The Fate of Nitrogen Fixed by Diazotrophs in the Ocean

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The fate of nitrogen fixed by diazotrophs in the ocean

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Abstract. While we now know that N₂ fixation is a significant source of new nitrogen (N) in the marine environment, little is known about the fate of this N (and associated C), despite the importance of diazotrophs to global carbon and nutrient cycles. Specifically, does N fixed during N₂ fixation fuel autotrophic or heterotrophic growth and thus facilitate carbon (C) export from the euphotic zone, or does it contribute primarily to bacterial productivity and respiration in the euphotic zone? For *Trichodesmium*, the diazotroph we know the most about, the transfer of recently fixed N₂ (and C) appears to be primarily through dissolved pools. The release of N varies among and within populations and as a result of the changing physiological state of cells and populations. The net result of trophic transfers appears to depend on the co-occurring organisms and the complexity of the colonizing community. In order to understand the impact of diazotrophy on carbon flow and export in marine systems, we need a better understanding of the trophic flow of elements in *Trichodesmium*-dominated communities and other diazotrophic communities under various defined physiological states. Nitrogen and carbon fixation rates themselves vary by orders of magnitude within and among studies of *Trichodesmium*, highlighting the difficulty in extrapolating global rates of N₂ fixation from direct measurements. Because the stoichiometry of N₂ and C fixation does not appear to be in balance with that of particles, and the relationship between C and N₂ fixation rates is also variable, it is equally difficult to derive global rates of one from the other. This paper seeks to synthesize what is known about the fate of diazotrophic production in the environment. A better understanding of the physiology and physiological ecology of *Trichodesmium* and other marine diazotrophs is necessary to quantify and predict the effects of increased or decreased diazotrophy in the context of the carbon cycle and global change.

1 Introduction

Although we now know that dinitrogen (N₂) fixation is a significant source of new nitrogen (N) fueling primary production in the marine environment (sensu Dugdale and Goering, 1967), little is known about the fate of this production, whether it is exported or stimulates remineralization (e.g., Eppley and Peterson, 1979), despite the importance of diazotrophs to global carbon and nutrient cycles (Karl et al., 2002; LaRoche and Breitbarth, 2005). A variety of marine cyanobacteria and bacteria are now known to fix N₂ in marine environments, however, *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 they 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 N₂ fixation (Mahaffey et al., 2005). In addition to *Trichodesmium*, pelagic nitrogen fixers include other filamentous cyanobacteria, unicellular cyanobacteria, bacterioplankton, and cyanobacterial endosymbionts (Carpenter et al., 1999; Zehr et al., 2001; Montoya et al., 2004; Carpenter and Capone, 2007).

Global estimates of N₂ fixation and possible controls on marine N₂ fixation, at least by *Trichodesmium*, have been recently summarized and reviewed (LaRoche and Breitbarth, 2005; Mahaffey et al., 2005; Carpenter and Capone, 2007) and so will not be re-reviewed here. Growth rates of these organisms vary by orders of magnitude as do rates of N₂ and carbon fixation (see Mulholland et al., 2006) and reasons for this variability are not well understood. Inputs of N and carbon (C) via diazotrophic growth have been measured directly or extrapolated in a variety of systems, however, the quantification of loss terms for N and C (e.g., export) are 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
Pelagic N\textsubscript{2} fixation is an important source of new N to otherwise oligotrophic 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\textsubscript{2} fixation by *Trichodesmium* has been estimated to be 60 (Mahaffey et al., 2005) to 80 Tg N year\textsuperscript{-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\textsubscript{2} fixation rates by *Trichodesmium*, N\textsubscript{2} fixation by these species alone is comparable in magnitude to the estimated diffusive 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\textsubscript{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\textsubscript{2} fixation that may yet reconcile measurements with geochemically predicted rates of N\textsubscript{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, fixes significant amounts of nitrogen where diatom/*Richelia* 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\textsubscript{2} fixation are now between 100 and 200 Tg N year\textsuperscript{-1} (Karl et al., 2002; Galloway et al., 2004).

Extrapolation of N\textsubscript{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\textsubscript{2} fixation rates. For example, rates of N\textsubscript{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\textsubscript{2} fixation vary with environmental factors and according to physiological state (e.g., Mulholland et al., 1999, 2001; Mulholland and Capone, 2001; Mulholland and Bernhardt, 2005; Breitbarth et al., 2006; Hutchins et al., 2007) and yet the physiological state of natural populations is impossible to assess at the time of sampling. It is thought that rates of N\textsubscript{2} fixation and growth by *Trichodesmium* are limited by phosphorus (P), iron (Fe), or light (Šaňudo-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).

