizes ammonium with nitrite yielding N_2 (Thamdrup and Dalsgaard, 2002; Dalsgaard and others, 2003; Kuypers and others, 2005). Since P is neither lost, nor gained, during either N_2 fixation or denitrification, these processes must alter the nitrate (NO_3^-) to phosphate (PO_4^{-3}) ratio in the marine environment if they are quantitatively important (see section on Denitrification, N_2 fixation, dust and ice for a global perspective).

On a local or basin scale, deviations from the canonical 16:1 ratio between nitrate and phosphate have been exploited to define a quasi-conservative tracer, N*, which describes the production or consumption of nitrate relative to that expected from the remineralization of phosphate at Redfield stoichiometry (16:1). Michaels and others (1996), were the first to explore the Bermuda Atlantic Time-Series (BATS) and TTO data set in the Atlantic Ocean and define N* as

$$N^* = [NO_3^-] - 16[PO_4^{3-}] + 2.7\ \mu mol\ kg^{-1} \quad (1)$$

where $[NO_3^-]$ and $[PO_4^{3-}]$ are the concentrations of nitrate and phosphate, respectively. Using the extensive GEOSECS nutrient data set and the stoichiometric balance between nitrification, denitrification and remineralization of N rich organic matter from N_2 fixation (Gruber and Sarmiento, 1997), N* was redefined as:

$$N^* = (0.87[NO_3^-] - 16[PO_4^{3-}] + 2.9)\ \mu mol\ kg^{-1} \quad (2)$$

The constants employed in equations (1) and (2) simply drive the global N:P ratio to 16:1 and the intercept to zero, implying net global denitrification. Despite the various derivations of N* (Gruber and Sarmiento, 1997; Deutsch and others, 2001) or comparatively, excess nitrate (described by DIN_{xs}; Hansell and others, 2004) which have evolved since (Michaels and others, 1996), the interpretation of N* and its application exploit gradients in this metric, the actual N* value being arbitrary. A non-conservative positive gradient in N* (defined as N* when N:P = 16:1, >2.5 μmol kg^{-1}; Gruber and Sarmiento, 1997) in excess of mixing implies net N_2 fixation. Likewise, a negative gradient in N* (<2.5 μmol kg^{-1}), again that does not stem from mixing, implies net denitrification. N*, defined by Gruber and Sarmiento (1997), will be employed throughout.

N* is highest in the main thermocline and is transported along density surfaces (σ_θ) away from the region of formation by isopycnal mixing. Positive gradients in N* along density surfaces represent an accumulation of remineralized N from the

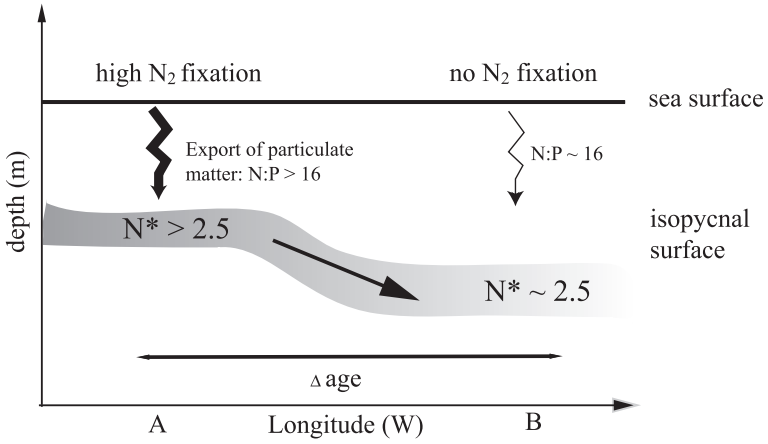

Fig. 1. Schematic illustrating the formation of N* from N_2 fixation and its transport along an isopycnal surface (σ_θ, kg m^{-3}). The gradient in N* and change in age (Δage) between point A and point B are used to derive rate of formation of excess nitrate, translated to a geochemically derived N_2 fixation rate.

overlying water column (fig. 1). Using water mass age data, determined using tracers such as helium/tritium (^{3}He/^{3}H) and CFCs (Jenkins, 1988; Jenkins and Doney, 2003), one can calculate the rate of accumulation of excess nitrate formation that would be required to maintain this gradient in the face of that rate of mixing (Michaels and others, 1996). Assuming that this excess nitrate formation is from N_2 fixation, this translates to a net rate of N_2 fixation over some assumed source area (fig. 1). Calculating gradients, estimating mixing rates and interpreting the spatial extent of the process are all areas where this approach requires often difficult assumptions.

Excess Nitrate and Derived Rates of N_2 Fixation

Although anomalous nutrient ratios have long been recognized in the Atlantic and Pacific Oceans (Fanning, 1992), their potential in estimating basin scale biological processes has only been recently appreciated. Four separate studies have provided distributions of N* and estimates for N_2 fixation in the north Atlantic and Pacific Oceans, using independent nutrient data sets collected between 1972 and 1997, and thermocline ventilation of isopycnal surfaces (σ_θ) (Michaels and others, 1996; Gruber and Sarmiento, 1997; Deutsch and others, 2001; Hansell and others, 2004), (table 1). Depth distributions of N* in the western and eastern tropical Atlantic (fig. 2) show excess nitrate between 70 and 700 m, which correspond to σ_θ layers between 26 and 27 σ_θ. Elevated N* anomalies are observed in the tropical and subtropical north Atlantic (>2.5 μmol kg^{-1}), N* being more pronounced in the western Atlantic basin than the eastern Atlantic (fig. 2). This implies net N_2 fixation over denitrification in the northern basin, which is in contrast to the South Atlantic where low N* anomalies are found across the entire basin (<1.5 μmol kg^{-1}; Gruber and others, 1998). Using the distribution of excess nitrate in the thermocline, Michaels and others (1996) initially estimated the areal integrated rates of N_2 fixation over the North Atlantic to range between 500 to 2500 μmol N $m^{-2}d^{-1}$ (table 1). While this preliminary study served to bring geochemical approaches in deriving N_2 fixation rates into the limelight, the upper boundary for N_2 fixation exceeded estimates for the larger Pacific basin and indeed early estimates for the entire world's oceans by an order of magnitude (see

TABLE 1

Basin scale estimates of N_2 fixation based on various geochemical and direct approaches (Adapted and expanded from Capone and others, 2005)

Location	Comment	Areal Estimates avg μmol N m^{-2} d^{-1}	Domain Area $km^2 \times 10^6$	Areally Integrated Annual N_2 Fixation mol N $\times 10^{12}$ y^{-1}	References
ATLANTIC					
N. Atlantic	extrapolation	na	(19)[a]	0.09	Carpenter, 1983a
N. Atlantic	extrapolation	710 - 3600	7 - 19	2 - 25	Carpenter and Romans, 1991
N. Atlantic	extrapolation	160 -430	7 - 19	1.1	Lipschultz and Owens, 1996
N. Atlantic	N*, residence time	500 - 2500	7 - 19	3.7- 6.4	Michaels and others, 1996
N. Atlantic, 10°-50°N, 25°-90° W	Integrated N*, N:P	197 (315)[b]	27.8	2 (3.2)[b]	Gruber and Sarmiento, 1997
BATS	C_t inventory	1006[c]	27.8	4.5	Gruber and others, 1998
Atlantic, 40°N-40°S	C_t inventory	134 (75.3)[c]	49.1	2.4 (1.35)[c]	Lee and others, 2002
BATS	C_t inventory	482[d]	27.8	1.1	Anderson and Pondaven, 2003
N. Atlantic, 15°-25°N, 25°-75°W	Excess nitrate	70-208 (105- 312)[b]	6.8	0.15 - 0.46 (0.9 - 2.8)[b, e]	Hansell and others, 2004
N. Atlantic, 10°-40°N, 35°-75°W	Excess nitrate	45 - 259[f]	16.0	0.06 - 0.34	Bates and Hansell, 2004
N. Atlantic	^{15}N isotope mass balance[f]	850	17.8	5.5	Capone and others, 2005a
All N. Atlantic	*Trichodesmium* extrapolation	197[g]	17.8	1.3	Capone and others, 2005a
PACIFIC					
Station Aloha	MB/N:P	93[h]		na	Karl and others, 1997
Station Aloha	MB:15N	137[h]		na	Karl and others, 1997
Pacific, (N and S)	N*/mass balance	107		4.2 (± 1)[j]	Deutsch and others, 2001
N. Pacific only[i]	N*/mass balance			2.5	Deutsch and others, 2001
All N. Pacific	*Trichodesmium* extrapolation	93	43	1.5	Capone and others, 2005b

[a]Inferred.
[b]Assuming an N:P ratio of 45 for diazotrophs rather than 125 as originally computed by Gruber and Sarmiento (1997).
[c]Gruber and others (1998) determined a net community production rate of 40 ± 4 μmol C kg^{-1} for the summer/fall period, inferring a nitrogen demand of 0.16 mol N m^{-2} over a period of 159 days (summer/fall).
[d]Model output indicates that N_2 fixation is responsible for 3.25 g C $m^{-2}y^{-1}$ at BATS. However, we know that N_2 fixation at BATS only takes place over the summer months (82 days) and therefore we can assume that the C production rate is for 82 days rather than 365 days (Orcutt and others, 2001).
[e]Assuming a domain area of 27.8×10^6 km^2 (Gruber and Sarmiento, 1997).
[f]Assuming nitrogen fixation occurs for only 82 days rather than 365 days.
[g]Used average from table 2 (201 observations) rather than the 6 N. Atlantic cruises used in Capone and others, unpublished manuscript.
[h]Assuming N_2 fixation occurs over 365 days per year.
[i]Using the SST scaling for the N. Pacific and S. Pacific from table 5.
[j]Error given by Deutsch and others (2001) for their N_2 fixation estimate.

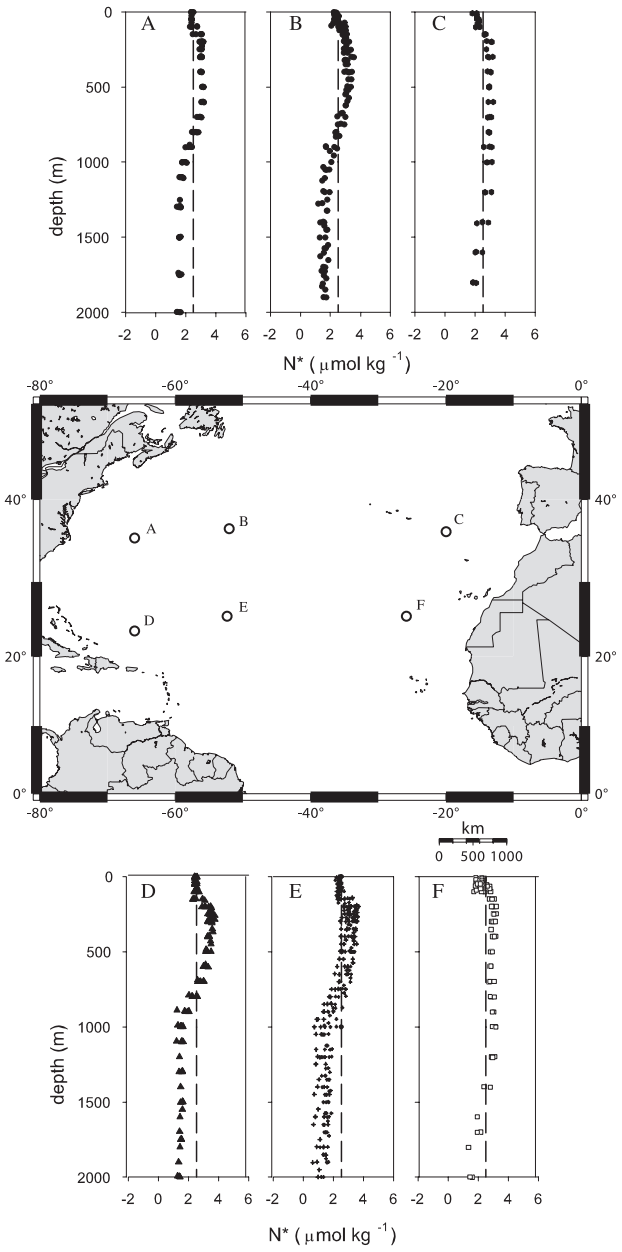

Fig. 2. Depth variation (0-2000m) in $N^* = 0.87[NO_3^-] - 16[PO_4^{3-}] + 2.9$ at 6 locations in the North Atlantic Ocean. Nutrient data extracted from the World Ocean Circulation Experiment data set (http://whop.uscd.edu) (A) A04 and A20 (B) A03 and A20, (C) A16N and A03, (D) A22 and AR01, (E) A20 and AR01 and (F) A16N. Map created using www.aquarius.geomar.de.

Capone and Carpenter, 1999). This high estimate is due to the choice of end member N* values at the area of ventilation of the isopycnal layers, which are likely too low and therefore create inflated gradients. More recent estimates of areal N_2 fixation in the N. Atlantic have ranged from 70 to 208 μmol N $m^{-2}d^{-1}$ (Hansell and others, 2004) to 197

μmol N m^{-2} d^{-1} (Gruber and Sarmiento, 1997) (table 1), which translates to areally integrated rates of 0.15 to 0.46 × 10^{12} mol N y^{-1} (Hansell and others, 2004) and 2 × 10^{12} mol N y^{-1} (Gruber and Sarmiento, 1997) (table 1). As discussed by Capone and others (2005a), this divergence in estimates is partially due to the difference in the domain area chosen in each study. Earlier studies report positive N* anomalies (>2.5 μmol kg^{-1}) between 10 to 50°N and 10 to 90° W in the subtropical (σ_θ, 26.5) and subpolar (σ_θ 27.1) mode waters (Gruber and Sarmiento, 1997) and thus an area of ~27.8 × 10^6 km^2 was used to derive a basin wide rate of N_2 fixation (table 1, fig. 3A). In contrast, Hansell and others (2004) report positive gradients in N* anomalies between 15 to 25°N and 25 to 75°W, which represents ~6.8 × 10^6 km^2, only one quarter of the area covered by Gruber and Sarmiento (1997) (fig. 3A).

Using a two source mixing model to define contributions of different water masses to subtropical water, both Gruber and Sarmiento (1997) and Hansell and others (2004) agree that the northern and southern waters entering the North Atlantic gyre have low preformed N* and therefore elevated N* anomalies are created within the North Atlantic gyre. Basin scale N* distributions derived by Gruber and Sarmiento (1997) indicate development of excess nitrate along isopycnal surfaces across the entire North Atlantic and point to maximal N* anomalies in the western Atlantic, similar to that observed in figures 2 and 4. In contrast, Hansell and others (2004) assumed that excess nitrate only forms along isopycnals which fall within the depth of remineralization of N rich organic matter (150-400 m, represented by subtropical mode water, STMW) and report maximal positive N* anomalies in the eastern subtropical Atlantic. The approach used in this study diminishes any excess nitrate to a fine layer of the ocean and greatly reduces the gradients in N* along these surfaces, thus decreasing the derived rate of N_2 fixation.

An assumption in both the Gruber and Sarmiento (1997) and Hansell and others (2004) geochemical estimates of N_2 fixation is the choice of the N:P ratio of diazotrophs of 125 based on observations by Karl and others (1992). Field studies have reported N:P ratios of one prominent N_2 fixer, the cyanobacterium *Trichodesmium,* to be in the range of 40 to 50 (Mague, 1977; Letelier and Karl, 1996, 1998; Krauk, ms, 2000; Krauk and others, unpublished data), although laboratory studies have found much wider limits (Krauk, ms, 2000; Krauk and others, unpublished data). Applying an N:P ratio of 45 and a domain area of 28 × 10^6 km^2 (Gruber and Sarmiento, 1997; fig. 3A), areal estimates of N_2 fixation rates increase to 0.9 to 2.8 × 10^{12} mol N y^{-1} (from Hansell and others, 2004) and 3.2 × 10^{12} mol N y^{-1} (Gruber and Sarmiento, 1997) (table 1).

