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The fate of new production from N_2 fixation

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Abstract

While we now know that marine N_2 fixation is a significant source of new nitrogen (N) in the marine environment, little is known about the fate of this production, despite the importance of diazotrophs to global carbon and nutrient cycles. Specifically, does new production from N_2 fixation fuel autotrophic or heterotrophic growth, facilitate carbon (C) export from the euphotic zone, or contribute primarily to microbial productivity and respiration in the euphotic zone? For *Trichodesmium*, the diazotroph we know the most about, the transfer of recently fixed N_2 (and C) appears to be primarily through dissolved pools. The release of N appears to vary among and within populations and, probably as a result of the changing physiological state of cells and populations. The net result of trophic transfers appears to depend on the complexity of the colonizing community and co-occurring organisms. In order to understand the impact of diazotrophy on carbon flow and export in marine systems, we need a better assessment of the trophic flow of elements in *Trichodesmium* communities dominated by different species, various free and colonial morphologies, and in various defined physiological states. Nitrogen and carbon fixation rates themselves vary by orders of magnitude within and among studies highlighting the difficulty in extrapolating global rates of N_2 fixation from direct measurements. Because the stoichiometry of N_2 and C fixation does not appear to be in balance with the stoichiometry of particles, and the relationship between C and N_2 fixation rates is also variable, it is equally difficult to derive global rates of one from the other. A better understanding of the physiology and physiological ecology of *Trichodesmium* and other marine diazotrophs is necessary to understand and predict the effects of increased or decreased diazotrophy in the context of the carbon cycle and global change.

1 Introduction

Although a variety of marine cyanobacteria and bacteria are now known to fix dinitrogen (N_2) in marine environments, *Trichodesmium* spp. remain the most studied and most quantitatively significant pelagic nitrogen fixer based on available information. *Trichodesmium* spp. occur throughout the subtropical and tropical ocean where it can represent up to half of the primary production (Carpenter et al., 2004). Based on direct rate measurements, *Trichodesmium* accounts for a quarter to half of geochemically derived estimates of marine N_2 fixation (Mahaffey et al., 2005). In addition to *Trichodesmium*, pelagic nitrogen fixers include other filamentous cyanobacteria, unicellular cyanobacteria, bacterioplankton, and cyanobacterial endosymbionts (Carpenter and Capone, 2006)[1] Global estimates of nitrogen fixation and possible controls on marine nitrogen fixation have been recently summarized and reviewed (LaRoche and Breitbarth, 2005; Mahaffey et al., 2005; Carpenter and Capone, 2006[1]) and so will not be re-reviewed here. While we now know that marine N_2 fixation is significant source of new nitrogen (N) in the marine environment, little is known about the fate of this production, despite the importance of diazotrophs to global carbon and nutrient cycles (Karl et al., 2002; LaRoche and Breitbarth, 2005). Growth rates of these organisms vary by orders of magnitude as do rates of N_2 and carbon fixation (see Mulholland et al., 2006) and reasons for this variability are not well understood. Inputs of N and carbon (C) via N_2 fixation and associated carbon fixation have been measured directly or extrapolated in a variety of systems, however, the quantification of loss terms is poorly constrained. *Trichodesmium* are rarely found in sediment traps and are positively buoyant (Walsby, 1992) and so sinking appears to be a minor loss term compared with cell lysis (Ohki, 1999; Hewson et al., 2004), extracellular release (Capone et al., 1994; Glibert and Bronk, 1994; Mulholland et al., 2004a), and grazing (O'Neil et al., 1996; O'Neil, 1999)

[1] Carpenter, E. J. and Capone, D. G.: Nitrogen fixation in the marine environment, in: Nitrogen in the Marine Environment, edited by: Capone, D. G., Bronk, D. A., Mulholland, M. R., and Carpenter, E. J., in review, 2006.

each of which are discussed below. In this paper, the fate of production from N_2 fixation will be examined based on observations of N_2 fixation and associated carbon fixation rates, N and C release, and trophodynamics associated with nutrient cycling.

2 N_2 fixation

Pelagic N_2 fixation is an important source of new N to marine systems. The most widely studied pelagic marine diazotrophs, *Trichodesmium* spp., play a pivotal role in marine elemental cycles in otherwise oligotrophic tropical and subtropical seas (Capone et al., 1997; Karl et al., 2002; LaRoche and Breitbarth, 2005). Globally, based on direct measurements, oceanic N_2 fixation by *Trichodesmium* has been estimated to be 80 Tg N year^{-1} (Capone and Carpenter, 1999), and represents upwards of 50% of the new production in some oligotrophic tropical and subtropical oceans (Karl et al., 1997; Capone et al., 2005; Mahaffey et al., 2005). Based on observed and derived N_2 fixation rates by *Trichodesmium*, N_2 fixation by these species alone is comparable in magnitude to estimated nitrate flux across the base of the euphotic zone in tropical and subtropical systems (Karl et al., 1997; Capone, 2001; Capone et al., 2005).

However, *Trichodesmium* still represent only 40 to 59% of the geochemically inferred N_2 fixation for the North Atlantic and Pacific (Mahaffey et al., 2005). The recent discoveries of diazotrophic unicellular cyanobacteria and bacterioplankton in marine systems (e.g., Zehr et al., 2001; Falcón et al., 2004; Montoya et al., 2004) suggest that there are additional sources of N_2 fixation that may yet reconcile measured with geochemically predicted rates of N_2 fixation in the ocean. Although, the full range of diazotrophic marine organisms is as yet unknown, it is thought that unicellular diazotrophs may contribute up to 10% of global new production (Montoya et al., 2004). *Richelia intracellularis*, an endosymbiotic cyanobacterium that can inhabit a diverse group of diatoms, fix significant amounts of nitrogen where these associations occur (Carpenter et al., 1999). Based on the available rate measurements and regional and global abundance estimates in the euphotic zone, endosymbiotic and free-living unicellular cyanobacteria

and bacteria are now believed to fix at least as much nitrogen as *Trichodesmium* in the ocean (Table 1, Carpenter et al., 1999; Zehr et al., 2001; Montoya et al., 2004). As a result, recent estimates for total pelagic marine N_2 fixation are now estimated to be between 100 and 200 Tg N year^{-1} (Karl et al., 2002; Galloway et al., 2004).