Besides real physiological variability, rates vary depending on the method used to estimate N\textsubscript{2} fixation. The two most commonly used methods are the acetylene reduction method and 15\textsubscript{N}N\textsubscript{2} uptake; the former measures gross N\textsubscript{2} fixation and the latter measures net N\textsubscript{2} uptake into biomass (Gallow et al., 2002; Mulholland et al., 2004, 2006). The acetylene reduction method relies on a conversion factor to convert moles of acetylene to moles N\textsubscript{2} reduced and the value of this conversion factor has been a matter of debate (see Capone, 1993; Mulholland et al., 2004, 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\textsubscript{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\textsubscript{2} fixation.

In addition to extrapolations from direct measurements of N\textsubscript{2} fixation rates, global estimates of marine N\textsubscript{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\textsubscript{2} fixer, *Trichodesmium*, discussed below. The physiology of more recently identified N\textsubscript{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.
Table 1. Ranges of water column N\textsubscript{2} fixation rates. Rates are presented as hourly rates because it is unclear whether N\textsubscript{2} fixation by unicellular diazotrophs exhibits diel periodicity. For comparison, rates of N\textsubscript{2} fixation by *Trichodesmium* range from <0.01 to 2.14 nmol N col\(^{-1}\) h\(^{-1}\) (N\textsubscript{2} fixation is confined to the light period) and colony abundance can range from <1 to >1000 colonies L\(^{-1}\) (Mulholland et al., 2006).

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Depth</th>
<th>N\textsubscript{2} fixation (nmol L(^{-1})h(^{-1}))</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001–2003</td>
<td>Gulf of Mexico</td>
<td>Surface</td>
<td>0.011–0.23</td>
<td>(^{15})N\textsubscript{2}</td>
<td>Mulholland et al. (2006)</td>
</tr>
<tr>
<td>2002</td>
<td>New Caledonia</td>
<td>Surface</td>
<td>0.044–0.063</td>
<td>(^{15})N\textsubscript{2}</td>
<td>Mulholland et al. (2006)</td>
</tr>
<tr>
<td>2002</td>
<td>N Atlantic</td>
<td>25 m (nighttime)</td>
<td>0.23–0.85</td>
<td>(^{15})N\textsubscript{2}</td>
<td>Mulholland et al. (2006)</td>
</tr>
<tr>
<td>2001</td>
<td>N. Atlantic</td>
<td>Upper 100 m</td>
<td>0.025–0.045</td>
<td>(^{15})N\textsubscript{2}</td>
<td>Falcon et al. (2004)</td>
</tr>
<tr>
<td>2002</td>
<td>N Pacific</td>
<td>Upper 100 m (nighttime)</td>
<td>~0.003</td>
<td>(^{15})N\textsubscript{2}</td>
<td>Falcon et al. (2004)</td>
</tr>
<tr>
<td>2000–2001</td>
<td>Station ALOHA &amp;</td>
<td>25 m &amp; Surface</td>
<td>0.01–0.15</td>
<td>(^{15})N\textsubscript{2}</td>
<td>Montoya et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Kaneohe Bay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>Eastern N. Pacific</td>
<td>Mixed layer &amp; pigment maximum</td>
<td>0.047–1.85 (0.72)</td>
<td>(^{15})N\textsubscript{2}</td>
<td>Montoya et al. (2004)</td>
</tr>
<tr>
<td>1999</td>
<td>Arafura Sea</td>
<td>Pigment maximum</td>
<td>20–62</td>
<td>(^{15})N\textsubscript{2}</td>
<td>Montoya et al. (2004)</td>
</tr>
<tr>
<td>2000</td>
<td>Station ALOHA</td>
<td>25 m</td>
<td>0.010–0.016</td>
<td>(^{15})N\textsubscript{2}</td>
<td>Zehr et al. (2001)</td>
</tr>
<tr>
<td>2002</td>
<td>Tropical Atlantic</td>
<td>Upper 100 m</td>
<td>up to 3.1</td>
<td>(^{15})N\textsubscript{2}</td>
<td>Voss et al. (2004)</td>
</tr>
<tr>
<td>2000–2001</td>
<td>Station ALOHA</td>
<td>Upper 100 m</td>
<td>0–0.09</td>
<td>(^{15})N\textsubscript{2}</td>
<td>Dore et al. (2002)</td>
</tr>
</tbody>
</table>

\(^1\)Converted from daily rate assuming N\textsubscript{2} fixation persisted for 24 h per day.