Unfortunately, we do not know the N:P ratio of *Trichodesmium* and other diazotrophs leaving the euphotic zone as they rarely show up in sediment traps. There is evidence to suggest that leakage of organic compounds after degradation or lysis of *Trichodesmium* cells provides nutrients for other organisms (Hewson and others, 2004), and contribute to "echo-blooms" (interestingly, modeling efforts also predict echo-blooms, see below and Hood and others (2004). It is possible that one pathway of the export of recently fixed N comes via these "echo-blooms" with a lower N:P when converted to sinking material. If the average N:P of export and remineralization of diazotrophs is closer to 20, comparable to the ratio of nitrate to phosphate in those waters, then the rates of N_2 fixation would need to be correspondingly higher.

The BATS site in the subtropical Sargasso Sea has been a test-bed for early studies of excess nitrate (Michaels and others, 1996) and stable N isotopes (Altabet, 1988; see below). Ironically, direct observations of diazotrophic activity at BATS, at least by the cyanobacterium *Trichodesmium,* show that N_2 fixation is relatively low (0.015 mol N $m^{-2}y^{-1}$), is restricted to the summer and fall months and is highly variable from year to year (Orcutt and others, 2001; Bates and Hansell, 2004). At BATS, positive N*

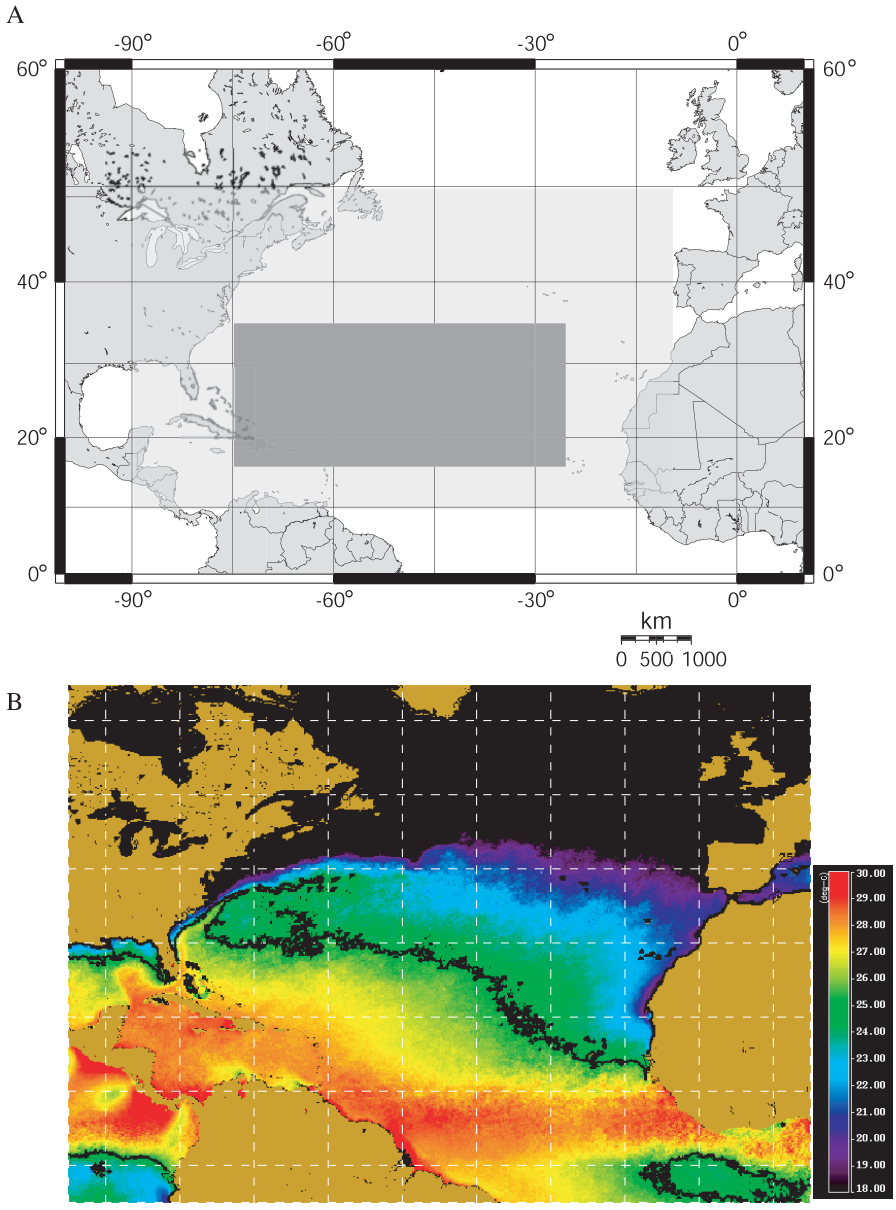

Fig. 3. Map of the North Atlantic Ocean illustrating the domain areas over which geochemical and direct estimates of areal rates of N_2 fixation are scaled to estimate basin-scale rates: (A) light gray shading represents an area of ~27.8×10^6 km^2 (10° to 50°N, 10° to 90°W; Gruber and Sarmiento, 1997) and dark gray shading represents 6.8×10^6 km^2 (15° to 25°, 25° to 75° W; Hansell and others, 2004) (B) sea surface temperature over the North Atlantic, the black lines indicating the 25° C isotherm over which direct estimate of N_2 fixation are scaled to represent basin scale rates.

anomalies (2.95-5.13 μmol kg^{-1}) are observed throughout the subtropical mode water (STMW, σ_θ = 26.4-26.6; Bates and Hansell, 2004). Large seasonal and inter-annual variations in N* (as much as 5 μmol kg^{-1}) are observed at BATS over a 13 year period (1988-2001). While analytical precision and accuracy can account for 5 to 15 percent

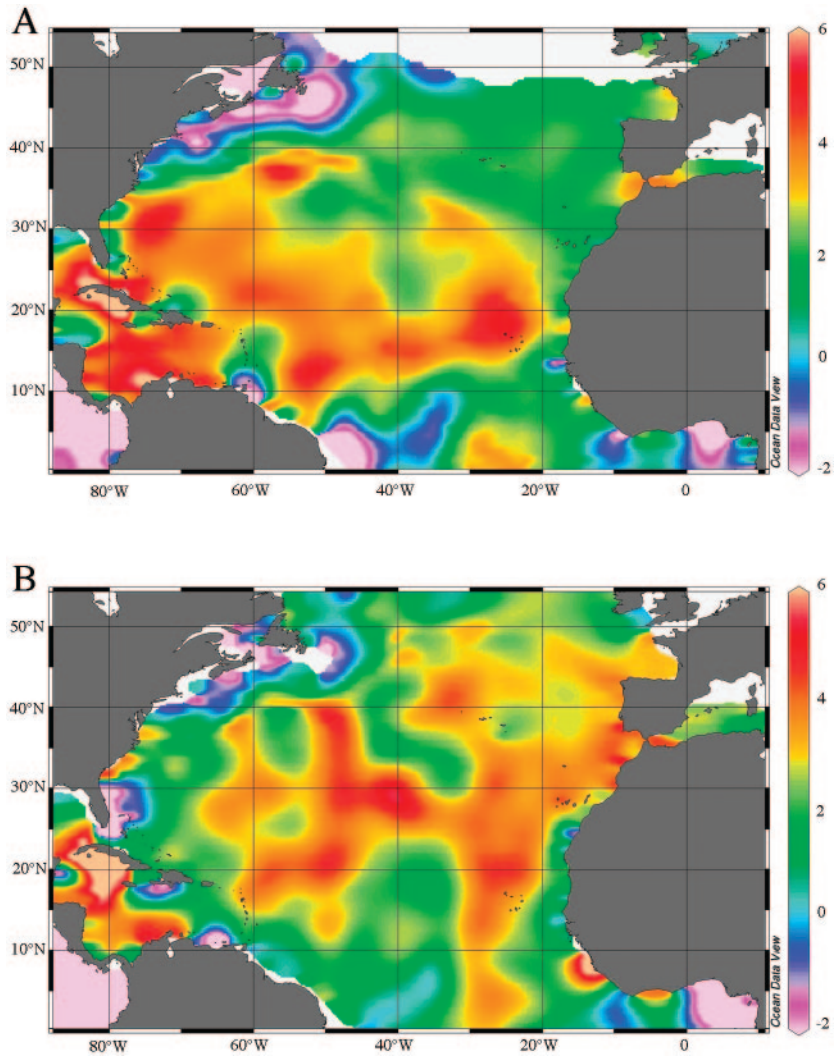

Fig. 4. Distribution of N* [Gruber and Sarmiento, 1997] in the tropical and subtropical North Atlantic. N* was derived according to Gruber and Sarmiento [1997] using objectively analyzed 1° nutrient fields from the World Ocean Atlas 2001 (http://www.nodc.noaa.gov/OC5/WOA01/) for the North Atlantic. (A) N* (μmol/kg) on the isopycnal surface (σ_θ) = 26.5 kg/m^3 representing the Subtropical Mode (18°) Water. (B) N* (μmol/kg) distribution on the isopycnal surface (σ_θ) = 27.1, representing the Subpolar Mode water. Figure was created by J. Montoya using Ocean Data View (Schlitzer, 2004) from Capone and others, 2005a.

(2.56-2.65 μmol kg^{-1}) of the variability in N*, between cruise variability was higher (2.9 μmol kg^{-1}) and therefore temporal changes in N* have been interpreted as a reflection in the strength of N_2 fixation at BATS or the surrounding subtropical waters (Bates and Hansell, 2004; see also Pahlow and Riebesell, 2000). However, due to the lag time in the remineralization of surface organic matter in the thermocline, as well as both horizontal and vertical fluxes promoting mixing, no direct link exists between the "event," N_2 fixation, and the "signal," excess nitrate in the thermocline (Lipschultz and others, 2002; Bates and Hansell, 2004). Therefore, it is not possible to infer N_2 fixation in the surface ocean overlying a region of excess nitrate in the thermocline, but rather,

it is the gradient in N*, created by horizontal transport and mixing process, which will be indicative of the source region. Bates and Hansell (2004) attempt to reconcile the temporal variation in N* and the mismatch in surface processes and thermocline properties by proposing a complex series of interactions between N_2 fixation, atmospheric dust, variability in the North Atlantic Oscillations (NAO) and formation of STMW. They suggest that during positive NAO years (1989-1994, 1997-2000), enhanced stratification stimulates N_2 fixation and thus excess nitrate production (N* >3.83 μmol kg^{-1}), in contrast to negative NAO years (1995-1996, 2001), when N* is generally lower (<3.39 μmol kg^{-1}). Integrated excess nitrate accumulation at BATS suggests that N_2 fixation contributes <0.06 to 0.34×10^{12} mol N y^{-1} (Bates and Hansell, 2004), similar to estimates by Hansell and others (2004).

Comparison of meridional transects (Bates and Hansell, 2004; Hansell and others, 2004) and modeling efforts (Siegel and Deuser, 1997) infer that elevated N* anomalies observed at BATS are being advected along isopycnal surfaces from the south, most likely from the Caribbean and western tropical Atlantic, a region where dense populations of *Trichodesmium* (Carpenter and others, 2004) and prolific N_2 diazotrophic activity has been observed, (Montoya and others, 2002; Capone and others, 2005a). Water arriving at BATS has a preformed N* value of 2.9 μmol kg^{-1} (Bates and Hansell, 2004) and thus any variation at BATS may be more intrinsically linked to variability in the N_2 fixation in the Caribbean or the strength of the horizontal transport processes between the Caribbean and BATS. Northward advection of DON has been suggested as a likely vehicle for this excess nitrate signal. DON consists of a continuum of reactive pools, which are remineralized on timescales of hours to days for the more labile fractions, to months to years for the more recalcitrant fractions, thus surviving remineralization during transport through a nutrient depleted gyre. Indeed, elevated N* is observed in the Caribbean and western Tropical Atlantic (Capone and others, 2005a) (fig. 4).

In contrast to the Atlantic basin, negative N* anomalies are generally observed across the entire Pacific basin, with few exceptions (Deutsch and others, 2001). Strong east-west gradients in N* are maintained by negative N* anomalies (N* <-11 μmol kg^{-1}) in the eastern tropical north and south Pacific (ETNP and ETSP, respectively) and positive N* anomalies (~ 1 μmol kg^{-1}) in the north- and southwest of the basin. Exchange and mixing of these N* end members are facilitated by equatorial currents and isopycnal transfer across the basin (Gruber and Sarmiento, 1997; Deutsch and others, 2001). Negative N* anomalies are also observed in the Bering Sea and south Pacific (<-2 μmol kg^{-1}). This pattern in N* supports the co-occurrence of N_2 fixation and denitrification in the Pacific Ocean. Direct studies have demonstrated that the ETNP (Hattori, 1983) and ETSP (Codispoti and others, 1986) are sites of intense denitrification. Near zero oxygen concentrations and high vertical particulate fluxes fuelled by upwelling of nutrients creates a competitive advantage for denitrifying bacteria (Cline and Kaplan, 1975; Codispoti and Richards, 1976). The northwest Pacific and China Sea are regions of high diazotrophic activity (Carpenter and Capone, 1992; Liu and others, 1996; Hansell and Feely, 2000; Wu and others, 2003). Such patterns require deconvolution of N* on a local rather than basin scale. Deutsch and others (2001) used the segregation of denitrification and N_2 fixation (east versus west basin, oxygenated versus anoxic waters) to quantify N inputs and losses in the west and east Pacific, respectively. Using a similar approach to Gruber and Sarmiento (1997), denitrification was estimated to remove 3.4×10^{12} mol N y^{-1} in the eastern tropical Pacific (Deutsch and others, 2001). However, this approach could not be applied to regions of positive N* anomalies in the western Pacific, due to difficultly in estimating water mass ages containing elevated N*, which appeared at the surface

rather than deeper in the water column. A N budget box model revealed that N_2 fixation in the entire Pacific ocean accounted for 4.2×10^{12} mols N y^{-1} (Deutsch and others, 2001; table 1). In the Pacific Ocean, the conservative behavior of N* implies that N_2 fixation is low over the entire Pacific Ocean in comparison to denitrification, or that denitrification completely masks the excess nitrate signature of N_2 fixation. This is in stark contrast to the Atlantic, where the non-conservative behavior of N* implies that biological process have a greater effect on the distribution of excess nitrate than physical processes.

At station ALOHA, the Hawaii Ocean Times-series (HOT) site in the tropical North Pacific, an invaluable data set collected for a period of 15 years reveals that the N:P ratio in the dissolved inorganic and total dissolved pools is highly variable (Karl and others, 2001b). In contrast to N:P ratios at BATS, which usually exceed 16:1 above 1000 m (Cavender-Bares and others, 2001), the ratio between nitrate and phosphate rarely reaches 16:1 at the HOT site, station ALOHA, but varies between ~10 to 15 below 100 m. The ratio of total dissolved N to P (TDN:TDP) varies between ~8 to 30 in surface waters and is less than 16 below 500 m.

Temporal variations in the N:P ratio at HOT are explained by a combination of uncoupling between nitrate and phosphate, intense periods of N_2 fixation, and shifts in ENSO periods (Karl and others, 1997, 2002). Karl and others (1997, 2001a) propose that there has been a decadal increase in the abundance and activity of diazotrophs due to increased stratification in response to climate change. Using an independent two-component N source model based upon variations of the N:P ratio of inputs and exports of the dissolved inorganic and particulate pools, Karl and others (1997) estimated that 32 percent of the particulate N, equivalent to 93 μmols N m^{-2} d^{-1} (table 1) is from N_2 fixation at this Pacific site on the cusp of the tropics. Indeed, the increase in N_2 fixation is potentially responsible for the 35 percent increase in export production over the past 8 years in the Pacific Ocean (Emerson and others, 2001).