In addition to extrapolations from direct measurements of N_2 fixation rates, global estimates of marine N_2 fixation have been inferred based on geochemical arguments that rely on elemental stoichiometry of particles and dissolved nutrients in the ocean (see Mahaffey et al., 2005, for a more complete discussion). There are limitations to both of these approaches, however, because of methodological constraints and the physiological peculiarities of the dominant marine N_2 fixer, *Trichodesmium*, discussed below. The physiology of more recently identified N_2 fixers is still being elucidated and so it is premature to speculate on how these groups may influence estimates of global new production and carbon export.

Extrapolation of N_2 fixation rates made in laboratory or field populations of *Trichodesmium* to the world's ocean can yield a wide range of global marine N_2 fixation rates. For example, rates of N_2 fixation by *Trichodesmium* from field populations vary by six orders of magnitude (LaRoche and Breitbarth, 2005; Mulholland et al., 2006). Laboratory estimates vary only by about 4 orders of magnitude, but still, which rates do we choose for our global estimate? Based on laboratory studies, rates of N_2 fixation vary according to physiological state and yet the physiological state of natural populations is impossible to assess at the time of sampling. It is thought that rates of N_2 fixation and growth by *Trichodesmium* are limited by phosphorus (P), iron (Fe), or light (Sañudo-Wilhelmy et al., 2001; Mills et al., 2004; Fu et al., 2005; Mulholland and Bernhardt, 2005). However, the range of responses to these variables and their interactions is unknown (Mulholland and Bernhardt, 2005).

In addition to real physiological variability, rates vary depending on the method used to estimate N_2 fixation. The two most commonly used methods are the acetylene reduction method and $^{15}N_2$ uptake; the former measures gross N_2 fixation and the latter measures net N_2 uptake into biomass. The acetylene reduction method relies

on a conversion factor to convert moles of acetylene to moles N_2 reduced and the value of this conversion factor has been a matter of debate (see Mulholland et al., 2006; LaRoche and Breitbarth, 2005). Paired comparisons between the two methods, used to calibrate one against the other, demonstrate that the ratio between acetylene reduction and N_2 uptake varies widely both within and among systems and studies (Table 2). Consequently, we are left with an unsatisfying set of data with which to make direct estimates of global N_2 fixation.

3 Carbon fixation

Global estimates of carbon fixation by marine diazotrophs based on direct measurements have not been attempted to my knowledge. There are fewer published estimates of carbon fixation by *Trichodesmium* and global carbon fixation by this genus is generally estimated by multiplying the nitrogen fixation rate by some average C:N for *Trichodesmium* biomass. Modeling results assume N_2 fixation equals denitrification, and this corresponds to 480 to 960 Tg C $year^{-1}$ (Mahaffey et al. 2005). Fortunately, the C:N ratio of *Trichodesmium* biomass, unlike the N:P ratios, fall within a narrow range (e.g., 4.7 to 7.3; LaRoche and Breitbarth, 2005) with an average value of 6.3, very near the Redfield ratio. Unfortunately, as for N_2 fixation, direct rate measurements of carbon fixation and carbon specific turnover times by *Trichodesmium* vary by orders of magnitude (Mulholland et al., 2006). Further, there is no consistent stoichiometric relationship between the ratio of C to N_2 fixation. Available paired estimates of N_2 and C fixation suggest that in general, C:N fixation ratios far exceed the C:N ratio of cells (Table 3; Mulholland et al., 2006). Consequently, geochemical estimates that rely on elemental stoichiometry to extrapolate of N_2 fixation from observations of carbon drawdown or the carbon cycle in general may be grossly in error (Mahaffey et al., 2005). For example, at the Bermuda Atlantic Time-Series Study (BATS) site, the observed rates of C drawdown were much higher than that which can be accounted for based on the observed rates of N_2 fixation and Redfield stoichiometry (e.g., C:N of 6.6:1).

However, when the average observed ratio of carbon to N_2 fixation rates measured at BATS (C:N_2 fixation rates of 128) were used, the observed low rates of N_2 fixation could indeed account for the observed carbon drawdown at BATS (Orcutt et al., 2001). Interestingly, the extrapolation of N_2 fixation rates necessary to close C budgets may be seriously biased (overestimated) if the actual rate relationships between N_2 and carbon fixation are not considered. The relationship between N and P may be even more complex.

There are a variety of reasons why there may be higher-than-stoichiometrically-expected carbon to N_2 fixation ratios in nature; these include factors resulting in underestimates of N_2 fixation rates and rationalizations as to why *Trichodesmium* may have unusually high carbon fixation rates. Gross N_2 fixation rates can be underestimated in $^{15}N_2$ incubations if there is substantial N release (Glibert and Bronk, 1994; Mulholland et al., 2004a, 2006; see Sect. 4 below) or gross N utilization may be underestimated if alternative N sources are taken up (Mulholland and Capone, 1999; Mulholland et al., 1999a, 1999b). On the other hand, carbon fixation rates may be stoichiometrically higher than expected, based on the elemental ratio of cells, if carbon is used as ballast for vertical migration (Villareal and Carpenter, 1990; Romans et al., 1994; Gallon et al., 1996), if substantial carbon is excreted as mucilage or extracellular polymeric substances (Stal, 1995; Sellner, 1997), to support the high observed respiration rates by *Trichodesmium* (Carpenter and Roenneberg, 1995; Kana, 1993), or if cells "over-fix" carbon to support Mehler reactions to reduce cellular oxygen concentrations or support the production of ATP (Kana, 1992, 1993). Kana (1993) estimated that 48% of gross photosynthetic electron flow went to oxygen reduction. *Trichodesmium* also make poly-beta-hydroxybutyric acid as a storage product (Siddiqui et al., 1992) and this may be important in carbohydrate ballasting (Romans et al., 1994) but would require additional cellular carbon reserves. In addition to these physiological reasons why carbon might be "over-fixed" relative to nitrogen, active release of carbon compounds and photosynthate has been observed and will be discussed below in Sect. 5. Alternatively, N and C uptake may not be tightly coupled in diazotrophic cyanobacteria (Gallon et al., 2002).