*Conundrums of N**

N* anomalies are apparently formed by the downward flux and remineralization of N rich particulate organic matter or the removal of nitrate by denitrification. Process other than N_2 fixation can potentially increase the N:P ratio of the dissolved inorganic nutrient pool (Karl and others, 2002) and thus, interpretation of N* has its limitations. Differential remineralization of organic P relative to organic N can act to retain P within the mixed layer and export N rich organic matter into the thermocline, therefore providing a source of excess nitrate. This differential lability should create shallow areas of low nitrate: phosphate as the more labile P is removed preferentially, followed by sequentially higher N:P in the sinking pools with depth. This pattern is not obvious in flux or nutrient data. This scenario is also difficult to constrain due to our lack of knowledge regarding the bioavailability and rates of remineralization of the largely uncharacterized organic pool. The critical factor in determining the potential of DON as a source of excess nitrate is knowledge of the timescales of DON export versus the residence time of the organic pool in the mixed layer. If DON is exported into the thermocline faster than it can be remineralized in the mixed layer, then its potential in creating an excess nitrate signal is high. On the other hand, if it is retained in the mixed layer and remineralized, excess nitrate will not be created in the thermocline. Gruber (2004) refutes this scenario, arguing that preferential remineralization of P would alter the N* profile, causing a minima in the surface layers, but a decrease with depth. In addition, horizontal advection and remineralization of dissolved organic matter with an elevated N:P ratio (Karl and others, 2002; Mahaffey and others, 2004; Abell and others, 2005) may be important in generating a positive N* anomaly in the upper thermocline, but there are few basin scale studies of the total

dissolved N and P pools to qualify the role of dissolved organic matter in excess nitrate formation.

An assessment of the atmospheric deposition of N not only revealed that the atmospheric input of N in the North Atlantic (17-21 mmol N m^{-2} y^{-1}; Prospero and others, 1996) was similar to that supplied by N_2 fixation but that the N:P ratio of these atmospheric inputs can be as high as 175. Similar elevated N:P ratios have been observed in dust events to the Mediterranean, the N:P ratio ranging from 18 to 288 (Duce, 1986; Herut and others, 2002) and in aerosols over the Atlantic Ocean (Baker and others, 2003). Although deposition and dissolution of atmospheric fallout with elevated N:P ratios provide an alternative source of excess nitrate in the ocean, the exact mechanism that transports these atmospheric inputs from the surface into the main thermocline is largely unknown.

While basin scale studies in the Atlantic and Pacific Oceans have used nutrient data sets collected over decades, temporal variation in the data sets, similar to that observed at BATS and HOT, is proscribed and "data consistency" corrections applied so that the spatial distribution covered during these globally extensive studies is much greater than the temporal variation expected (compare, Pahlow and Riebesell, 2000) and therefore considered negligible (Gruber and Sarmiento, 1997). The conceptual theories and insight the oceanographic community gains from N* is only as reliable as the nutrient data set used to derive this tracer, which relies on isolating small deviations in a ratio between two significantly and globally correlated elements. Gruber and Sarmiento (1997) and Deutsch and others (2001) applied "corrections" to nutrient data and (Hansell and others, 2004) omitted large sections of WOCE data to maintain what they thought was the appropriate data quality. In addition, estimates of N_2 fixation are highly sensitive to the determination of water mass age and identification of the site of isopycnal outcropping (Doney and others, 1997; Jenkins and Doney, 2003).

Stable Nitrogen Isotopes

Natural abundance stable isotopes [^{15}N:^{14}N ratio, generally expressed as $\delta^{15}N$ in permil (‰)] can provide an independent and integrative measure of the contribution of N_2 fixation to the upper ocean N budget (Liu and others, 1996; Carpenter and others, 1997; Karl and others, 1997; Capone and others, 2005a). Principally, two factors determine the $\delta^{15}N$ of living phytoplankton; (1) the $\delta^{15}N$ of the nutrient source and (2) the isotopic discrimination between ^{15}N and ^{14}N during the uptake and assimilation of the nutrient (Mariotti and others, 1981). The enrichment factor, "ε", a manifestation of isotope discrimination is defined as $(R_p/R_s - 1) * 1000$ where R_p and R_s are the isotopic ratios of the products and substrates, respectively. It is usually a negative value. The $\delta^{15}N$ of dissolved N_2 is ~0.6 permil, near that of atmospheric N_2 (~0‰). Little isotopic fractionation occurs during the fixation of N_2 (-2.6 ± 1.3 ‰; Hoering and Ford, 1960; Delwiche and others, 1979; Beaumont and others, 2000; Brandes and Devol, 2002), resulting in the $\delta^{15}N$ of organic matter derived from N_2 fixation being close to zero (-2.1 to $+2.9$; Wada and Hattori, 1976; Saino and Hattori, 1987; Carpenter and others, 1997). In contrast, the $\delta^{15}N\text{-}NO_3^-$ of deep ocean nitrate (>800 m) is reported to range between 5 and 6.5 permil (Sigman and others, 1999; Montoya and others, 2002; Knapp and others, 2005; Sigman and others, 2005). Nitrate from the deep ocean may be introduced into the surface ocean by vertical diffusion, upwelling or through episodic injections such as eddies. While under nutrient replete conditions, isotopic discrimination by assimilatory nitrate uptake may result in the biomass produced being slightly depleted relative to the source, complete utilization of this nitrate pool in a "closed" system results in the $\delta^{15}N$ particulate organic nitrogen

(PON) reflecting that of the injected $\delta^{15}N\text{-}NO_3^-$ (Waser and others, 1998a, 1998b, 2000). Numerous studies have used a two-end member source model of $\delta^{15}N\text{-}N_2$ (close to 0 permil) and $\delta^{15}N\text{-}NO_3^-$ (5-6.5 ‰, representing the mean deep ocean $\delta^{15}N\text{-}NO_3^-$) to determine the relative contribution of N_2 fixation and nitrate to the N supply of phytoplankton in the surface ocean (Karl and others, 1997; Dore and others, 2002; Mahaffey and others, 2003; Capone and others, 2005a).

In surface waters, catabolism of organic N by metazoans (Checkley and Miller, 1989) and bacterial degradation (Altabet, 1988) causes ^{15}N enrichment of residual PON, and thus retains ^{14}N in the surface ocean. Sinking and remineralization of ^{15}N-enriched PON from the euphotic is therefore responsible for the near constant $\delta^{15}N\text{-}NO_3^-$ in the deep ocean (>800 m; Sigman and others, 1999). However, Liu and others (1996) observed ^{15}N-depleted nitrate (as low as −0.5 ‰) in the upper layers (<500m) of the Kuroshio Current northeast of Taiwan, an area of significant *Trichodesmium* abundance and activity (Wu and others, 2003). Sinking and remineralization of ^{15}N depleted organic matter derived from N_2 fixation is a potential substrate for such observations. Indeed, this phenomenon of ^{15}N depleted nitrate has also been observed in the eastern Pacific and Arabian Sea (Brandes and others, 1998) and at BATS in the Sargasso Sea (Knapp and others, 2005) (see below).

Do $\delta^{15}N\text{-}NO_3$ distributions support excess nitrate distributions?—The inherent problems with the interpretation of N* (see above) may be unraveled by the parallel analysis of $\delta^{15}N\text{-}NO_3^-$ (Brandes and others, 1998; Karl and others, 2002; Gruber, 2004; Sutka and others, 2004). While elevated N* anomalies and ^{15}N depleted NO_3^- infer regions of N_2 fixation, low N* anomalies and ^{15}N enriched NO_3^- are potentially diagnostic of regions of denitrification (Gruber, 2004). Using a similar approach, rates of denitrification have been estimated in the Santa Barbara Basin (Sigman and others, 2003) and in the Cariaco Basin (Thunell and others, 2004).

Elevated N* anomalies and ^{15}N depleted nitrate (to ~ 1.5 ‰), indicative of sinking and remineralization of ^{15}N depleted organic matter, have been observed in mode waters of the Sargasso Sea (Altabet and others, 1999). If the temporal variation in N* at BATS is reflecting the effects of climatic forcing on the seasonal and interannual variations in the intensity of N_2 fixation (see "*N_2 Fixation Perturbs Redfield Stoichiometry*" above, and Hansell and others, 2004), one would expect the $\delta^{15}N\text{-}NO_3^-$ to change in a similar manner. While no parallel data set for $\delta^{15}N\text{-}NO_3^-$ exists at BATS for this time period (1988-2001), other studies have provided concurrent $\delta^{15}N\text{-}NO_3^-$ and $\delta^{15}N$ of particulate and total organic N measurements (Altabet, 1988; Knapp and others, 2005). Isotope mass balance between the $\delta^{15}N$ of exported PON ($\delta^{15}N$ ~3.7‰) and $\delta^{15}N\text{-}NO_3^-$ ($\delta^{15}N$ ~3.5 ‰) provided the first stable isotopic evidence that N_2 fixation rates were sufficiently low not to be recorded in the water column N isotope budget at BATS (Altabet, 1988). One significant caveat for this observation is that the estimates of sinking fluxes with sediment traps of the type used by Altabet (1988) and others are complicated by the presence of so-called "swimmers" (Michaels and others, 1990). These zooplankton tend to have elevated $\delta^{15}N$ signatures which may therefore bias the $\delta^{15}N$ estimate for the total flux. If the true export flux had a lower $\delta^{15}N$, it would proportionally increase the required amount of N_2 fixation in the budget.

More recently, Knapp and others (2005) have attempted to reconcile the seasonal differences between biological and geochemical estimates of N_2 fixation at BATS by determining the ^{15}N signatures in the nitrate and total N (TN) pools over a one year period (June 2000 to May 2001). Vertical profiles reveal ^{15}N-depleted nitrate in the shallow thermocline (~1.8 to 4 ‰) relative to the global average at depths greater than 800m (5 to 6 ‰; Sigman and others, 1999; Knapp and others, 2005). More importantly, over the time period of the study, the surface $\delta^{15}N\text{-}NO_3^-$ (<250m)

co-varied with nitrate concentrations, ^{15}N enriched nitrate (>2.5 ‰) being observed during winter months and ^{15}N depleted nitrate (<2.5 ‰) being observed during summer months. Winter-time ventilation and possibly eddy-induced injections of sub-euphotic zone ^{15}N-enriched nitrate into surface waters (McGillicuddy and others, 1999) are thought to be responsible for winter time observations. Any contributions of ^{14}N from N_2 fixation in the summer months would be rapidly dissipated during winter ventilation. To account for the source of ^{15}N depleted nitrate at the BATS site, Knapp and others (2005) used a simple mass balance model of $\delta\ ^{15}N$ of total organic N (TON) in the surface and deep ocean. They conclude that oxidation of a ^{15}N depleted semi-labile component of the TON pool was sufficient to maintain a flux of ^{15}N depleted N to the thermocline at BATS and that again, N_2 fixation was not required to balance the isotope budget. In a similar manner, Sutka and others (2004) used nitrification to explain ^{15}N-depleted nitrate on a west-east transect in the north Pacific. Multi-year investigation of ^{15}N dynamics at a site of intense diazotrophic activity would be an ideal platform from which to investigate the contribution of N_2 fixation to the water column N isotope budget.

As mentioned above, the proximal co-occurrence of N_2 fixation and denitrification can confound the interpretation of N*. This can also be a factor in the intepretation of $\delta^{15}N$ in nitrate as each process drives the $\delta^{15}N$ signature in different directions. The development of new analytical techniques for the measurement of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O$ in seawater (Sigman and others, 2001; Casciotti and others, 2002) provide a means for resolving the confounding effects that N_2 fixation and denitrification can have on N* as well as on the $\delta^{15}N$ of NO_3^- (Sigman and others, 2005). While the isotopic discrimination by nitrate assimilation for N and O in nitrate are equivalent, as are the ε for N and O by denitrification, nitrification only affects N and can thereby leave an isotopic imprint. Where recently fixed N is being nitrified this can result in an isotopic anomaly between the $\delta^{18}O$ and $\delta^{15}N$ in nitrate. For a set of stations taken between Santa Barbara and the southern tip of Baja California, Mexico (Sigman and others, 2005), the antagonistic effects of N_2 fixation and DIN loss on $\delta^{15}N\text{-}NO_3^-$ were disentangled by this dual isotope analysis of NO_3^-. N_2 fixation manifested itself as a negative anomaly (lighter ^{15}N than would be predicted based on ^{18}O), resulting from the different effects nitrification of N_2 fixer biomass has on $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O$, as described above.

N_2 fixation and dissolved organic nitrogen.—DON dominates the total dissolved nitrogen pool in the surface waters of the open ocean, representing 80 to almost 100 percent of the surface total dissolved N pool (Karl and others, 2001b; Mahaffey and others, 2004). ^{15}N tracers experiments reveal that N_2 fixers such as *Trichodesmium* can contribute significantly to the DON pool (Glibert and Bronk, 1994; Mulholland and others, 2004), exuding 50 to 100 percent of their fixed N as amino acids (Capone and others, 1994). Robust changes in $\delta^{15}N$ of individual amino acids between a producer or "source," and the consumer or "sink" (McClelland and Montoya, 2002), have been used to investigate the direct link between N_2 fixation and zooplankton (250-500 μm size class) in the tropical North Atlantic (McClelland and others, 2003). In laboratory and field populations of *Trichodesmium* spp., McClelland and others (2003) found that the $\delta^{15}N$ of all amino acids were <0 permil (except aspartic acid) and the biomass $\delta^{15}N$ of *Trichodesmium* spp. ranged from −2.2 to +0.5 permil. The authors found that the contribution of N_2 fixation can be traced using the $\delta^{15}N$ of phenylalanine, while the trophic position can be determined from the difference between the $\delta^{15}N$ of glutamic acid (glut) and phenylalanine ($\Delta\ \delta^{15}N$ glut-phe, respectively). In the tropical North Atlantic, McClelland and others (2003) observed little change in the trophic level of the zooplankton (constant $\Delta\ \delta^{15}N$ glut-phe), but a decrease in the $\delta^{15}N$ of the N

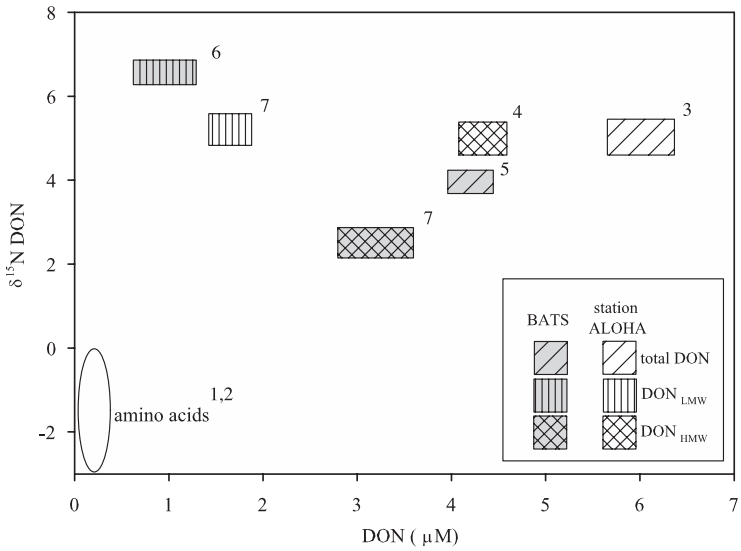

Fig. 5. A comparison of the concentration of dissolved organic nitrogen (DON; μM) and the stable nitrogen (N) isotopic composition (δ^{15}N; permil) of the bulk dissolved organic nitrogen pool (diagonal stripes) and its high molecular (checked) and low molecular weight (vertical stripes) components (DON_{HMW} and DON_{LWM}, respectively) at the Bermuda Atlantic Time Series in the Sargasso Sea (shaded) and (b) station ALOHA in the tropical North Pacific (unshaded). The δ^{15}N DON_{LMW} is derived by isotope mass balance. Sources: 1.) Mahaffey and others, 2004; 2.) McClelland and others, 2003; 3.) Angela Knapp, personal communication; 4.) Claire Mahaffey, unpublished data; 5.) Knapp and others, 2005; 6.) Benner and others, 1997; 7.) δ^{15}N DON_{LMW} calculated by isotope mass balance.

source, derived from the decrease in δ^{15}N of phenylalanine. This reinforces previous observations of the increasing westward importance of N_2 fixation in the tropical Atlantic (Montoya and others, 2002).

The DON pool is not only composed of amino acids but an array of molecules which are defined along a continuum of size, structure and biological availability (Benner, 2002; Bronk, 2002). For studies focusing on the isolation of high molecular weight dissolved organic matter ($DOM_{HMW;}$ for example, >1000 daltons) by tangential flow ultrafiltration, molecular and age characterization have revealed that the DOM_{HMW} pool is the younger and more biologically reactive than low molecular weight DOM (DOM_{LMW}), which is more aged and recalcitrant (Guo and other, 1996; Loh and others, 2004). Thus, one would expect the isotope signature of recently exuded DON from N_2 fixers to be recorded in the DOM_{HMW}. During a cruise in the tropical north Pacific in summer 2003, the δ^{15}N of TON and DON_{HMW} was determined to be ~5 permil (Angela Knapp, personal communication) and 5.2 ± 0.5 permil (unpublished data, Claire Mahaffey), respectively, in the surface ocean (0-20m). Assuming that HMW DON represents 20 to 36 percent of the total DON pool (McCarthy and others, 1996; Benner and others, 1997), the remaining 74 to 80 percent of the DON pool would have a δ^{15}N value of 4.9 permil (fig. 5). A similar isotope mass balance approach can be applied at BATS in the Sargasso Sea. The concentration and δ^{15}N of TON and DON_{HMW} is reported to be 4.2 ± 0.5 μM and 3.5 to 4.5 permil (Knapp and others, 2005), and 0.96 μM and 6.6 permil (Benner and others, 1997) in the surface ocean, respectively. Thus, the remaining 77 percent of the DON pool has a δ^{15}N signature of 2.57 to 3.74 permil at BATS (fig. 5). Therefore, at both these sites in the tropical North Pacific and subtropical North Atlantic, the ^{15}N-depleted signature of N_2 fixation is not

recorded in either the total DON pool, DOM_{HMW} or the DOM_{LMW} pool, but instead, this complex organic pool appears to be significantly enriched in ^{15}N compared to DON directly associated with N_2 fixers (McClelland and others, 2003). One explanation for this observation is that amino acids, which represent a small fraction of the total DON pool in the open ocean (dissolved free amino acids <1 percent, total hydrolysable amino acids <10 percent (Mahaffey and others, 2004) are highly labile with turnover times on the scale of hours to days (Fuhrman and Ferguson, 1986) and thus are rapidly assimilated by heterotrophs. In addition, McCarthy and others (2004) suggest that there exists a non-discriminating pathway by which recently formed autotrophic biomolecules are shunted into a recalcitrant pool. The effects of such processes on the ^{15}N:^{14}N exchange are presently unknown.

While rates of N_2 fixation cannot be directly gleaned from the stable isotope studies of the DON pool, such studies allow a more comprehensive assessment of the inputs and pathways of ^{15}N depleted N from N_2 fixation through the marine N cycle. A great deal of laboratory and field work investigating the ^{15}N dynamics of this complex N pool is required before $\delta^{15}N$ DON can be employed in isotope budgets of the N cycle.

A conundrum exists in the comparison of DON dynamics between station ALOHA in the tropical North Pacific and BATS in the subtropical North Atlantic. While no seasonal variability in surface DON concentrations exist in the surface waters (0-175m) at station ALOHA, a 9 percent increase in the DON inventory has been observed over an 11 year period (Karl and others, 1997; Church and others, 2002). In comparison, DON concentrations appear to be highly conservative at BATS in comparison (Carlson and others, 1994; Hansell and Carlson, 2001), with seasonal rather than interannual variability being observed. At station ALOHA, a decadal enhancement in N_2 fixation, as well as the lack of seasonal pumping has been deemed responsible for the accumulation of surface water DON (Karl and others, 1997, 2001b; Church and others, 2002). In contrast, winter convective mixing at BATS causes a seasonal downward flux of DON from the surface to the deep ocean, thus resetting the surface net seasonal accumulation of DON to zero each year (Carlson and others, 1994).

Distribution of $\delta^{15}N$ of particulate matter.—Extensive regions of ^{15}N depleted suspended PON and zooplankton in the tropical north Atlantic are in agreement with elevated N* anomalies indicative of N_2 fixation (Montoya and others, 2002; Mino and others, 2002; Mahaffey and others, 2003). In the central and western tropical Atlantic, an east-west depletion in ^{15}N of suspended PON (1-2 ‰) and zooplankton in the mixed layer was concomitant with increases in *Trichodesium* spp. abundance, suggesting that N_2 fixation may be more intense in the western rather than eastern tropical Atlantic during this cruise (Montoya and others, 2002). Latitudinal variations in surface $\delta^{15}N$ PON between 50°N and 50°S along 20°W in the eastern Atlantic Ocean reveal ^{15}N depleted PON between 0 to 15°N during a fall occupation (Mino and others, 2002) and between 25 and 30°N during a spring occupation (Mahaffey and others, 2003), supporting the seasonal and interannual variability in *Trichodesmium* spp. abundance in the eastern tropical Atlantic from direct observations (Tyrrell and others, 2002) and elevated N* anomalies (Hansell and others, 2004). Using similar isotope mixing models as above, N_2 fixation was estimated to supply 13 to 63 percent of the N as above input to the surface ocean in these studies (Montoya and others, 2002; Mino and others, 2002; Mahaffey and others, 2003).

^{15}N depleted PON in surface waters is also indicative of assimilation of ^{15}N depleted ammonium excreted by zooplankton (Mullin and others, 1984; Checkley and Miller, 1989; Montoya and others, 2002) and in the open ocean, must be considered as an alternative explanation for ^{15}N depleted PON. Therefore, being able to decipher if

^{15}N-depleted PON signatures are due to assimilation of regenerated N or N_2 fixation would be an invaluable tool in the open ocean.

Methodological advancements in Gas Chromatography/Combustion/Isotope Ratio Mass Spectrometer (GC/C/IRMS) (McClelland and Montoya, 2002) and preparative High Performance Liquid Chromatography (HPLC) techniques (Sachs and others, 1999) have prompted a revolution in the analysis of compound specific stable isotopes to track N from its source to sink. Three recent studies employing compound specific δ^{15}N signatures of chlorophyll *a* (Beaumont and others, 2000; Pantoja and others, 2002) and amino acids (McClelland and others, 2003; see above) have provided conclusive evidence of N_2 fixation as the N source.

The δ^{15}N of chlorophyll *a* is an isotopic proxy for photoautotrophs (Sachs and Repeta, 1999) and thus eliminates isotopic effects of heterotrophs and detrital material. A near constant offset is thought to exist between cellular N and chlorophyll *a* in various phytoplankton and bacteria cultures (5.27-7.5 ‰; Sachs and others, 1999; Beaumont and others, 2000; Pantoja and others, 2002), such that the δ^{15}N of chlorophyll *a* allows the δ^{15}N of algal or bacterial biomass to be specifically determined. In a study in the Mediterranean, Pantoja and others (2002) observed a west-east decrease in the δ^{15}N chlorophyll *a* from -3.3 ± 1.8 to -7.1 ± 1.3 permil, translated to 1.8 ± 1.8 permil and -2.0 ± 1.3 permil for δ^{15}N of algal biomass, implying that diazotrophic activity was the dominant N source in the eastern Mediterranean (see below). Beaumont and others (2000) have taken this molecular specific isotope approach one step further by analyzing the bacteriochlorophyll *a,* chlorophyll *a* and lipid extracts from two N_2 fixing bacteria, *Rhodobacter capsulatus* and *Anabaena cylindrical.*

While surface particles record nutrient utilization, as well as isotopic fractionation during assimilation and degradation, sinking particles represent the δ^{15}N of the new nutrient source (Altabet and others, 1991). At station ALOHA in the tropical North Pacific, a mean δ^{15}N sinking PON of 3.5 permil was observed at 1500m, ^{15}N increasing between the summer (1.53 ‰) and winter (4.83 ‰) fluxes. Again, assuming a simple two end member mixing model in which δ^{15}N NO_3^- is 6.5 permil, Karl and others (1997) estimate that N_2 fixation accounts for 48 percent of the N demand at this site (increasing from 20%-75% between winter and summer, respectively), which equates to an areal rate of N_2 fixation of 137 μmol N m^{-2} d^{-1} (table 1).

Conundrums in the $^{15}N/^{14}N$ distribution.—Stable isotopes are a robust and reliable technique for investigating the importance and contribution of N_2 fixation in the marine environment. In contrast to N*, which uses a series of assumptions during its derivation, stable isotopes applications may be flawed in their interpretation rather than derivation.

Estimates of N_2 fixation based on the ratio of ^{15}N to ^{14}N are highly sensitive to the choice of end member values. Accumulating evidence suggests that δ^{15}N NO_3^- between 200 and 400m, the depth interval that is responsible for a diffuse or rapid injection of nitrate in the tropical ocean, is depleted in ^{15}N (δ^{15}N NO_3^- ranging from ~1 to 4 ‰; Altabet, 1988; Liu and others, 1996; Montoya and others, 2002; Sutka and others, 2004; Knapp and others, 2005). If the δ^{15}N NO_3^- at station ALOHA is less than 4 permil, rather than 6.5 permil assumed by Karl and others (1997), then from a two end member source model and a δ^{15}N of 3.5 permil for sinking matter at station ALOHA, N_2 fixation would account for less than 15 percent of the N budget at station ALOHA . In the same way, if δ^{15}N NO_3^- at BATS was assumed to equal the global average of 5 to 6 permil, the estimated contribution of N_2 fixation would be roughly 50 percent of the N demand in this region. One interesting aspect of the box models that use the δ^{15}N balance in the upper ocean is that the choice of boundaries influences the outcome. The light δ^{15}N-NO_3 just below the euphotic zone is what is upwelled and, if it is similar

to the sinking material, it implies little N_2 fixation. Yet, some process must create and maintain this vertical gradient in $\delta^{15}N\text{-}NO_3$ below this arbitrary boundary and the number of candidate processes below the surface is small.

There is accumulating evidence that the $\delta^{15}N$ of inorganic and organic N compounds in dry particles and rain are ^{15}N depleted, the $\delta^{15}N$ ranging from -12 permil to $+5$ permil (Wada and Hattori, 1991; Cornell and others, 1995; Hastings and others, 2003) with organic N deposition accounting for up to one third of the total N deposition (Cornell and Jickells, 1999; Cornell and others, 2001). As mentioned above, the biological availability of this atmospheric N source is largely unknown and thus, the ability of phytoplankton to exploit such a N source in the open ocean is difficult to constrain. While earlier studies concluded that atmospheric N deposition was, in general, relatively minor quantitatively in ocean areas removed from continents (Knap and Jickells, 1986; Duce and others, 1991; Michaels and others, 1993), increasing source strength with human population growth will increase the impact of this input in the upcoming decades (Galloway and others, 2004).

Carbon Anomalies and N_2 Fixation

The oceans "biological pump" removes carbon from the ocean-atmosphere interface by the fixation of carbon into particles, the downward flux of these particles from the sunlit surface to the deep ocean, where they are remineralized to CO_2. In nutrient replete regions of the ocean (for example, upwelling regions), allochthonous nutrient inputs are accompanied by respired CO_2 (Eppley and Peterson, 1979; Lewis, 1992), leading to a net CO_2 flux to the atmosphere. In the stratified subtropical gyres, complete nutrient consumption leads to a net CO_2 flux from the atmosphere to the ocean. On the global scale, this leads to a global zero balance of the biologically induced air-sea flux of CO_2 (Murnane and others, 1999; Sarmiento and Gruber, 2002), implying that in a steady state ocean, export production fuelled appears to require little interaction with atmospheric CO_2.

The emerging awareness of a major ongoing perturbation of the global C cycle (Siegenthaler, 1986) and an anthropogenically induced increase in atmospheric carbon dioxide (CO_2) [Intergovernmental Panel on Climate Change (IPCC), 2001] has prompted further studies of the controls and constraints on primary production in the oceans. The strength and efficiency of the biological pump can be dramatically altered by the availability of nutrients and thus dramatically alter the air-sea flux of CO_2 (for example, Emerson and others, 1997, 2001; Dore and others, 2003; Sabine and others, 2004).

In the tropical ocean, export production is limited by the supply of nutrients to the surface layer. The potential for N_2 fixation to relieve nutrient limitation and fuel significant C fixation and particulate export (Eppley and Peterson, 1979; Lewis, 1992) has only been recently fully recognized (Michaels and others, 1994; Karl and others, 1997). In the tropical north Atlantic, carbon fixation rates by *Trichodesmium* sp. were found to exceed 67 mmols C m^{-2} d^{-1} and accounted for 8 to 47 percent of the water column primary production (Carpenter and others, 2004). Such estimates exceed primary production rates typically quoted for nutrient-impoverished environments (10-44 mmol C m^{-2} d^{-1}; Eppley and Peterson, 1979; Neuer and others, 2002). Subsequently, N_2 fixation has been deemed a potential cause of significant dissolved inorganic C (DIC) drawdown in the absence of nitrogenous nutrients, the DIC anomalies providing an indirect means of estimating local and basin scale estimates of N_2 fixation in the tropical Atlantic (Gruber and others, 1998; Lee and others, 2002; Anderson and Pondaven, 2003).

Derivation of new production from the annual decrease in inorganic C (C_t inventory) in nitrate-depleted waters of the Atlantic Ocean (40°N to 40°S) reveals that 1.6×10^{13} mol organic carbon are produced in excess of that accounted for by the

supply of nitrate from the sub-euphotic zone (Lee and others, 2002). Assuming that the observed C production was fuelled by diazotrophic activity, then 2.4×10^{12} mol N y^{-1} (or an areally integrated rate of 134 μmol N m^{-2} d^{-1}) would be required to meet this demand (assuming a C:N ratio of 7). A number of studies at BATS have used a similar approach using C budgets (Michaels and others, 1994; Gruber and others, 1998) or ecosystem models (Anderson and Pondaven, 2003) to explain the inorganic C drawdown paradigm (in summer/fall) and dissolved organic C (DOC) accumulation (spring) at this subtropical site (Carlson and others, 1994). Using a 5 year time series of the inorganic C budgets, Gruber and others (1998) estimate a summer/fall (April to October, 156 days) drawdown of C of 40 ± 4 μmol kg^{-1} over the surface mixed layer in which nitrate was undetectable. Again, assuming diazotrophs are responsible for this C consumption, areally integrated N_2 fixation rates at BATS are computed to be 1006 μmol N m^{-2} d^{-1}. Using an ecosystem model embedded on a one-dimensional physical model, Anderson and Pondaven (2003) were able to capture the seasonal dynamics of C and N at the BATS site and estimated that an areal N_2 fixation rate of 482 μmol N m^{-2} d^{-1} could complete the C budget at BATS during the summer months (Anderson and Pondaven, 2003). These estimates by Gruber and others (1998) and (Anderson and Pondaven, 2003) are double to one order of magnitude greater than N_2 fixation rates estimated by (Lee and others, 2002) because in the former two studies, N_2 fixation is assumed to occur in the summer/fall months at BATS (82 to 159 days) whereas in the latter study, N_2 fixation is assumed to occur over the entire year. Using a domain area of 27.8×10^6 km^2 (Gruber and Sarmiento, 1997) (fig. 3A), C based estimates of areally integrated N_2 fixation range from 1.1 to 4.5×10^{12} mol N y^{-1}. Such extrapolations represent a higher bound compared to nutrient derived estimates (Gruber and Sarmiento, 1997; Hansell and others, 2004) and consider only one atypical subtropical site in the North Atlantic.

A Case Study: Excess Nitrate and Isotopes in the Mediterranean

The Mediterranean Sea provides an intriguing case study for the relative importance of N_2 fixation. Anomalous nutrient ratios have long been recognized in this system, with the highest N:P ratios of nitrate to phosphate in the eastern Mediterranean being observed during summer stratification (Krom and others, 1991; Marty and others, 2002). Nutrient budgets, phytopigment distributions and stable N isotope studies strongly imply that N_2 fixation is the likely source of this excess nitrate to the Mediterranean (Bethoux and Copin-Montegut, 1986; Marty and others, 2002; d'Alcala and others, 2003), although some studies argue for preferential input of N enriched atmospheric or continental sources (Coste and others, 1988; Herut and others, 1999; Kress and Herut, 2001).

In the Mediterranean, floristic studies do not indicate large populations of *Trichodesmium* or other planktonic macrodiazotrophs (Margalef, 1969) and there are few studies on diazotrophic activity. However, sedimentary records (Kemp and others, 1999; Sachs and Repeta, 1999) as well as seagrass studies (Bethoux and Copin-Montegut, 1986) provide evidence for the significance of N_2 fixation in the present and recent geological timeframe in this region.