Another interesting genomic finding is that *Trichodesmium erythraeum* is unusual among cyanobacteria in that it lacks any genes encoding known high-affinity carbon concentrating mechanisms (Badger and Price, 2003). While it is not clear how this affects photosynthetic C acquisition by *Trichodesmium*, it suggests this species is vulnerable to C limitation. Continuous carbon fixation or storage of fixed carbon compounds, even in excess of their growth requirements, may protect them from carbon limitation in nature. Although carbon limitation has not been demonstrated for these species in nature, the interior of *Trichodesmium* colonies have been shown to exhibit oxygen dynamics that may be an important for aerobic N_2 fixation (Paerl and Bebout, 1988; Carpenter et al., 1990; Gallon, 1992).

Although the ultimate biogeochemical fate of *Trichodesmium*-fixed elements is not fully understood (Mahaffey et al., 2005; Mulholland et al., 2006), any fraction of new production from diazotrophy that is exported to underlying waters will contribute to sequestering atmospheric carbon and so it is important that we gain a better understanding of the coupled N and C cycles for these organisms.

4 N release

Trichodesmium sp. can fix N_2 at high rates, thereby introducing new nitrogen N into nutrient impoverished areas of the tropical and subtropical ocean. Because *Trichodesmium* also release fixed N as dissolved organic N (DON) (Glibert and Bronk, 1994), amino acids (Capone et al., 1994), and ammonium (Mulholland and Capone, 2001; Mulholland et al., 2004a), they may contribute to regenerated production where they occur. Elevated NH_4^+ and/or DON concentrations have also been observed in and around *Trichodesmium* blooms in the Pacific (Devassy et al., 1978, 1979; Karl et al., 1992, 1997), the Gulf of Mexico (Lenes et al., 2001), along the coast of Australia (Glibert and O'Neil, 1999) and in aging *Trichodesmium* cultures (Mulholland and Capone, 2001). However, nutrient concentrations within and around blooms may not always be high if the released N is rapidly taken up by organisms growing on and

around colonies (e.g., see Sellner, 1992, 1997) or co-occurring in the water column (Mulholland et al., 2006).

In addition to direct release of labile N, viral cell lysis (Ohki, 1999; Hewson et al., 2004), grazing (O'Neil et al., 1996), and cell death (Berman-Frank et al., 2004) may contribute dissolved or particulate N to the available N pool. *Trichodesmium* biomass may also be degraded via bacterial activity and extracellular enzymes thereby rendering large organic compounds into smaller utilizable compounds (Paerl et al., 1989; Nausch, 1996)

N release from *Trichodesmium* was first suggested by Devassy et al. (1978) who observed substantial enrichment of phosphate, nitrate and ammonium within, during, and after *Trichodesmium* blooms and relative to non-bloom sites or at times prior to blooms. Based on changes in nutrient concentrations within incubations, Devassy et al. (1978) estimated 1.5 μmol N (as inorganic N) and 6.8 μmol inorganic P was released per g of *Trichodesmium*. These authors also suggested that release rates would have been much higher if DOP, urea and amino acids had been measured.

N release has been difficult to estimate using isotopic tracer or other methods for a number of reasons:

(1) release products may be diverse and so it is often difficult to isolate and measure all relevant dissolved pools, (2) release products are rapidly taken up by organisms in oligotrophic environments, and (3) intracellular pools of intermediate metabolites can accumulate before their release for variable amounts of time.

To get around these problems, it has been suggested that the difference between net and gross N_2 fixation measured using $^{15}N_2$ uptake and acetylene reduction techniques, respectively, might be a good metric of N release (Gallon et al., 2002; Mulholland et al., 2004a, 2006).

High N release rates would seem to argue for high cellular N turnover, however, if N is released prior to its assimilation into biomass, this would contribute to gross N_2 fixation (e.g., reduction of N_2 to NH_4^+) but not net uptake into biomass. In numerous paired comparisons between acetylene reduction and $^{15}N_2$ uptake, ratios of acetylene

(C_2H_2) reduced to N_2 taken up have varied by at least an order of magnitude (Table 2). Because C_2H_2 reduction measures just the reduction step, it is a measure of gross N_2 fixation while movement of $^{15}N_2$ from the dissolved to the particulate pool measures net N assimilation (see Gallon et al., 2002; Mulholland et al., 2004a, 2006; Mulholland and Bernhardt, 2005). Release of recently reduced N_2 and the difficulty in chemically recovering all possible dissolved pools into which products of N_2 fixation might be released, may make intercalibration between the two methods impossible. However, the difference between N_2 reduction (gross N_2 fixation) and net N_2 assimilation may prove to be an excellent index of the release of recently fixed N_2 (Mulholland et al., 2004a, 2006; Mulholland and Bernhardt, 2005). If this is the case, and the theoretical ratio, three, is assumed to be correct (see Mulholland et al., 2006, for a discussion of this assumption) in estimating N_2 fixation from C_2H_2 reduction, paired comparisons in which C_2H_2:N_2 reduction ratios of approximately 3:1 are observed, would indicate no N release while C_2H_2:N_2 reduction ratios of six would translate into a release rate of about 50%, and so on.

Examining paired comparisons of C_2H_2 reduction and $^{15}N_2$ uptake from recent studies and the literature (Table 1), rates of release of recently fixed N can be compared across systems and with respect to temperature. Results demonstrate that release rates are highly variable on a variety of temporal and spatial scales. Release rates appeared to be high in populations collected from a South Pacific lagoon, varied seasonally in the North Atlantic along a latitudinal gradient, and on a daily and interannual basis in the Gulf of Mexico (Table 2). In cultures, release rates varied with the growth rate (Mulholland and Bernhardt, 2005) and with the time of day (Table 2). Based on various studies where $^{15}N_2$ and acetylene reduction were compared directly, it appears that N release from *Trichodesmium* is common but varies with physiological state and among environments. One paired comparison is available for a diatom/*Richelia* association (Table 2; Carpenter et al., 1999) and suggests that there was N release from N_2 fixation for this association as well. No published paired comparisons of C_2H_2:N_2 reduction for unicells are available and so release by these organisms cannot be as-

sessed at this time.

While there have been observations that *Trichodesmium* release recently fixed N_2 as DON in natural populations (Capone et al., 1994; Glibert and Bronk, 1994) and as NH_4^+ in cultures of *Trichodesmium* IMS101 (Mulholland et al., 2004a; Mulholland and Bernhardt, 2005) it is unclear why cells do this. Previous speculation suggested that this is a mechanism for the extracellular transfer of fixed N between cells that fix N_2 and those that do not have that capability (Mulholland and Capone, 1999, 2000). Another possible fate for regenerated N is co-occurring organisms (O'Neil et al., 1996; Mulholland et al., 2004b, 2006). Release rates averaged about 52% of the recently fixed N_2 or 0.29 nmol col^{-1} h^{-1} in a recent study in the Gulf of Mexico (Mulholland et al., 2006), much of this may have fueled production of co-occurring phytoplankton (Mulholland et al., 2004b).

N release from cellular material can also be mediated through cell lysis. Viruses and a lytic cycle have been observed in natural populations and cultures of *Trichodesmium* (Ohki, 1999). Hewson et al. (2004) estimated lysis rates of 0.3 to 6.5% trichomes d^{-1}, representing a release of 3 to 65% of the production for *Trichodesmium* growing at 0.1 d^{-1}. While these authors report this as 3 to 65% of recently fixed N d^{-1}, this also applies to C (see below). At an average rate of 43 pmol N fixed $trichome^{-1}$ d^{-1}, this represents a release rate of 1.3 to 28 pmol $trichome^{-1}$ d^{-1} or (using an average colony size of 100 trichomes col^{-1}) 13 to 280 pmol col^{-1} d^{-1}. For C, this could be substantially greater than a factor of 6 if stoichiometrically more C is fixed per day. These estimates agree well with calculated mortality rates for *Trichodesmium* derived from models at BATS and in the equatorial Atlantic estimate 2.1 to 2.5% d^{-1} (Hood et al., 2002, 2004).

5 C release

Dissolved organic N also contains C and so it is therefore logical to assume that *Trichodesmium* also release substantial amounts of photosynthetic products as dissolved organic carbon (DOC). In fact, Shimura at al. (1978) first quantified the extracellular re-

lease of photosynthate from ^{14}C incubations and calculated that about 8% of the total photosynthetic products were released during incubation experiments (range 0–18%). Similarly, Gallon et al. (1996) estimated that DOC excretion by *Trichodesmium* in the western North Atlantic and eastern Caribbean Sea represented only 7% of the primary productivity. As for N, the amount of C released changed depending on light conditions and the physiological status of cells. More recently, Renaud et al. (2005) estimated a much lower value (1%) for DOC release by *Trichodesmium*. However, they suggested that tight coupling between organisms in the *Trichodesmium* consortium might cause underestimates of actual release rates. Thus, the same methodological limitations that make it difficult to estimate N release from tracer studies make it difficult to make estimates of C release; just as $^{15}N_2$ uptake can underestimate gross N_2 fixation, ^{14}C (or ^{13}C) incorporation can underestimate the gross rate of photosynthetic carbon fixation (Gallon et al., 2002).

Cyanobacteria release C compounds such as glycolate (Renström-Kellner et al., 1989), as well as, amino acids which contain N and C (Capone et al., 1994). Amino acid release as glutamine and glutamate (molar C:N ratios of 5:2 and 5:1, respectively) represented only 3% of the C fixed by *Trichodesmium* (Capone et al., 1994). However, *Trichodesmium* have a carbohydrate mucoid matrix, which is colonized by other organisms (Stal, 1995; Sellner, 1997; Sheridan et al., 2002) and so there is a constant production of glucose- and mannose-rich mucilage that could account for more DOC release (e.g., Sellner, 1997). Cyanobacteria in general can exude as much as 80% of the CO_2 they fix as extracellular polymeric substances (mainly polysaccharides).

Production of colored dissolved organic matter (CDOM) by *Trichodesmium* was recently been observed (Steinberg et al., 2004). Production of DOC ranged from 0.04 to 0.32 μg C col^{-1} h^{-1}. Assuming an average of 11.3 μg C col^{-1} (McCarthy and Carpenter, 1979), this represents between 0.4 and 2.8% h^{-1} or up to 67% d^{-1}, although it is unclear whether this production is confined to the dark or light periods. The CDOM had absorption spectra similar to microsporin-like amino acids, compounds that *Trichodesmium* are know to have and serve in photoprotection (Subramaniam et

al., 1999).

Although globally, we are interested in the fate of new production from N_2 fixation as a means to export C, little has been done to quantify or characterize DOC release from *Trichodesmium* or other marine N_2 fixers. If we are to extrapolate export from production of N_2 fixers, it will be important to determine the primary pathways of C flow through these organisms.

6 Trophic interactions

It is impossible to discuss the fate of new production by diazotrophs without discussing trophic interactions. Colonies of *Trichodesmium* provide stable "homes" for a numerous and diverse association of organisms (Siddiqui et al., 1992; Sellner, 1997; O'Neil, 1999; Sheridan et al., 2002). This creates a complex microenvironment with multifarious pathways for internal nutrient cycling. Sheridan et al. (2002) estimated that 85% of *Trichodesmium* colonies were inhabited by other organisms. Colonizing organisms include bacteria, other cyanobacteria, fungi, pennate and centric diatoms, heterotrophic and autotrophic dinoflagellates, chrysophytes, ciliates, amoebae, hydroids, different life stages of harpacticoid copepods and juvenile decapods. Bacteria and dinoflagellates were the most common associates. Despite the fact that colonies are rich microenvironments, there is a variety of evidence suggesting that *Trichodesmium* themselves go largely ungrazed and so viral cell lysis and decomposition are the likely fates for many of these populations (nutrient accumulation), and that the importance of higher trophic levels in processing *Trichodesmium* biomass is minimal as compared to recycled primary production and bacterial productivity.