The Mediterranean circulation is driven by excess evaporation over precipitation (Hecht and others, 1988), as well as an exchange of surface water (100-300m) with eastward flowing Atlantic Water at the Strait of Gibraltar (Prieur and Sournia, 1994). The importance of N_2 fixation in the Mediterranean is manifested as a strong positive N* anomaly in the main thermocline (value at 800-1000m) of the mid Atlantic. These waters are of Mediterranean origin and are estimated to have a source N* value of 4 μmol kg^{-1} (Gruber and Sarmiento, 1997).

A survey of the ^{15}N distribution in the Mediterranean Sea reveals an eastward decrease in surface $\delta^{15}N$ suspended PON (2.7 ± 1.2‰ to −0.2 ± 0.7‰), $\delta^{15}N$

chlorophyll *a* (2.6 ± 2.3‰ to −7.1 ± 1.3‰) and deep-water nitrate (3.4 ± 0.5‰ to 2.5 ± 0.1‰), implying an eastward increase in the contribution of N_2 fixation to the water column N budget (Pantoja and others, 2002). Using a two-end member source model (where $\delta^{15}N\text{-}NO_3^-$ is 3.4‰ and 2.5‰ for the west and east budgets, respectively, and $\delta^{15}N\text{-}N_2$ is −2.6‰) Pantoja and others (2002) estimate that N_2 fixation accounts for 20 to 90 percent of the N supply to the western and eastern Mediterranean, respectively, exceeding previous estimates (7-41%) based on nutrient budgets (Bethoux and Copin-Montegut, 1986).

In the eastern Mediterranean, new production is estimated to be 300 mmol N m^{-2} y^{-1} (Krom and others, 1991). Winter-time mixing and atmospheric inputs are estimated to supply 60 mmol N m^{-2} y^{-1} (Kress and Herut, 2001) and 17 mmols N m^{-2} y^{-1} (Herut and others, 1999) of new N to the eastern Mediterranean, respectively. Thus, 223 mmols N m^{-2} y^{-1} or 74 percent of new production remains unaccounted for and may be fuelled by N_2 fixation, which is agreement with Pantoja and others (2002).

direct observations and quantification of marine diazoptrophs

N_2 fixation was discovered in terrestrial ecosystems in the middle of the 1800's (Boussingault, 1838). Ironically, and as mentioned above, the earliest observations of N_2 fixers in the sea, and specifically *Trichodesmium* spp., lacked the knowledge of the functional significance of the organisms under study. For example, Wille (1904) mapped the distribution of this interesting organism on the Humboldt Expedition through the central Atlantic Ocean in 1899, over 60 years before the first reports of its diazotrophic ability (Dugdale and others, 1961; see above). Subsequent to Wille (1904), numerous other surveys provided information on the global distribution of *Trichodesmium* (summarized in Carpenter, 1983a) although quantitative studies of the biomass and distribution have only been recent (Carpenter and McCarthy, 1975; Carpenter and Price, 1976; Carpenter and others, 2004).

Other N_2 fixers have been identified in the sea. In the 1960's, N_2 fixing bacteria were first isolated from seawater (for example, Pshenen, 1963; see Capone, 1988 for a summary; also Carpenter, 1983a) and Japanese workers quantified densities of some heterotrophic diazotrophs (Maruyama and others, 1970). Using the $^{15}N_2$ technique, many sites of shallow benthic N_2 fixation were also identified (Stewart, 1965; Jones, 1974; Capone, 1983; Howarth and others, 1988b), with the advent of the sensitive C_2H_2 reduction method dramatically increasing the region over which benthic N_2 fixation was recognized (Capone, 1983).

In the planktonic realm, cyanobacterial symbionts of certain open ocean diatoms (Carpenter, 1983a; Villareal, 1994) and other phytoplankters (Carpenter and Foster, 2002) (figs. 6A-C) were also recognized as a potential source of recently fixed N. In addition, a number of studies found N_2 fixing bacteria associated with the intestinal flora of zooplankton (Proctor, 1997; Braun and others, 1999).

The discovery of large populations of coccoid cyanobacteria in the sea (Johnson and Sieburth, 1979; Waterbury and others, 1979; fig. 6D), coupled to the emergent recognition that some coccoid cyanobacteria are diazotrophic (Rippka and Waterbury, 1977) prompted speculation on their potential role in marine N_2 fixation (Carpenter, 1983a). Several groups reported isolation of marine coccoid diazotrophs (Mitsui and others, 1986; Waterbury and others, 1988).

Perhaps the most exciting recent development in marine N_2 fixation is the demonstration that diazotrophy potentially occurs in the smaller size (<10 μm) fraction of open ocean marine waters (Zehr and others, 2001). Transcripts of *nif* H genes associated with both coccoid cyanobacteria and heterotrophic alpha and gamma proteobacteria have been detected in tropical oligotrophic waters around Hawaii (Zehr and others, 2001; Falcón and others, 2004; Church and others, 2005) and in the N. Atlantic (Falcón and others, 2004). *Nif* H sequences from divergent proteobacteria

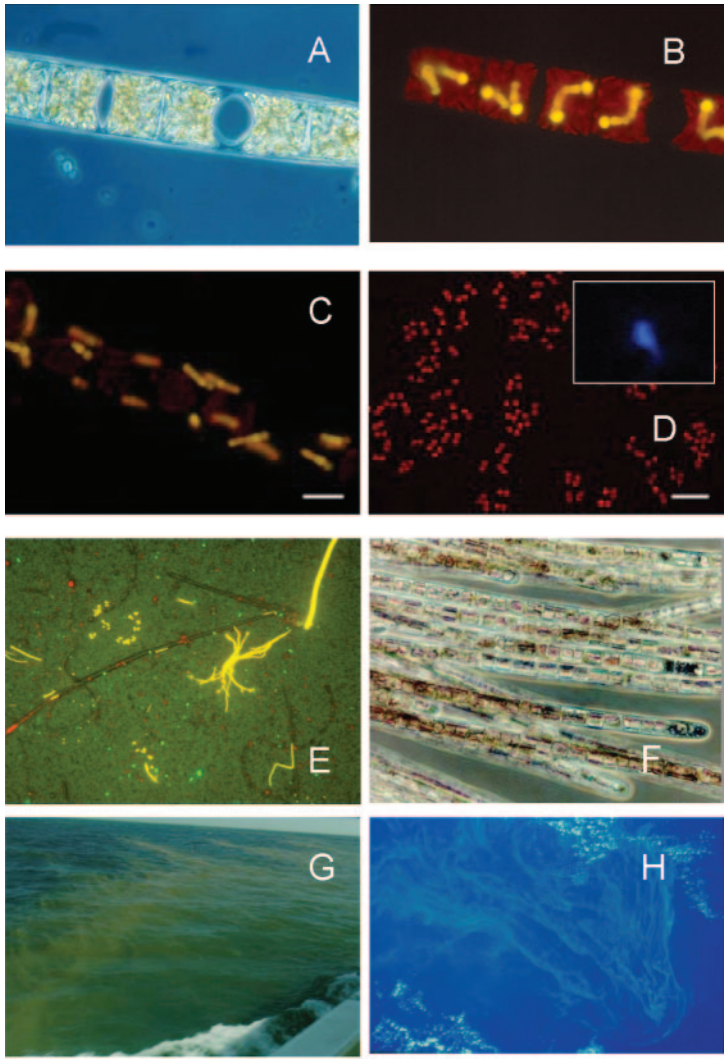

Fig. 6. Examples of photoautotrophic marine diazotrophs. (A) Light microscope and (B) epifluorescent images of *Rhizosolenia* spp. revealing its cyanobacterial endosymbiont, *Richelia intracellularis* (photos by Dave Caron); (C) epibiotic heterocystous cyanobacteria (*Calothrix* spp.) on a diatom (*Chaetoceros* spp.). Scale bar = μm (R. Foster); (D) Coccoid cyanobacteria illuminated by natural fluorescence of phycoerythrin. Scale bar = 50μm. Insert: coccoid cyanobacteria conjugated with a fluorescent antibody to nitrogenase (E. J. Carpenter); (E) Field of diazotrophic organisms including a small colony of *Trichodesmium,* the large filamentous cyanobacterium, *Katagnymene* (upper right), *Rhizosolenia* with symbiotic *Richelia* (in center, the red dots are the *Richelia*) (R. Foster). (F) Light micrograph of *Trichodesmium* trichomes each about 5 μm in diameter (photo by K. Ohki); (G) surface slick of *Trichodesmium* in the Capricorn Channel, southern great barrier reef (photo by D. Capone). (H) U.S. Space Shuttle photograph of the same region showing extensive surface slicks. The photo encompasses about 100 km across.

and Archaea have also been identified in deep sea hydrothermal vent and non-vent environments (Mehta and others, 2003).

Trichodesmium, the Obvious Source

Complementing the geochemical approaches, and analogous to the widely used procedures for estimating primary production (Steeman Nielsen, 1952; Koblentz-

Mishke and others, 1970), direct quantitative estimates of N_2 fixation have been undertaken for some time by various approaches. The most widely reported estimates have been those focused on the cosmopolitan, macroscopic forms of *Trichodesmium* (figs. 6E–H) using the C_2H_2 reduction method. Under typical field conditions in highly oligotrophic tropical waters *Trichodesmium* is relatively dilute (100-1000s trichomes L^{-1}; Carpenter and others, 2004). The colonial forms are easily concentrated using gentle plankton tows or filtration methods and isolated before tracer or enzymological assay (Goering and others, 1966; Capone and others, 2005a). N_2 fixation by *Trichodesmium* occurs only in the daylight (Saino and Hattori, 1978; Capone and others, 1990) and experiments are generally conducted under conditions of natural light in on deck incubators or *in situ*.

Trichodesmium may also occur as free trichomes to varying degrees in different systems (fig. 6E), adding to the challenge of accurately quantifying its contribution. Letelier and Karl (1996) noted at Station ALOHA that about 50 percent of the population occurred as free trichomes and contributed about half the overall N_2 fixation associated with this organism. Similarly, Orcutt and others (2001) reported a preponderance of free trichomes in a seasonal study at the BATS station, with colony abundances increasing sharply on transects to the south. They speculated that inclusion of free trichome N_2 fixation would increase their colony-based estimate by about 4-fold. In contrast, Carpenter and others (2004) found the bulk of *Trichodesmium* to occur in colonial form in studies focused in the tropical N. Atlantic.

Observations in the sub-tropics indicate very strong seasonality in *Trichodesmium* biomass and N_2 fixation: the bulk of the annual input of N through *Trichodesmium* N_2 fixation occurs in the summer (for example, Orcutt and others, 2001). Studies earlier in the year, and further to the east, find much lower abundances and rates of activity (McCarthy and Carpenter, 1979) (see for example, Tyrell and others, 2003; Voss and others, 2004; Mills and others, 2004). In the tropics, however, as *Trichodesmium* biomass increases (Orcutt and others, 2001; Carpenter and others, 2004), rates of N_2 fixation are substantially higher, and appear more spatially and temporally uniform [table 2, (Capone and others, 2005a)]. Whilst many studies report wide ranges, mean N_2 fixation rates from the various discrete studies in the N. Atlantic and N. Pacific are generally in the range of about 100 to 300 μmol N m^{-2} d^{-1}.

Trichodesmium can also form dense, near surface accumulations often referred to as "blooms" (figs. 6G and H). Reports at the high end of the observed range (greater than 500-1000 μmol N $m^{-2}d^{-1}$) are typically associated with such surface accumulations (Carpenter and Capone, 1992). As stochastic, largely unpredictable events, blooms have not been routinely or frequently sampled. When they are encountered, however, they can be relatively easily sampled and surface waters assayed without the need for concentration (Goering and others, 1966). Active surface accumulations can often account for as much, if not more, N_2 input than that through the rest of the water column attributable to *Trichodesmium* (Capone and others, 1998, 2005b) (table 3).

Less Obvious Agents of N_2 Fixation

Numerous other potential agents of N_2 fixation have been recognized, although quantification of their input has been a greater challenge. While the isolation of diazotrophic heterotrophic bacteria and coccoid cyanobacteria led to speculation that these components of the oligotrophic food web might also contribute to aggregate N_2 fixation (Carpenter, 1983a), initial attempts to quantify activity through concentration were unsuccessful (Carpenter, Capone and Zehr, unpublished data), possibly because of their rarified densities and concentration artifacts. The detection of *nifH* transcripts

TABLE 2

Summary of direct, areal estimates of N_2 fixation by Trichodesmium, as originally reported or as derived

Location	Date	Comment	Areal Estimates avg	se	min	max	Number of Stations	Method	References
			μmol N m^{-2} d^{-1}						
SUBTROPICAL									
N. Pacific, 28°N, 155°W	Aug-73		33	-			1	AR, 3:1[a]	Mague and others, 1977
W. Sargasso , ~ 27°-34°N	Sep-Oct 73		1.4	0.47			9	AR, 3:1	Carpenter and McCarthy, 1975
Sargasso, 22°-36°N	Aug-74		13	8.4			7	AR, 3:1	Carpenter and Price, 1977
Caribbean Passages, 22°-23°N	Feb-Mar 74, Aug 74		8.8	8.4			10	AR, 3:1	Carpenter and Price, 1977
Atlantic transect, 30°N	May-Jun 75		0.61	0.27			5	AR, 3:1	McCarthy and Carpenter, 1979
BATS, 32°N	Jun-Sep 1995-97		41				36	$^{15}N_2$AR	Orcutt and others, 2001
TROPICAL ATLANTIC									
SW N. Atlantic	Fall 64		41	7.6			19	$^{15}N_2$ tracer	Goering and others, 1966
0°-24°N, 45°-66°W	Spr 65		108	24			15	$^{15}N_2$ tracer	Goering and others 1966
Caribbean, 12°-22°N	Feb-Mar 74, Aug 74		161	20			12	AR, 3:1	Carpenter and Price, 1977
SW N Atlantic, 14°-26°N	May-94		898	234	3.8	3542	18	AR, 3:1	Capone and others, 2005a
N.E. Caribbean									
SW N Atlantic, 0°-17°N	Apr-96		163	58	0.48	1317	29	AR, 3:1	Capone and others, 2005a
SW N Atlantic, 7°-27°N	Oct-96		300	71	0.7	1577	27	AR, 3:1	Capone and others, 2005a
SW N Atlantic	Jan-01		161	46	0	893	24	AR, 3:1	Capone and others, 2005a
SW N. Atlantic	Jul-01		59	21	0	540	29	AR, 3:1	Capone and others, 2005a
SW N. Atlantic	Apr-May 03		85	23	0.5	439	28	AR, 3:1	Capone and others, 2005a
N. Atlantic Average			220						
N. Atlantic Weighted Average			197				201		
TROPICAL N PACIFIC									
N. Pacific, 21°N, 159°W	Oct, Dec 72		134	-			2	AR, 3:1	Gunderson and others, 1976
SE E. China Sea , 10°-25° N	summer		126	na			32	AR, 3:1	Saino, 1977
Station Aloha			84	(49)[b]			3	AR, 3:1	Karl and others, 1997
N. Pacific	Oct-02	total	143	21	0.15	389	23	AR, 3:1	Capone and others, 2005b
	Oct-02	colonies	112	21	8	214	10	AR, 3:1	Capone and others, 2005b
	Oct-02	free	89	26	0	274	11	AR, 3:1	Capone and others, 2005b
N. Pacific	Aug-04	total	53	14	2	194	14	AR, 3:1	Capone and others, 2005b
N. Pacific Average			142						
N. Pacific Weighted Average			93				96		
OTHER TROPICAL									
S.W Pacific	Apr-98	total/ col	22	20	0.01	425	22	AR, 3:1	Capone and others, 2005b
Arabian Sea, 7-10°N	May-95	Depth int	36	10	0	97	12	AR, 3:1	Capone and others, 1998
Arabian Sea @ 10°N	"	surface blooms	129	23	18	170	7	AR, 3:1	Capone and others, 1999
		sum	165					AR, 3:1	
N Australian Coast	Nov-99	Depth int	353	164	4	3145	19	AR, 3:1	Capone and others, 2005b
		surface blooms	691	428	12	5207	12	AR, 3:1	
		total (avg)[c]	714	390	21	8361	21		

[a]AR 3:1 = acetylene reduction method assuming a 3:1 molar conversion ratio between acetylene reduced and N_2 fixed.
[b]Not stated if standard error or standard deviation.
[c]Averaged from summed totals at individual station. Several stations had no surface accumulation.