Consistent with this idea is the observation that a variety of phytoplankton, bacteria, and higher trophic levels co-occur or occur in the water column subsequent to blooms of *Trichodesmium* sp. (Devassy, 1978, 1979; Revelante et al., 1982; Furnas and Mitchell, 1996; Walsh and Steidinger, 2001; Mulholland et al., 2006). It is thought that these communities are relieved from N limitation as a result of N release from *Trichodesmium*.

Trichodesmium occur as variously sized and shaped aggregates or colonies but also as free filaments or trichomes. Large colonies may contain hundreds of trichomes. However, the average colony size and colony abundance can vary from day to day (Devassy et al., 1978). Colonies take the form of bundles with trichomes arranged in parallel (tufts) or radially (puffs). Little is known about the causes of bundle formation but, the distributions of free filaments and bundles vary regionally and apparently with the degree of turbulence (Bryceson and Fay, 1981; Mahaffey et al., 2005). The purpose of bundle formation is unclear but, there has been speculation that it may be a behavioral strategy for minimizing the exposure of nitrogenase (an oxygen sensitive protein) to oxygen (e.g., Paerl et al., 1989; Gallon, 1992). Regardless of the reasons colonies form, the trophodynamics of *Trichodesmium* varies depending on the form it takes and the amount of stable surface area and interfilamental space available for colonization.

There are few direct measurements of the trophic transfer of recently fixed N or C. Bryceson and Fay (1981) first demonstrated that the trophic transfer of recently fixed N_2 might be important in communities dominated by *Trichodesmium* and they subsequently demonstrated enrichment in non-*Trichodesmium* size fractions after incubation of *Trichodesmium* and natural marine communities with $^{15}N_2$. They did not have control incubations to account for N_2 fixation by smaller diazotrophic cyanobacteria and bacterioplankton but, nevertheless, they report enrichment in the 2 to 30 μm and 0.2 to 2.0 μm size fractions (Bryceson and Fay, 1981). Subsequently, the only other direct estimates of the trophic transfer of recently fixed N_2 demonstrated that up to 11% of recently fixed N_2 was transferred to non-N_2 fixing cells in whole water samples even in short (2 h) incubations (Mulholland et al., 2004b). This suggests that *Trichodesmium* may support community productivity in the upper water column and the growth of co-occurring organisms, including heterotrophs, rather than substantial direct sinking flux.

Despite the idea that dissolved nutrients may be the primary route of trophic transfer of recently fixed N_2, isotopically "light" zooplankton were collected from the tropical Atlantic (Montoya et al., 2002) and isotopically light sediment trap material was collected under a station experiencing a *Trichodesmium* bloom in the Indian Ocean (Capone et

al., 1998) indicating that recently fixed N_2, which has an isotopic signature similar to atmospheric N, is being transferred to higher trophic levels.

6.1 Bacteria

Bacterial associates with *Trichodesmium* colonies have been widely observed (Paerl et al., 1989; Nausch, 1996; Sheridan et al., 2002; Renaud et al., 2005; Mulholland et al., unpublished data). *Trichodesmium* colonies are inhabited by both rod-shaped and filamentous bacteria, as are many other filamentous cyanobacteria (Paerl et al., 1989). Bacteria associates, including heterotrophic N_2 fixers, were located around and within aggregates where they took up carbohydrates and amino acids.

Varying degrees of enrichment of bacteria have been found on and around colonies. Nausch (1996) reported that bacteria were 2 to 5 times higher on colonies of *Trichodesmium* than in the surrounding water, however, during her study, *Trichodesmium* were not abundant, the water column was turbulent, and colonies were small. At BATS, Sheridan et al. (2002) report that bacteria were enriched on average 401 and 1709 times on *Trichodesmium* puffs and tufts, respectively. Carpenter and Price (1977) found that up to 8.3% of *Trichodesmium* were populated by bacteria in the Sargasso Sea. So, it appears that there is high variability in the degree of colonization of bacterial colonization of *Trichodesmium* aggregates.

In terms of their productivity, Nausch (1996) found thymidine incorporation to be enhanced in colonies of *Trichodesmium* relative to that of the water column and comparable to the enrichment found in marine snow. However, productivity per unit bacteria appeared to be lower in colonies than in the surrounding water. In the Gulf of Mexico, leucine uptake increased by up to 72% in association with *Trichodesmium* colonies relative to the surrounding water column (Mulholland et al., unpublished data). Similarly, Tseng et al. (2005) found that bacterial productivity and abundance was higher but productivity per unit bacterial biomass was lower, in association with *Trichodesmium* populations. In addition, they found that populations became more autotrophic during times of the year when *Trichodesmium* was abundant (lower bacterial productiv-

ity: primary productivity ratio). The authors attribute this to N release and alleviation of competition between bacteria and phytoplankton for scarce NH_4^+. However, they also note that *Trichodesmium* occurred as free filaments in the Kuroshio and therefore lacked harpacticoid grazer populations and associated organisms observed in other communities (Tseng et al., 2005).

High rates of amino acid oxidase activity (Mulholland et al., 1998; Glibert and O'Neil, 1999), peptide hydrolysis (Mulholland et al., unpublished data), and hydrolytic enzyme activity have also been found in association with *Trichodesmium* colonies, suggesting bacteria and organisms associated with colonies actively cycle nutrients. Nausch (1996) calculated C and N release rates between 30.5 and 1086 ng C col^{-1} h^{-1} and 4.6 to 209 ng N col^{-1} h^{-1}, respectively, based on hydrolytic enzyme activities associated with *Trichodesmium* colonies.

6.2 Phytoplankton

In some coastal systems, blooms of dinoflagellates and diatoms have been observed during and subsequent to *Trichodesmium* blooms (Devassy et al., 1978; Revelante et al., 1982; Furnas and Mitchell, 1996). For example, Devassy et al. (1979) found that as blooms of *Trichodesmium* decayed, *Chaetoceras* populations increased, followed by a succession of cladocerans, dinoflagellates, green algae (*Noctiluca*), copepods, and finally, carnivores. On the West Florida shelf, dense *Karenia brevis* blooms occur during and subsequent to *Trichodesmium* blooms and it has been hypothesized that they provide a source of new N to fuel destructive red tides (Walsh and Steidinger, 2001, Mulholland et al., 2006). Based on direct estimates of N_2 fixation, N release, and in situ water column N uptake rates, *Trichodesmium* produced ample dissolved N to fuel *K. brevis* population growth in the Gulf of Mexico (Mulholland et al., 2006).

Experiments suggest that *Tetraselmis* grew well on decaying *Trichodesmium* (Devassy et al., 1978). Similarly, *Karenia brevis* cultures grew well on culture medium enriched in *Trichodesmium* exudates as the sole source of nitrogen (Mulholland et al., unpublished data). While direct evidence of trophic transfer from *Trichodesmium*

to phytoplankton in nature are lacking, Bryceson and Fay (1981) and Mulholland et al. (2004b), demonstrated that ^{15}N derived from $^{15}N_2$ additions moved into the co-occurring plankton, which presumably included a variety of phytoplankton.

6.3 Zooplankton and higher trophic levels

The fate of recently fixed N_2 and transfer of *Trichodesmium* biomass to higher trophic levels is poorly understood. Although a variety of herbivores are thought to graze on *Trichodesmium* (e.g., Sellner, 1997), *Trichodesmium* sp. are not grazed by many of the dominant zooplankton in marine systems and are toxic to many copepods (Hawser and Codd, 1992; O'Neil, 1999). Some specialized harpacticoid copepods do graze on and inhabit *Trichodesmium* colonies but these do not produce fecal pellets that would rapidly remove grazed material from the euphotic zone (O'Neil and Roman, 1994; O'Neil et al., 1996).

O'Neil et al. (1996) estimated that the harpacticoid copepod, *Macrosetella*, could consume 33–45% of total colony N, or 100% of the new N_2 fixed each day. The copepod then excretes 48% of its body N per day, mainly as NH_4^+, thereby recycling much of the N in the water column (O'Neil et al., 1996). Further, Roman (1978) found that *Macrosetella* could ingest from 90 to 126% of its body carbon per day when feeding on *Trichodesmium*. Based on stoichiometric arguments, O'Neil (1999) calculated 30% of the recently fixed C from *Trichodesmium* flowed into grazers and because *Macrosetella* appear to have a higher C:N ratio than the *Trichodesmium* themselves, they are likely to excrete excess N. Therefore, the major flux of recently fixed N and C through zooplankton may also be through extracellular release and dissolved nutrient pools. In addition to excretory release, zooplankton grazers can mediate the transfer of N through additional release from sloppy feeding (O'Neil and Roman, 1996).

Not much is known about higher trophic levels, although isotopic evidence suggests that there are other grazers of *Trichodesmium*. In general, it has been observed that there is a low quality of fish associated with blooms although *Trichodesmium* do not appear to be directly toxic to fish (Devassy et al., 1978). Fish and some other higher

trophic levels have been observed to graze on *Trichodesmium* (see Carpenter and Capone, 2006[1]).

7 Implications

While we appear to be making strides in our ability to derive global estimates for marine N_2 fixation, we have a long way to go before we understand the role of N_2 fixation in the context of C dynamics in the ocean. Because many direct estimates of global N_2 fixation are based on highly spatially, temporally, and physiologically variable data, and because many geochemical estimates rely on stoichiometric relationships of nutrient standing stocks without considering the imbalances between rate estimates of C and N_2 fixation we should proceed cautiously when inferring one from the other (see examples in Sect. 3 above). Based on the observed C drawdown from the atmosphere, we may be trying to find "too much" N_2 fixation if we use Redfield stoichiometry versus the observed relative rates of C and N_2 fixation.

It is also difficult to determine the effect of N_2 fixation on system trophic status. In some systems *Trichodesmium* appears to fuel primary productivity and make the system more autotrophic (e.g., Tseng et al., 2005). In other systems dominated by *Trichodesmium*, heterotrophic processes appear to dominate (Fig. 1; also see Sect. 6.3 above). For the Kenyan coast, primary productivity, even during *Trichodesmium* blooms, could barely sustain the observed bacterial productivity (Kromkamp et al., 1997).

The preponderance of filaments versus colonial morphology can also seriously bias our understanding of trophodynamics associated with *Trichodesmium* and the net outcome of elemental cycling (e.g., recycling and respiratory losses versus export). Not only would it influence colony specific estimates of N and C fixation, but the degree of aggregation and size of colonies affect the degree to which *Trichodesmium* is colonized and thereby recycled to bacteria and copepods, and possibly other taxa. Free filaments often dominate populations in the Pacific (Saino and Hattori, 1978, 1980; Letelier and

Karl, 1996; Tseng et al., 2005) and the Atlantic, at least seasonally (Orcutt et al., 2001) although in many systems, colonies appear to be more common (Capone et al., 1997; Carpenter et al., 2004).

Finally, diazotrophs may have different fates. Diatom/*Richelia* assemblages may be prone to gravitational settling while unicellular cyanobacteria may be more readily grazed. Not enough is known about community dynamics associated with these populations to speculate at this time.

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Table 1. Ranges of water column N_2 fixation rates. Rates are presented as hourly rates because it is unclear whether N_2 fixation by unicellular diazotrophs exhibit diel periodicity. For comparison, rates of N_2 fixation by *Trichodesmium* range from <0.1 to 21.4 nmol N col^{-1} d^{-1} (N_2 fixation is confined to the light period) and colony abundance can range from <1 to >1000 colonies L^{-1} (Mulholland et al., 2006).