TABLE 3

Comparison of bloom (surface accumulation) and non-bloom (background) observations for Trichodesmium and Richelia/Hemiaulus associations

	Areal Estimate μmol N m^{-2} d^{-1}				
	Background > 0.5 m	**Surface Accum. 0-0.5m**	**n**	**Comment**	**References**
Trichodesmium					
Tropical SW NorthAtlantic	13	231[a]	1	Sta. 484, $15N_2$	Goering and others, 1966
Tropical North Pacific	134	319	1		Gunderson and others, 1976
Arabian Sea,	36 ± 10[c]	129 ± 23[b]	12, 7		Capone and others, 1998
Tropical Atlantic	136	879[b]	2		Capone and others, 2005a
Australia, North coast	538 ± 301	816 ± 508[b]	10		Capone and others, 2000b.
	Outside	**Within**			
Richelia					
SW NorthAtlantic	216	3110 ± 1315	14		Carpenter and others, 1999

[a]Extrapolated over top 1 m.
[b]For surface blooms, rates extrapolated over top 0.5 m.
[c]Error values are standard errors of the mean.

in the small (<10 μm) fraction (Zehr and others, 2001) has prompted renewed interest in assessing the quantitative significance of microbial diazotrophs. Indeed, application of such molecular techniques have revealed pronounced diel periodicity in *nif* H gene expression among cyanobacterial phylotpes at station ALOHA in the North Pacific subtropical gyre (Church and others, 2005).

Several reports in varied locations have used the ^{15}N tracer technique (Montoya and others, 1996b) to quantify the rate of N_2 fixation by the small size fraction of the plankton (typically < 10 μm). Montoya and others (2004) found highly variable but at times, extremely high, rates of N_2 fixation in studies in the sub-tropical N. Pacific and along the north coast of Australia (table 4). Average rates (excluding one extreme value) for 10 stations on the N. Pacific transect north of the Hawaiian Islands were about 520 μmol N m^{-2} d^{-1} (table 4). Curiously, rates of N_2 fixation were considerably and consistently lower at station ALOHA in the vicinity of the Hawaiian Islands. These findings were also concordant with an earlier study (Dore and others, 2002) and a recent study that employed plankton concentrates (Falcón and others, 2004; table 4). Several studies have reported N_2 fixation rates for microbial diazotrophs in the tropical North Atlantic as well, where results to date seem relatively low (Falcon and others, 2004; Capone and others, 2005b; table 4).

The endosymbiont of certain diatoms can also contribute a significant input of N_2 through N_2 fixation in the upper water column. During a cruise off the NE coast of South America, Carpenter and others (1999) encountered an extensive monospecific bloom of the diatom, *Hemiaulus* with endosymbiotic *Richelia.* They gently concentrated surface water by reverse filtration, and reported rates of N_2 fixation exceeding 1000 μmol N m^{-2} d^{-1}. During a more recent occupation of this region, similar ^{15}N derived N_2 fixation rates were observed during a period of high Amazon river flow and significant populations of the diatom *Hemiaulus* with the symbiotic cyanobacteria, *Richelia,* at many stations (Subramaniam and others, unpublished data).

Several studies have examined N_2 fixation rates of unconcentrated bulk water using a $^{15}N_2$ tracer approach. Goering and others (1966) first reported on unconcentrated, bulk water $^{15}N_2$ uptake studies off the northeast coast of South America. They observed mean N_2 fixations rates of approximately 86 (n = 2) and 271 (n = 9) μmol

TABLE 4

Summary of direct, areal estimates of N_2 fixation by Richelia or nanoplankton, as originally reported or as derived

Location	Date	Method	Comment	Areal Estimates avg	Areal Estimates se	Number of Stations	References
				μmol N m^{-2} d^{-1}			
TROPICAL ATLANTIC							
Bulk water							
WTNA	Fall -64	$^{15}N_2$ tracer	to 40m	86		2	Goering and others, 1966
WTNA	Spr-65	$^{15}N_2$ tracer	to 40m	271	89	9	Goering and others, 1966
WTNA	Jan-01	$^{15}N_2$ tracer		16	10	7	Capone and Montoya, unpublished data
WTNA	Oct-Nov 2002	$^{15}N_2$ tracer		24	18	2	Voss and others, 2004
ETNA	Oct-Nov 2002	$^{15}N_2$ tracer		140	78	6	Voss and others, 2004
WTNA	Apr-May 2003	$^{15}N_2$ tracer	*Richelia/ Hemi*	1309	412	22	Subramaniam and others, unpublished data
WTNA	Jul-01	$^{15}N_2$ tracer	*Richelia/ Hemi* bloom	3499		1	Subramaniam and others, unpublished data
Nanoplankton							
WTNA	Aug-01	$^{15}N_2$ tracer	conc, < 10 um	47			Falcon and others, 2004
WTNA	Apr-May 02	AR[a]	conc, < 10 um	37			Falcon and others, 2004
WTNA	Jan-01	$^{15}N_2$ tracer	< 10 uM	6	2.0	8	Capone and Montoya, unpublished data
WTNA	Jul-01	$^{15}N_2$ tracer	< 20 uM	29	5.9	17	Capone and Montoya, unpublished data
Richelia/ Hemiaulus							
WTNA	Oct-96	conc/ AR	3:1	3110	1315	14	Carpenter and others, 1999
TROPICAL PACIFIC							
Bulk water							
Station Aloha	Jul, Nov 2000; Apr, Jun 2001	$^{15}N_2$ tracer	[b]	69	12.5	4	Dore and others,2002
Nanoplankton							
Station Aloha	Jul, Nov 2000; Apr, Jun 2001	$^{15}N_2$ tracer	< 10 um	24	6	3	Dore and others, 2002
Station Aloha		$^{15}N_2$ tracer	< 10 um	66	19	7	Montoya and others, 2004
Station Aloha	Oct-02	$^{15}N_2$ tracer	conc, < 20 um	2.2			Falcon and others, 2004
Kaneohe Bay		$^{15}N_2$ tracer	< 10 um	24	6	12	Montoya and others, 2004
Pacific Transect, Along ~30°N	Jun-Jul 2002	$^{15}N_2$ tracer	< 10 um	520	160	10	Montoya and others, 2004
Pacific Transect, Along ~30°N	Jun-Jul 2002	$^{15}N_2$ tracer	< 10 um	2800[c]	na	1	Montoya and others, 2004
N Australian Coast	Nov-99	$^{15}N_2$ tracer	< 10 um	126	47	7	Montoya and others, 2004
N Australian Coast	Nov-99	$^{15}N_2$ tracer	< 10 um	3955[c]	na	2	Montoya and others, 2004

[a]Acetylene reduction.
[b]Our calculation, integrated to 100m.
[c]Stations with exceptionally high rates were not factored in the mean (see text).

N_2 m^{-2} d^{-1} in the fall of 1966 and spring of 1967, respectively, with an appreciable biomass of *Trichodesmium* present in their samples. Voss and others (2004) recently reported relatively high N_2 fixation rates on the eastern side of the basin off NW Africa, with somewhat lower rates on the western boundary, and unmeasurable activity in the central portion of the Atlantic basin (table 4).

Importantly, the fate of recently fixed N may be quite distinct among the various potential agents of N_2 fixation. Whereas *Trichodesmium* is not readily grazed by planktonic copepods (O'Neil and Roman, 1992) and its recently fixed N may enter food webs as DON after exudation (Capone and others, 1994; Glibert and Bronk, 1994) or lysis (Hewson and others, 2004), coccoid cyanobacteria and heterotrophic bacteria are directly grazed by protists (Caron and others, 1991). In contrast to *Trichodesmium*, which is buoyant and resides in the upper layers of the water column, the endosymbiotic species, *Richelia*, may be more prone to gravitational settlement to deeper layers in their diatom hosts (Scharek and others, 1999a, 1999b).

N_2 Fixation by Trichodesmium Versus Nanoplankton

While coccoid cyanobacteria such as *Synechococcus* are reported to occur at densities of about $\sim 10^4$ ml in oligotrophic waters, presumptively diazotrophic coccoids appear to occur at much lower densities of 10s to 100s of cells per ml (Montoya and others, 2004; Falcón and others, 2004). No information is currently available on the density of diazotrophic bacterioplankton in these systems. It is clear from tables 2, 3 and 4 that areal rates of activity are highly variable for both *Trichodesmium* and microbial diazotrophs, with extremes in the mmol N m^{-2} d^{-1} range and with a greater spread of values overlapping in the 10 to 100 μmol N_2 m^{-2} d^{-1}.

As has been pointed out by Carpenter and others (2004), while *Trichodesmium* cells may be relatively rare compared to *Synechoccocus* and *Prochlorococcus*, they are also relatively large cells (up to 10 μm in diameter). Even a single colony of 200 trichomes in a liter of water would represent about 20,000 cells and could account for ~50 percent of all autotrophic biovolume and carbon. Given the presumed lower densities of diazotrophic coccoid cyanobacteria, at the same density, *Trichodesmium* would also account for about 50 percent of coccoid plus *Trichodesmium* biovolume assuming a cell diameter for the coccoids of about 5 μm. Also, maximum cell specific rates of N_2 fixation appear higher in *Trichodesmium* (10-30 fmol N $cell^{-1}$ h^{-1}; Capone and others, 2005a) than those reported for diazotrophic coccoid cyanobacteria (~4 fmol N $cell^{-1}h^{-1}$; see Mitsui and others, 1986; Grobbelar and others, 1991; Gallon, 1992; Reddy and others, 1993).

In any event, results from the field (tables 2 and 4) would suggest that for both of these components of the diazotrophic flora of the sea, as well as for the symbiotic associations, the relative intensity of N_2 fixation is highly variable and heterogeneous (Montoya and others, 2004; Subramaniam and others, unpublished data). On a local basis, each component may make substantial contributions. A critical evaluation of the relative global importance of each awaits more extensive and robust estimates of the N input by symbiotic associations and the microbial diazotrophs.

COMPARISON OF GEOCHEMICAL VERSUS DIRECT ESTIMATES

Current global or basin-scale geochemical estimates, be they based on N excesses or isotopic mass balances, represent net fluxes integrated over relatively large spatial and temporal scales. Direct estimates, on the other hand, are snaphshots and will reflect natural variability occurring over shorter (for example, daily, seasonal and interannual) time scales. Furthermore, depending on the nature of the direct estimate, they may only capture specific components of the diazotrophic flora (see above).

TABLE 5

Globally scaled rates of N_2 fixation by Trichodesmium based on basin specific rates and the area of SST greater than or equal to 25 °C as a proxy for highly oligotrophic waters

	Seasonally Average SST Area km²					N_2 Fixation mol N x 10^{12} y^{-1}		
	≥ 25°C	≥ 20°C	Areal rate N_2 Fixation μmol N m^{-2} d^{-1}	# obs	notes	Direct ≥ 25°C	Geochem	Direct/ Geochem
N Atlantic	17795376	28103436	197	201		1.28	3.2	40%
S Atlantic	7438716	18282348	98.5	na	(½N Atl.)[a]	0.27	1.8	15%
N Pacific	43205400	57356748	93	98		1.47	2.5	59%
S Pacific	29011284	49651704	46.5	na	(½N Pac.)[a]	0.49	1.9	26%
Indian	30391524	41253624	55	13		0.61	1.5	41%
Med	349920	1610604	100			0.01		
Total						4.1	10.9	38%

[a]Assumed areal rates of ½ those in the northern basin of lower iron flux.

In order to accurately quantify N_2 fixation by direct or geochemical methods, an understanding of the temporal variation in N_2 fixation, residence time of the tracer, and ventilation timescales of the water column is vital.

Currently, several geochemical estimates are available for the North Atlantic (Michaels and others, 1996; Gruber and Sarmiento, 1997; Lee and others, 2002; Hansell and others, 2004) and the Pacific Oceans (Deutsch and others, 2001), while the greatest density of direct observations are for *Trichodesmium* in the tropical North Atlantic and North Pacific (table 2). We have recently scaled globally averaged rates of *Trichodesmium* N_2 fixation to seasonally averaged waters greater than or equal to 25°C as a proxy for highly oligotrophic waters (Galloway and others, 2004; fig. 3B). Given the basin-specific averages weighted for the extent of each study, *Trichodesmium* N_2 fixation scaled to waters of 25°C and above can explain about 40 percent and 59 percent of the geochemically inferred N_2 fixation in the north Atlantic and north Pacific, respectively (tables 1 and 5, fig. 7).

We believe this is remarkable in explaining a substantial fraction of the geochemically derived estimate. The difference is likely a function of input from *Trichodesmium* blooms (Carpenter and Capone, 1992; Capone and others, 1996), microbial diazotrophs (Montoya and others, 2004) and symbiotic associations (Carpenter and others, 1999). As noted, all three can contribute intense amounts of N, although the broader inputs over larger time and space scales are not currently well constrained.

Physical flux of nitrate versus N_2 fixation.—In order to provide a context for evaluating the importance of N_2 fixation, it is useful to compare it to the estimated physical flux of nitrate. The vertical flux of nitrate from below the thermocline through eddy diffusion and turbulent mixing has generally been considered the main source of new nitrogen in highly stable non-upwelling open-ocean regions (Lewis and others, 1986; Platt and others, 1992) (table 6). Nitrate flux from depth is often estimated as the product of the eddy diffusivity (K_z) and the observed nitrate gradient. Early estimates for eddy diffusivities for open ocean systems ranged from 0.05 to 1 cm^2 sec^{-1} (Munk, 1966; Lewis and others, 1986), with an extreme estimate of 7 cm^2 sec^{-1} found near Bermuda based on ^3H anomalies. Much of the discrepancies between the early estimates of K_z may be explained by regional differences in hydrography and nitrate gradients (Oschlies, 2002). However, extensive experimental evidence conducted at a site 1200 km west of the Canary islands revealed that K_z was much lower

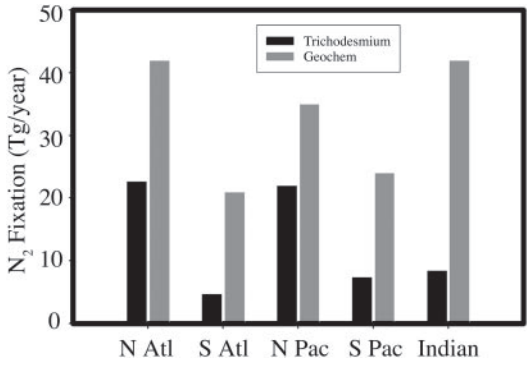

Fig. 7. Basin scaled estimates of *Trichodesmium*-based and geochemically-inferred N_2 fixation. Data from table 5. Approach for *Trichodesmium* scaling as in Galloway and others (2004) but using basin specific averages for the north Atlantic and north Pacific. Similarly, geochemical estimates for N_2 fixation for the Pacific from Deutsch and others (2001) ($4.2 \pm 1.0 \times 10^{12}$ mol N y^{-1}; table 1) were scaled to the north Pacific based on the relative area of SSTs 25°C in each sub-basin. The value for the north Atlantic from Gruber and Sarmiento (1997) was adjusted upwards to account for a lower N:P ratio of diazotrophs (see table 1 and text).

than previously cited, ranging from 0.11 ± 0.02 to 0.17 ± 0.02 cm^2 sec^{-1} (Ledwell and others, 1993, 1998).