Date	Location	Depth	N_2 fixation	Method	Reference
			(nmol $L^{-1}h^{-1}$)		
2001–2003	Gulf of Mexico	Surface	0.011–0.23	$^{15}N_2$	Mulholland et al. (2006)
2003	Gulf of Mexico	Pigment maximum	0.044–0.063	$^{15}N_2$	Mulholland et al. (2006)
2002	New Caledonia	Surface	0.23–0.85	$^{15}N_2$	Mulholland et al. (unpublished)
2002	N Atlantic	25 m (nighttime)	~0.147	AR	Falcon et al. (2004)
2001	N. Atlantic	Upper 100 m	0.025–0.045	$^{15}N_2$	Falcon et al. (2004)
2002	N Pacific	Upper 100 m (nighttime)	~0.003	AR	Falcon et al. (2004)
2000–2001	Station ALOHA & Kaneohe Bay	25 m & Surface	0.01–0.15	$^{15}N_2$	Montoya et al. (2004)
2002	Eastern N. Pacific	Mixed layer & pigment maximum	0.047–1.85(0.72)	$^{15}N_2$	Montoya et al. (2004)
1999	Arafura Sea	Pigment maximum	20–62	$^{15}N_2$	Montoya et al. (2004)
2000	Station ALOHA	25 m	0.010–0.016	$^{15}N_2$	Zehr et al. (2001)
2002	Tropical Atlantic	Upper 100 m	up to 3.1	$^{15}N_2$	Voss et al. (2004)
2000–2001	Station ALOHA	Upper 100 m	0–0.09	$^{15}N_2$	Dore et al. (2002)[1]

[1]Converted from daily rate assuming N_2 fixation persisted for 24 h per day.

Table 2. Results from paired comparisons of C_2H_2:N_2 measurements. Numbers are reported as molar ratios and N release is estimated as the observed molar ratio minus the theoretical ratio (3) divided by the observed molar ratio. Modified and expanded from Mulholland et al. (2006).

Location	C_2H_2:N_2 (molar ratio)		Average N release %	Reference
	Range	Average		
Trichodesmium:				
Gulf of Mexico	3.3–15.8	7.3	52	Mulholland et al. (2006)
North Atlantic (latitudinal gradient) – Aug	3.1–7.5	4.6	29	Mulholland et al. (unpublished)
North Atlantic (latitudinal gradient) – Mar	6.3–52.7	28.3	79	Mulholland et al. (unpublished)
North Atlantic	0.9–7.3	3.6	17	Capone et al. (2005)
Sargasso Sea		6.0	50	Carpenter and McCarthy (1975)
Sargasso Sea		6.3	52	Carpenter and Price (1977)
Sargasso Sea		2.9		Scranton (1984)
Caribbean and Sargasso Seas		4.1	27	Scranton et al. (1987)
Caribbean Sea		3.4	12	Glibert and Bronk (1994)
BATS (net tows)		4.9	39	Orcutt et al. (2001)
BATS (SCUBA)		1.4		Orcutt et al. (2001)
BATS		3.0		Orcutt et al. (2001)
North Pacific		1.9		Mague et al. (1977)
Bay of Bengal & South China Sea	3–10	2.9		Saino (1977)
New Caledonia lagoon – S Pacific	4.8–19.5		21–97	Mulholland et al. (in prep.)
Trichodesmium IMS101 (batch)	1.7–9.8	5.6	46	Mulholland et al. (2004)
Trichodesmium IMS101 (continuous)	3.0–22.2	1.4	74	Mulholland and Bernhardt (2005)
Trichodesmium IMS101 (semi-continuous) – morning	4.9–14.9	8.9	59.7	Mulholland et al. (unpublished)
Trichodesmium IMS101 (semi-continuous) – afternoon	14.3–45.6	23.5	81.6	Mulholland et al. (unpublished)
Trichodesmium GBRTRLI101 – afternoon	8.8–24.9	16.7	76.5	Mulholland et al. (unpublished)
Other marine cyanobacteria:				
Rhizosolenia/Richelia association		9.3		Mague et al. (1974)
Mixed cyanobacteria – *Nodularia spumigena/Anabaena/Aphanizomenon*		3.8–20		Gallon et al. (2002)

Table 3. Paired comparisons of C and N_2 fixation. Modified and expanded from Mulholland et al. (2006).

Location	C:N_2 fixation Range	Average	Reference
Trichodesmium:			
Gulf of Mexico	5.4–42.7	13.1	Mulholland et al. (2006)
New Caledonia (lagoon) – morning	3.7–51.3		Mulholland et al. (unpublished)
North Atlantic (latitudinal gradient) – Aug	11.0–30.6	20.1	Mulholland et al. (unpublished)
North Atlantic (latitudinal gradient) – Mar	5.2–22.4	12.8	Mulholland et al. (unpublished)
North Pacific	1.2–2.1		Mague et al. (1977)
Sargasso Sea		16	Carpenter and Price (1977)
Sargasso Sea	1.5–87		McCarthy and Carpenter (1979)
BATS (puffs)	13–437	128	Orcutt et al. (2001)
BATS (tufts)	15–703	198	Orcutt et al. (2001)
N. Atlantic (May–June 1994)		47.1	Carpenter et al. (2004); Capone et al. (2005)
N. Atlantic (April 1996)		37.7	Carpenter et al. (2004); Capone et al. (2005)
N. Atlantic (October 1996)		24.6	Carpenter et al. (2004); Capone et al. (2005)
Indian Ocean (Tanzania)		20	Lugomela et al. (2002)[1]
Trichodesmium IMS101 (batch)	4.6–132.5	28	Mulholland and Capone (2001)[2]
Trichodesmium IMS101 (batch)	6.5–15.2	9.6	Mulholland and Capone (2001)[3]
Trichodesmium IMS101 (continuous)	13.4–20.0		Mulholland and Bernhardt (2005)[4]
Trichodesmium IMS101 (semi-continuous) – morning	3.2–10.0	5.4	Hutchins et al. (unpublished)
Trichodesmium IMS101 (semi-continuous) – afternoon			Hutchins et al. (unpublished)
Trichodesmium GBRTRLI101	2.0–12.4	6.5	Hutchins et al. (unpublished)
Other marine cyanobacteria:			
Hemiaulus/Richelia association		12.5	Carpenter et al. (1999)[5]
Mixed cyanobacteria – Nodularia *spumigena/Anabaena/Aphanizomenon*		17.6 (0–7 m) 5.1 (7–14 m) 1.5 (14–21 m)	Gallon et al. (2002)

[1]Using study averages and recalculating with a conversion factor of 3:1. [2]Mid-day estimate during exponential growth. [3]Cumulative estimate over a growth or diel cycle. Ratio increased during stationary phase growth. [4]Lower at faster growth rates. [5]Calculated using average N_2fixation rate of 0.2 mg N m^{-3} h^{-1} and average C fixation rate at bloom stations of 2.14 mg C m^{-3} h^{-1}.