Field data from the tropical N. Atlantic would suggest vertical nitrate fluxes in the range of 20 to upwards of 800 μmol N $m^{-2}d^{-1}$ (McCarthy and Carpenter, 1983; Planas and others, 1999; Capone and others, 2005a) (table 6) and higher values in the N. Pacific resulting from the larger nitrate gradients (McCarthy and Carpenter, 1983). The convective supply of nitrate into the euphotic zone is estimated to range from 50 mmol N m^{-2} y^{-1} (equivalent to 140 μmol N m^{-2} day^{-1}) over the subtropical Atlantic (Williams and others, 2000) to 170 ± 50 mmol N m^{-2} y^{-1} (500 umol N m^{-2} day^{-1}) at BATS (Michaels and others, 1994). Over the north Atlantic, horizontal Ekman transfer of nitrate from nutrient rich subpolar and upwelling regions into the subtropical gyre is estimated to supply 30 to 60 mmol N m^{-2} y^{-1} (80-160 m^{-2} day^{-1}) (Williams and Follows, 1998). Mesoscale eddies are estimated to supply between 190 to 350 mmol N m^{-2} y^{-1} (520-950 m^{-2} day^{-1} (McGillicuddy, Jr. and Robinson, 1997; McGillicuddy, Jr. and others, 1998; Oschlies and Garcon, 1998).

Hence, areal rates of N_2 fixation by *Trichodesmium* alone (for example, a weighted average for the Atlantic of about 200 μmol N $m^{-2}d^{-1}$, table 2) can provide an input comparable to vertical nitrate flux. In fact, regions where the highest N_2 fixation rates by *Trichodesmium* spp. are reported (southwestern region of the subtropical gyre) (table 2) and where the models predict the very lowest nitrate fluxes (0-27 μmol $N*m^{-2}d^{-1}$) (Oschlies, 2002). Addition of other sources of diazotrophy (for example, *Richelia*/diatoms, microbial diazotrophs) will further increase the relative importance of this process with respect to vertical nitrate flux. Initial indications suggest that in the N. Pacific, microbial diazotrophy can, at times, greatly exceed vertical nitrate flux (Montoya and others, 2004).

CONUNDRUMS: WHAT CONTROLS N_2 FIXATION IN THE SEA?

Any number of factors, physical, chemical or biotic, can affect the extent of N_2 fixation in a system (Capone, 1988; Howarth and others, 1988a; Karl and others, 2002). Since nitrogenase is very O_2 sensitive (Gallon, 1992), physical mixing in planktonic systems has been recognized as a potentially important factor in regulating N_2 fixation,

TABLE 6

Estimates of vertical NO_3^- flux into the euphotic zone of the tropical and sub-tropical Ocean

Location		NO_3^- gradient mmol m^{-4}	K_z cm^2 sec^{-1}	se	N Flux μmol N m^{-2} d^{-1}		Comment	References
ATLANTIC								
Sargasso Sea	32°10' N, 64°30' W	0.02 - 0.03	[7.6][a]		1644	548 (sd)	^{3}He excess	Jenkins, 1988
Sargasso Sea	31°50' N, 64°10' W	0.03	0.4		100	-	Fickian	Michaels and others, 1996
Olig. E. Atlantic	28.5°N, 23°W,	0.045	0.37 (0.006-2.3)[b]		139 (2.7-1035)[b]	2-890 (95% CI)	tked/bfm[c]	Lewis and others, 1986
Olig. E. Atlantic	26°N 28°W	0.03	0.11		27	-	SF_6 tracer	Ledwell and others, 1993
Central Atlantic	34°S to 27°N	0.092	0.29	±1.2	380	± 180 (se)(n=14)	tked/bfm[c]	Planas and others, 1999
	3°N to 27°N	0.152	0.58	±2.9	838	± 344 (se)(n=6)	tked/bfm[c]	Planas and others, 1999
Trop/sub-trop N. Atlantic	25°-30°N, 70°-75°W	na	na		137		Convective model	Williams and others, 2001
Trop/sub-trop N. Atlantic		na	na		137		er/cecm[c]	Oschlies, 2002
Tropical Atlantic		0.05 - 0.023	0.11		46 - 228		Fickian	Capone and others, 2005a
Tropical Atlantic		0.05 - 0.024	0.37		169 - 736		Fickian	Capone and others, 2005a
PACIFIC								
Trop. N. Pacific	5°-20°N, 125°-150°W	0.15	0.5-5.1		180		Fickian	Anderson, 1978
E. trop. N. Pacif	5°-20°N, 125°-150°W	na	-		380-1760		pnd[e]	King and Devol, 1979
Central N. Pacific		0.08	1-3.6		800		Fickian	Platt and others, 1984
N. Pacific		0.035-0.05	0.2, 1.2		60.5-520		Fickian	McCarthyand Carpenter, 1982
Station Aloha	22.75°N, 158°W	0.03 - 0.07	.37		112-259		Fickian	Karl and others, 1992

[a]Apparent K_2 back-calculated from Jenkins 1988 assuming a NO_3 gradient of 0.025 mmol m^{-4}.
[b]Range.
[c]Turbulent kinetic energy diffusion/buoyancy frequency model.
[d]Eddy-resolving coupled ecosystem circulation model.
[e]Phytoplankton N demand.

particularly for non-heterocystous forms such as *Trichodesmium.* Carpenter and Price (1976) found agitation rapidly inactivates nitrogenase activity in *Trichodesmium,* and a strong inverse correlation between sea state and the extent of fixation by natural populations of this cyanobacterium. However, relatively dense populations of *Trichodesmium* are routinely encountered throughout the Trade Wind belts (Carpenter and others, 2004) with their steady 10 to 15 m s^{-1} breezes. How turbulence might affect planktonic microbial diazotrophs remains to be determined (for example see Howarth and others, 1995a, 1995b; Paerl and others, 1995).

While temperature is not necessarily an intrinsic constraint on nitrogenase activity [actively N_2 fixing populations (for example, marine cyanobacterial mats) are documented in Antarctic near 0°C; Vincent, 2000] for tropical planktonic diazotrophs, temperature may be an important consideration (Staal and others, 2003). While *Trichodesmium* has been reported in temperate waters below 20°C, it is generally only active at temperatures above 20°C (Carpenter, 1983b).

Since diazotrophs can be successful in oligotrophic regions due to their ability to utilize the largest reservoir of N, N_2 gas, their growth must necessarily be constrained by the availability of other nutrients. The chemical controls on oceanic N_2 fixation, and in particular iron (Fe) and P, have been the topic of much current research effort and debate. Fueling these efforts has been the observation of the extensive areas of positive N* anomalies in the north Atlantic that roughly correspond to regions which receive substantial dust input from aeolian deposition (Michaels and others, 1996; Gruber and Sarmiento, 1997; Gao and others, 2001). Due to the presumed elevated Fe requirement of photosynthetic diazotrophs (relative to non-diazotrophic phytoplankton—see below) and low atmospheric dust deposition to many oligotrophic areas (low nutrient, low chlorophyll, or LNLC), some have suggested that Fe limits N_2 fixation globally (Rueter and others, 1992; Falkowski, 1997). Combining data on *Trichodesmium* Fe quota and data on aeolian dust fluxes, Berman-Frank and others (2001) estimate that N_2 fixation is Fe limited in 75 percent of the world's oceans.

Substantial progress has been made in evaluating the role of Fe, at least with respect to *Trichodesmium* growth. Sañudo-Wilhelmy and others (2001) estimate that Fe requirements of N_2 fixing phytoplankton are only 2.5 to 5.2 times higher than for NH_4^+-assimilating phytoplankton, much less than previous estimates of 100 times higher (Raven, 1988). However, even an amplified cell quota of 2.5 to 5.2 is likely to be critical in waters where N_2 fixation occurs, as dissolved Fe levels are generally <1 nM (Wu and others, 2000). Indeed, there is experimental evidence that Fe can regulate *Trichodesmium* growth and nitrogenase activity. Rueter (1988) found increases in CO_2 and N_2 fixation rates and cellular chlorophyll a content in natural samples of *Trichodesmium* collected near Barbados when amended with Fe. Similarly, Paerl and others (1994) reported stimulation of growth and nitrogenase activity by Fe in cultures and natural samples of *Trichodesmium.* More recently, Fe limited cultures of *Trichodesmium* strain IMS101 were shown to have reduced N_2 fixation rates compared to Fe-replete cultures (Berman-Frank and others, 2001). Various natural organic compounds may facilitate, or impede, how accessible Fe is to diazotrophs (Achilles and others, 2003). No information is currently available on the Fe requirement of microbial diazotrophs.

Less is known about the role of P in controlling N_2 fixation in the open ocean. Obviously, the relative lability in the N:P stoichiometry of diazotrophs will influence their growth dynamics and the ability of most organisms to sequester P influences the interpretation of short-term measurements of N versus P dynamics. Sañudo-Wilhelmy and others (2001) found that N_2 fixation in *Trichodesmium* from the central Atlantic Ocean was tightly correlated to P content of the colonies, suggesting P limitation of N_2

fixation in this area. Inorganic P is not the only P source for *Trichodesmium* in the open ocean. *Trichodesmium* produces the enzyme alkaline phosphatase (APA), which cleaves PO_4^{-3} from dissolved organic P compounds to provide an additional P source to the organism. AP activity (APA) in natural populations of *Trichodesmium* can indicate P stress to some degree. APA was much higher in the North Atlantic, where inorganic P concentrations are extremely low, than off the northern coast of Australia where concentrations are perhaps 10-fold higher (Mulholland and others, 2002). APA was also found to be 10-fold higher in P-deplete cultures of *Trichodesmium* strain WH9601 compared to P-replete cultures (Stihl and others, 2001). However, APA is not a direct index of general P limitation, but an indication that *Trichodesmium* are experiencing low levels of inorganic P (Scanlan and Wilson, 1999).

Wu and others (2000) recently reported that the subtropical N. Atlantic is more depleted in P in comparison to the sub-tropical N. Pacific. They suggested that this occurs because more Fe reaches the subtropical north Atlantic than the subtropical North Pacific via aeolian dust deposition. They posit that the available Fe in the N. Atlantic increases N_2 fixation in the Atlantic, leading to a draw-down of all available P. With the accumulation of observations in the N. Atlantic and N. Pacific, there is indeed a suggestion of somewhat higher rates of N_2 fixation for *Trichodesmium* in the N. Atlantic (table 2), consistent with the hypothesis that P is more severely limiting for diazotrophs in the north Atlantic while Fe is more limiting in the north Pacific. Corroborating this suggestion, Sañudo-Wilhelmy and others (2001) examining the internal stoichiometry of field populations of *Trichodesmium* found a stronger relationship of N_2 fixation with cellular phosphate concentration compared to Fe content (Sañudo-Wilhelmy and others, 2001) indicating that *Trichodesmium* from the N. Atlantic are more likely limited by P. Dyhrman and others (2002) also provided evidence of P stress in populations of *Trichodesmium* from the Atlantic. However, the frequent occurrence of dense populations of *Trichodesmium* in areas of widespread phosphate depletion (Carpenter, 1983a; Carpenter and others, 2004) indicates that the local controls on this process are still not well understood (Karl and others, 2002). Correlation is not causation and the relationship between low P and N_2 fixation may be a consequence of their growth as a function of the availability of Fe rather than the control.

Wet and dry deposition of mineral aerosols fertilize the surface ocean with Fe (Mahowald and others, 1999), which is vital for the nitrogenase enzyme. A number of studies have speculated or observed a diazotrophic response to mineral dust Fe fertilization in the Atlantic (Mahaffey and others, 2003; Ramos and others, 2005), and Pacific Oceans (Johnson and others, 2003). Direct evidence for dust stimulation of *Trichodesmium* biomass has recently been reported. A 1999 Saharan dust event coincided with increases in dissolved Fe concentrations on the west Florida shelf and a 100-fold increase in *Trichodesmium* biomass. N_2 fixation rates were not measured, but DON concentrations doubled, presumably being exuded by N_2 fixers (Lenes and others, 2001). A recent mesocosm study off the coast of W. Africa found diazotrophs to be co-limited by both P and Fe (Mills and others, 2004). Note, however, analysis of aerosol dust shows that while providing Fe, it can also supply P and combined N (Ridame and Guieu, 2002; Baker and others, 2003; Mills and others, 2004).

Modeling and remote sensing of N_2 fixation

Representation of the flows and transformations of key nutrients such as N has been a goal of ecosystem and biogeochemical modelers going back to the work of Gordon Riley (Riley, 1967). Modeling approaches developed by Fasham and others (1990, 1993), who pioneered the widely used nutrient-phytoplankton-zooplankton—detritus (NPZD) framework, have greatly promoted our understanding of N dynamics

in planktonic systems. Ecosystem modeling has become an essential component of any large-scale ecosystem analysis, and is increasingly coupled with higher level general circulation models (GCMs).

It is only relatively recently, however, that N_2 fixation has been explicitly represented in ecosystem and biogeochemical models. Shaffer (1989) considered N_2 fixation in a coupled P-N-O-S biogeochemical model of the ocean he developed, exploring the linkages among the severity of P limitation and ocean anoxia, and the possible role of N_2 fixers in this interaction. Walsh and others (1999), while not specifically representing N_2 fixation in their C-N coupled model of the Cariaco Basin during spring upwelling, did conclude that N_2 fixation was a relatively minor source of new N during the spring, but was required for a more complete annual model. As mentioned above, Tyrrell (1999) explored the relationships among phytoplankton with regard to the relative importance of N versus P limitation in a biogeochemical box model with N_2 fixation explicitly represented, which resolved a role for N as a proximal limiting nutrient and P as the ultimate limiting nutrient.

A series of recent modeling efforts have benefited greatly from the wealth of field observations from the ocean time series stations, and the extensive physiological studies on *Trichodesmium.* Hood and others (2001) exploited the BATS data set, including observations of *Trichodesmium* abundance (Orcutt and others, 2001) in a one dimensional model. Tuning the model to the observed summertime drawdown of CO_2 yielded N_2 fixation rates compatible with some direct measurements as well as geochemically inferred rates. However, particulate N fluxes were overestimated. A solution tuned to the particulate N fluxes overestimated the drawdown of inorganic C. The model predicted strong interannual variability in N_2 fixation, with weaker populations of *Trichodesmium* developing after deeper winter convective mixing.

Using a simple biological model based upon the physiological responses of *Trichodesmium* to water column dynamics, Fennel and others (2002) were able to capture the seasonal and interannual cycle of *Trichodesmium* at station ALOHA in the tropical North Pacific, and explain the sometimes dramatic variation in the N: P ratios of the dissolved inorganic and organic nutrient pools which have been previously noted (Karl and others, 1997, 2001b).

As mentioned above, the recognition that *Trichodesmium* possessed highly reflective gas vacuoles and the phytopigment, phycoerythrin (Subramaniam and others, 1999) led to the specific detection of *Trichodesmium* chlorophyll at relatively high concentrations using ocean color remote sensing (for example, SeaWiFs) (Subramaniam and others, 2002; Westberry and othes, 2005). Hood and others (2002) coupled remote sensing data on *Trichodesmium* to predict areal rates of N_2 fixation, based on N_2 fixation versus irradiance relationships. However, the approach only works for relatively high concentrations of *Trichodesmium.*

Hood and others (2004) and Coles and others (2004a) built further on their work at BATS (above), and developed a climatologically-forced coupled, 3-dimensional, biological-physical model for the tropical North Atlantic basin, including a dynamic representation of *Trichodesmium.* The model captures and predicts the seasonal and spatial distribution of *Trichodesmium* in the basin. While there was a good correlation between the model derived distribution and seasonality and direct observations of *Trichodesmium,* (see table 1 of Hood and others, 2004), the model output also revealed persistently high *Trichodesmium* biomass and rates of N_2 fixation in the Gulf of Guinea off of Africa, a region where there are few direct observations of diazotrophs (Dandonneau, 1971). Thus, while benefiting greatly from direct field observations, these complex biological-physical models can also generate testable predictions that can be used to validate the model and to guide future field campaigns to investigate new and unexplored regions of N_2 fixation.

While it has been assumed that the basin scale spatial extent of *Trichodesmium* is largely controlled by temperature (>22-25 °C; Carpenter and others, 1992), model output also suggested that the depth and duration of winter mixing have a stronger control (Hood and others, 2004). Going one step further, Hood and others (2004) were able to capture the succession of phytoplankton species linked to the physical supply of nitrate. Model output describing a temporal progression from diatoms, to *Trichodesmium* in response to the drawdown of nitrate by diatoms, to *Trichodesmium* supported flagellate growth, exemplifies the impact of N_2 fixation on the plankton community.

The intensity of N_2 fixation predicted by the basin model using observed densities (Coles and others, 2004a) is comparable to recent direct estimates for *Trichodesmium* (for which it is tuned), but only 25 percent of geochemical estimates (Gruber and Sarmiento, 1997). In order to approach geochemical derived estimates of N_2 fixation (table 1), Coles and others (2004a) found that *Trichodesmium* biomass must be increased beyond that observed.

Ecosystem models have also been used to investigate factors that limit N_2 fixation in the world's oceans. Employing a global marine ecosystem mixed-layer model, Moore and others (2002b) and Moore and others (2002a) explored a wide variety of marine ecosystems, including N, P and Fe-limited systems, in which diazotrophs, as well as other phytoplankton were represented. Direct field data regarding the temperature constraints, growth rates, grazing and non-grazing mortality, photo-physiology, Fe requirements, elemental stoichiometry of diazotrophs, as well as atmospheric dust deposition model studies were used to parameterize these complex models. Indeed, Moore and others (2002a) found these models were able to reproduce the seasonality and biomass of diazotrophs at both BATS and station ALOHA and, largely in agreement with direct observations (Wu and others, 2001) and other projections (Berman-Frank and others, 2001), described P limitation at BATS and Fe limitation at station ALOHA.

In addition, complex ecosystem models have been used to examine the effect of N_2 fixation on the global C cycle. Using *Trichodesmium* and phytoplankton biomass and N_2 fixation rates from field data, Coles and others (2004a) concluded that N_2 fixation increases new production by 30 percent and total production by 5 percent. Moore and others (2002b) found that primary production, sinking particulate fluxes and chlorophyll concentrations were reduced by 40 to 50 percent if diazotrophs were excluded from their model runs.

With regard to the impact of N_2 fixation on the modeling of the C cycle, while few studies explicitly include this process in their modeling of the global C cycle, all C models make implicit assumptions about the existence of N_2 fixation. Any model that assumes a constant pool of nitrate implicitly assumes that the rate of N_2 fixation equals the rate of denitrification. This corresponds to a C fixation rate of order of $40-80 \times 10^{12}$ mol C y^{-1} (0.5-1 Gt C y^{-1}) in excess of the growth on nitrate. Conversely, if the C budget expressly assumes no N_2 fixation, they implicitly assume that the total stock of nitrate declines by 0.01 to 0.1 percent per year and more in shallow waters. In both cases, when the models are compared (or tuned) to observations and the authors find agreement, one or more explicit processes within the model must become a proxy for the activity of the diazotrophs.

Most recently, using monthly climatological satellite data of sea surface chlorophyll concentrations (from SeaWiFS) and sea surface height (from TOPEX/Poseidon altimeter), Coles and others (2004b) reported an anomalous summertime maximum in phytoplankton biomass in a region of highly stratified, nutrient-deprived water in the southwest tropical north Atlantic. Employing a climatological forced biological-physical model with a dynamic representation of *Trichodesmium,* Coles and others

(2004b) were able to simulate this chlorophyll maximum in the western tropical Atlantic, which was absent when *Trichodesmium* were omitted from the model. The authors concluded that N_2 fixation (*Trichodesmium* specifically in their model) was responsible for this summer time phytoplankton bloom in an otherwise nutrient starved region. Model and satellite derived estimates of N_2 fixation were 192 μmol N m^{-2} d^{-1} and 220 μmol N m^{-2} d^{-1} (Coles and others, 2004a), comparable to both direct (table 2) and geochemical observations (table 1).

Most models to date have focused on *Trichodesmium,* for which the most robust data sets for biomass and diazotrophic activity are available. The next phase of modeling of N_2 fixation should incorporate the novel sources of diazotrophy that may have distinct dynamics with respect to the pathways and fate of recently fixed N (see above).

N_2 fixation, denitrification, dust and ice sheets

Synchronized changes in the carbon dioxide (CO_2) content of the atmosphere with the advance and retreat of the high latitude ice sheets between the glacial and interglacial periods in earth's history (Neftel and others, 1982; Petit and others, 1999) has generated a plethora of research addressing the relationship between the ocean's "biological pump" and climate (Broecker, 1982; Sarmiento and Gruber, 2002). Continental margin erosion due to sea level changes (McElroy, 1983), increased polar ocean stratification (Sigman and Boyle, 2000), as well as perturbations in the oceanic silica cycle (Archer and others, 2000; Ridgwell and others, 2002) all have the potential to alter atmospheric CO_2 concentrations. However, the tenacious link between the N cycle, carbon and climate begs discussion.

In the ocean, N has a much shorter residence time (<3000 years; Codispoti, 1995; Gruber and Sarmiento, 1997) than P (50,000 years; Delaney, 1998) and therefore, any small changes in the N inventory will be unaccompanied by changes in the P inventory. Thus, relatively short term (thousands of years) oscillations between excess fixed N during glacial periods and fixed N deficits during interglacial periods is thought to have dramatically altered the "biological pump" and thus sequestration of atmospheric CO_2 by the oceans (McElroy, 1983; Falkowski, 1997; Devol, 2002). Has the fixed N inventory changed with glacial state and, if so, what has caused these changes in the fixed N inventory?

Evidence from Antarctic sea ice cores reveals that during interglacial periods, climate forcings decreased the supply of Fe reaching the oceans through aeolian dust (Duce and Tindale, 1991; Broecker and Henderson, 1998). Due to the high-Fe requirement of marine diazotrophs, this may have led to a decrease in marine N_2 fixation and thus the fixed N inventory, relaxing the oceans ability to sequester atmospheric CO_2 (Falkowski, 1997; Karl and others, 1997; Devol, 2002; Karl and others, 2002). From an alternative perspective, reconstruction of the N dynamics using sedimentary records of $\delta^{15}N$ in the Arabian Sea (Altabet and others, 1995, 2002), eastern tropical Pacific (Ganeshram and others, 1995, 2002) and Gulf of California (Pride and others, 1999) infers that a decrease in denitrification may have been responsible for an increase in the fixed N budget during glacial periods. However, Ganeshram and others (2002) propose that while the N inventory may have increased during glacial periods due to decreased denitrification, the P inventory may also have increased due to decreased phosphogenesis (Delaney, 1998). Differential rates of formation of N and P (P being slower) probably elevated the N:P ratio, causing P limitation and a subsequent decrease in N_2 fixation, thus decreasing the N inventory. In support of this scenario, Haug and others (1998) explain interglacial/glacial changes in N_2 fixation in the Cariaco Basin as a consequence of a change in the N:P of the nutrient supply, which was ultimately driven by denitrification.

There are inherent caveats to both of these scenarios (Sigman and Boyle, 2000; Gruber, 2004). A fixed P budget (with the source and sink of P being independent from N) compared to a dynamic N budget demands that the stoichiometric demands of phytoplankton deviate dramatically from Redfield ratios between glacial/interglacial time periods (Sigman and Boyle, 2000). Recent studies do indeed demonstrate that the N:P ratio of phytoplankton is much more elastic than previously thought (Cavender-Bares and others, 2001; Karl and others, 2001b). Moreover, the potential role of Fe in controlling the marine N inventory, on a cellular (Falkowski, 1997; Berman-Frank and others, 2001) and global scale (Mahowald and others, 1999; Lenton and Watson, 2000; Ridgwell, 2003) is becoming increasingly apparent. In fact, increased dust deposition over the high latitude nutrient-rich ocean has been posed as an alternative explanation for atmospheric CO_2 sequestration during glacial periods (Sarmiento and Toggweiler, 1994; Rosenthal and others, 2000; Sigman and Boyle, 2000), without the need for changes in N_2 fixation and denitrification.

There is a growing geochemical consensus that, while there may have been changes in the strength of N_2 fixation and denitrification throughout the geological record, the N cycle is close to a steady state (Brandes and Devol, 2002; Deutsch and others, 2004; Gruber, 2004). Through a series of feedback mechanisms involving fixed N, export production and oxygen-depletion (Codispoti, 1989), as well as the control of P on the N cycle (Tyrrell, 1999), Gruber (2004) describes a "N cycle homeostat," in which an internally stabilized system can interact, and potentially alter on short time scales, the global C cycle. Indeed, Michaels and others (2001) proposed a series of negative feedback mechanisms coupling increased aeolian dust supplies to N_2 fixation and climate. It appears that such intrinsic feedback mechanisms between N_2 fixation and denitrification (Gruber, 2004), as well as climate (Michaels and others, 2001) have retained the global N cycle within well-defined limits, even throughout glacial/interglacial timescales (Indermuhle and others, 1999), which according to Gruber (2004) diminishes the role of the N cycle in driving the large variations in atmospheric CO_2 between glacial and interglacial periods in earth's history.

So how does this steady state scenario compare with the present day marine N budget? According to Gruber (2004), the present-day marine global N cycle is close to balance, with inputs of N from N_2 fixation and other sources (18.9×10^{12} mol N y^{-1} or 265 Tg N y^{-1}) being approximately equal to losses from water column and sedimentary denitrification, and other processes (18.6×10^{12} mol N y^{-1} or 260 Tg N y^{-1}; see Brandes and Devol, 2002; and Gruber, 2004, for further details). However, re-evaluation of the magnitude of sedimentary denitrification ($\sim 21 \times 10^{12}$ mol N y^{-1} or $\sim$300 Tg N y^{-1}), (Codispoti and others, 2001; Brandes and Devol, 2002), reveals that this term is much greater than previous estimates ($<7.1 \times 10^{12}$ mol N y^{-1} or $<$100 Tg N y^{-1}, (Codispoti and Christensen, 1985), and almost three times greater than water column denitrification (10.7×10^{12} mol N $y^{-}1$ or 150 Tg N y^{-1}) (Codispoti and others, 2001; Brandes and Devol, 2002). If global N inputs are approximately 18×10^{12} mol N y^{-1} (or 250 Tg N y^{-1}), then N losses, including an increased estimate for sedimentary denitrification, almost reach 34×10^{12} mol N y^{-1} (or 482 Tg N y^{-1}), prompting the suggestion that the present day ocean is losing N (Codispoti, 1995). With a steady state N cycle in mind, a large N deficit implies that marine N_2 fixation may be drastically underestimated using both direct and geochemical methods, leaving the N_2 fixing community on a relentless search for those missing Tg's of N!

CONCLUSIONS

Historically, the biomass and contribution of N_2 fixers to the marine N cycle appears to have been chronically underestimated. Here, we have highlighted the recent and substantial advancements made in the study of N_2 fixation in the marine environment as a result of the development and application of modern techniques

from enzymology, stable isotope geochemistry and molecular biology, as well as the extensive and expanding field observations. With one exception, several geochemically based approaches of estimating basin scale rates of N_2 fixation have strongly pointed that direct methods may be missing a significant fraction of planktonic N_2 fixation.

Until very recently, field studies of N_2 fixation have mostly focused upon the prolific diazotroph, *Trichodesmium*. However, advances in molecular techniques reveal that the small ($<10\mu m$) fraction of the plankton that includes diazotrophic heterotrophic bacteria, and coccoid cyanobacteria (for example, *Synechococcus*), as well as symbiotic associations between diatoms (that is, *Hemiaulus*) and cyanobacteria (that is, *Richelia*) and zooplankton intestinal flora may contribute significantly to marine N_2 fixation. In fact, when quantification of these sources becomes available, their inclusion may help close the budget with respect to the higher geochemically derived N_2 fixation rates.

Comparison of geochemically derived N_2 fixation rates using N*, stable isotope budgets and C anomalies reveal a large divergence in basin scale areally-integrated estimates. These calculations are highly sensitive to the assumptions made regarding domain area over which N_2 fixation takes place, the stoichiometry between C, N and P, as well as the choice of end members in both N* and stable isotope studies. In addition, it appears that factors other than N_2 fixation may contribute to excess nitrate in the thermocline or to ^{15}N depleted signatures in the surface ocean. Deconvoluting the pathway of recently fixed N still remains a challenge. Stable N isotope techniques reveal that diazotrophs likely exuded ^{15}N-depleted amino acids, which are rapidly utilized by phytoplankton either directly or via the microbial loop. However, it appears that N_2 fixation may not have a significant isotopic impact on either the total organic nutrient pool or the DON_{HMW} pool, as least at BATS and station ALOHA. More importantly, there is accumulating evidence to suggest that the $\delta^{15}N\text{-}NO_3^3$ in the upper 500m of the water column is lower (3-4‰) than previously cited (5-6.5‰), which decreases the contribution of N_2 fixation in water column isotope budgets (which begs the question of how this layer of low $\delta^{15}N$-nitrate is maintained). Carbon-anomaly based estimates yield the highest areal N_2 fixation rates, possibly due to a discrepancy in the period that inorganic C drawdown actually occurs in the regions of study (summer/fall, 82-158 days) in comparison to the annually integrated C anomaly reported (365 days).

Experimental work in culture and in the field, along with the geographic distribution of diazotrophs, indicates that N_2 fixation is limited by Fe or P. Indeed, diazotrophic distributions may be intrinsically linked to the aeolian supply of Fe rich dust. However, elemental analysis of aeolian dust reveals that as well as supplying Fe, atmospheric dust deposition may also cause N and P fertilization, the stoichiometry of this supply being critical. The mechanism by which *Trichodesmium* and other diazotrophs living in the upper layers of the oligotrophic tropical ocean accesses phosphorus also remains an open question. Vertical migration from the P poor surface layers to P rich thermocline, as well as enzyme hydrolysis of organic P have been suggested as potential mechanisms.

We have herein provided a geochemical perspective on the significance of N_2 fixation in the Mediterranean Sea using a recent survey of stable isotope dynamics in this basin and outcrops of elevated N* anomalies of Mediterranean origin in the Atlantic ocean. There are few direct observations of diazotrophs in this region, yet geochemical evidence strongly suggests N_2 fixation is a significant process there. Using a crude box model, we estimate that N_2 fixation may account for about 223 mmols N $m^{-2}\,y^{-1}$ in the eastern Mediterranean Sea, although direct field surveys are required to confirm this.

There have been great advances in the ability to exploit the vast amount of data from field surveys to create realistic and explicit representations of diazotrophs, in particular, *Trichodesmium,* in complex biological-physical and ecosystem models. Not only has the seasonality and spatial distribution of *Trichodesmium* been captured in these complex models, but factors which may constrain their distribution and growth, such as Fe and P, have been explored. Models assessing the distribution of *Trichodesmium* in the North Atlantic identified the Gulf of Guinea as a region of extensive diazotrophic activity (Hood and others, 2004), which has been previously unrecognized, but that may represent a significant source of new N to the Atlantic Ocean. The next phase of model development incorporate diazotrophs other than *Trichodesmium.*

Oscillations between N_2 fixation and denitrification through geological time were thought to have contributed to the change in the ocean's biological pump and thus its ability to sequester atmospheric CO_2 through glacial and interglacial periods in Earth's history. More recent perspectives call for a balanced N budget in which N_2 fixation and denitrification are tightly coupled, thus diminishing the role of the N cycle in controlling over the longer term the atmospheric CO_2 content, but creating the requirement that there be powerful and rapid feedback mechanisms between the two processes. In the present day N budget, water column and sedimentary denitrification appear to exceed N_2 fixation, implying a large deficit and a gradual loss of N over timescales of thousands of years. Such an imbalance may infer either an overestimation of denitrification, or underestimation of global N_2 fixation, thus demanding a re-evaluation of techniques and scales used to extrapolate a discrete measurement to the basin or global scale.

Nonetheless, there is an increasing and compelling body of direct and model derived evidence to suggest that oceanic N_2 fixation does have a significant impact on the global carbon cycle. Model output suggests that primary production decreases by up to 50 percent when diazotrophs are omitted from complex ecosystems.

Oceanic N_2 fixation is a highly dynamic component of the marine N cycle system. For instance, in the tropical north Pacific time-series studies at station ALOHA, the abundance and activity of diazotrophs have undergone dramatic changes due to increased stratification in response to climate oscillations: these changes have been directly manifested in N and P pools, as well as being responsible for a sharp increase in export production over the past 8 years. In light of such findings, there is no better time than the present to assess and quantify the factors that control the temporal, basin scale and global distribution of N_2 fixers.

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