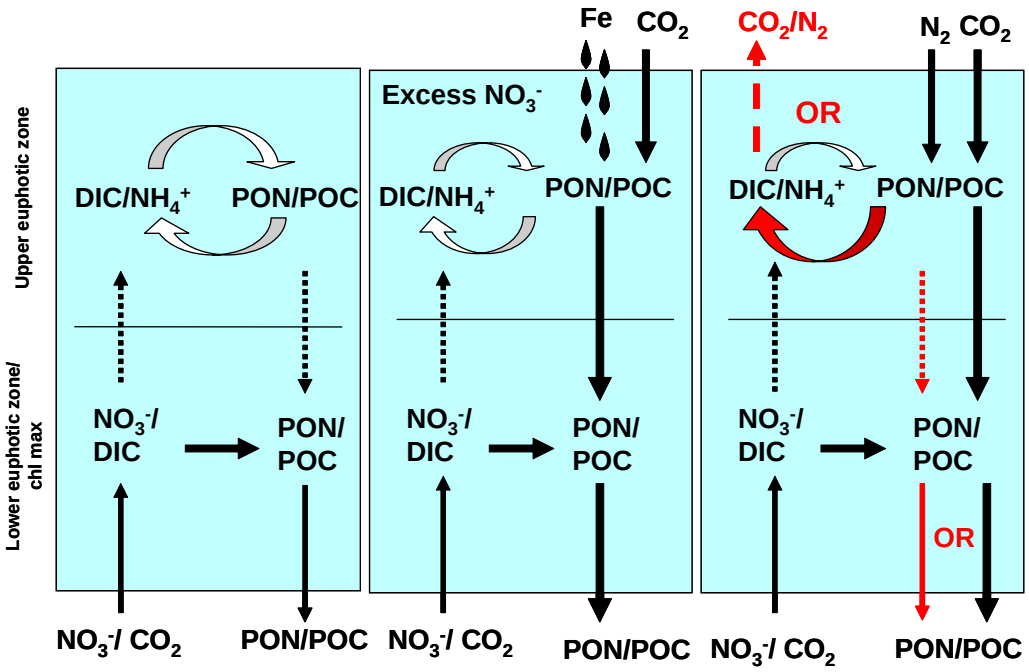

Fig. 1. Nitrogen and carbon cycling in the oligotrophic ocean with and without N_2 fixation. Panel **(A)** represents an ocean without N_2 fixation where new nitrate upwelled from below the euphotic zone balances export of material out of the euphotic zone. Panel **(B)** represents a low nutrient/high chlorophyll (HNLC) area fertilized with iron to stimulate drawdown and sequestration of atmospheric in the deep ocean. Panel **(C)** represents carbon drawdown from the atmosphere associated with N_2 fixation with alternative pathways whereby new production is respired in the euphotic zone resulting in no net drawdown or export of carbon from the euphotic zone (red) or drawdown and export of C from the atmosphere to the deep ocean. Redrawn and expanded from Hood et al. (2000).

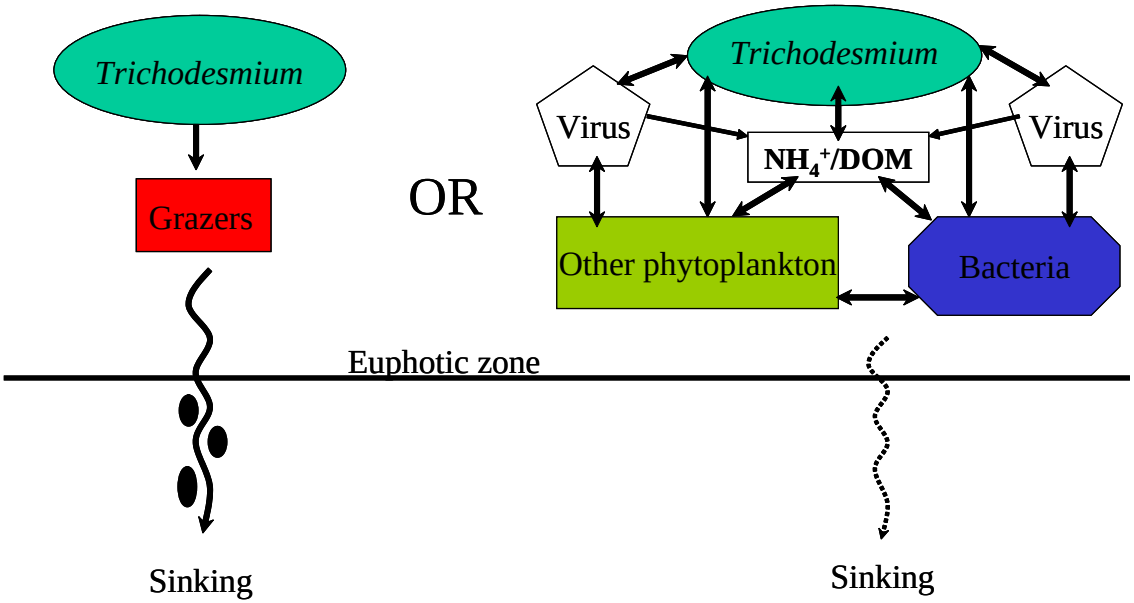

Fig. 2. Pathways of trophic transfer promoting **(a)** sinking flux and **(b)** carbon flow through a microbial loop with no net carbon export.