3 Carbon fixation

Global estimates of carbon fixation by marine diazotrophs based on direct measurements have not been attempted to the author’s knowledge. There are few published estimates of carbon fixation (compared to N\textsubscript{2} 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\textsubscript{2} fixation equals denitrification, which corresponds to 480–960 Tg C year\(^{-1}\) (Mahaffey et al., 2005). Fortunately, the C:N ratio of *Trichodesmium* biomass, unlike the N:P ratios, falls within a narrow range (4.7 to 7.3; LaRoche and Breitbarth, 2005) with an average value of 6.3, very near the Redfield ratio (6.6). Unfortunately, as for N\textsubscript{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\textsubscript{2} fixation (Table 3). Available paired estimates of N\textsubscript{2} and C fixation suggest that in general, C:N fixation ratios far exceed the C:N ratio of cells (see also Mulholland et al., 2006). Consequently, geochemical estimates that rely on elemental stoichiometry to extrapolate N\textsubscript{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\textsubscript{2} fixation and Redfield stoichiometry. However, when the average observed ratio of carbon to N\textsubscript{2} fixation rates measured at BATS (C:N\textsubscript{2} fixation rates of 128) were used, the observed low rates of N\textsubscript{2} fixation could indeed account for the observed carbon drawdown at BATS (Orcutt et al., 2001). Interestingly, the extrapolation of N\textsubscript{2} fixation rates necessary to close C budgets may be seriously biased (overestimated) if the actual rate relationships between N\textsubscript{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\textsubscript{2} fixation ratios in nature. These include: factors resulting in underestimates of N\textsubscript{2} fixation rates and rationalizations as to why *Trichodesmium* may have unusually high carbon fixation rates. Regarding the former, gross N\textsubscript{2} fixation rates can be underestimated in \(^{15}\)N\textsubscript{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, b). 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* (Kana, 1999; Carpenter and Roenneberg, 1995), 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.
Table 2. Results from paired comparisons of C\(_2\)H\(_2\):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.

<table>
<thead>
<tr>
<th>Location</th>
<th>C(_2)H(_2):N(_2) (molar ratio)</th>
<th>Average</th>
<th>N release</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trichodesmium:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gulf of Mexico</td>
<td>3.3–15.8</td>
<td>7.3</td>
<td>52</td>
<td>Mulholland et al. (2006)</td>
</tr>
<tr>
<td>North Atlantic (latitudinal gradient – Aug)</td>
<td>3.1–7.5</td>
<td>4.6</td>
<td>29</td>
<td>Mulholland et al. (unpublished data)</td>
</tr>
<tr>
<td>North Atlantic (latitudinal gradient – March)</td>
<td>6.3–52.7</td>
<td>28.3</td>
<td>79</td>
<td>Mulholland et al. (unpublished data)</td>
</tr>
<tr>
<td>North Atlantic</td>
<td>0.9–7.3</td>
<td>3.6</td>
<td>17</td>
<td>Capone et al. (2005)</td>
</tr>
<tr>
<td>Sargasso Sea</td>
<td>6.0</td>
<td>50</td>
<td></td>
<td>Carpenter and McCarthy (1975)</td>
</tr>
<tr>
<td>Sargasso Sea</td>
<td>6.3</td>
<td>52</td>
<td></td>
<td>Carpenter and Price (1977)</td>
</tr>
<tr>
<td>Sargasso Sea</td>
<td>2.9</td>
<td></td>
<td></td>
<td>Scranton (1984)</td>
</tr>
<tr>
<td>Caribbean and Sargasso Seas</td>
<td>4.1</td>
<td>27</td>
<td></td>
<td>Scranton et al. (1987)</td>
</tr>
<tr>
<td>Caribbean Sea</td>
<td>7.2</td>
<td></td>
<td></td>
<td>Carpenter and Price (1977)</td>
</tr>
<tr>
<td>Caribbean Sea</td>
<td>3.4</td>
<td>12</td>
<td></td>
<td>Glibert and Bronk (1994)</td>
</tr>
<tr>
<td>BATS (net tows)</td>
<td>4.9</td>
<td>39</td>
<td></td>
<td>Orcutt et al. (2001)</td>
</tr>
<tr>
<td>BATS (SCUBA)</td>
<td>1.4</td>
<td></td>
<td></td>
<td>Orcutt et al. (2001)</td>
</tr>
<tr>
<td>BATS</td>
<td>3.0</td>
<td></td>
<td></td>
<td>Orcutt et al. (2001)</td>
</tr>
<tr>
<td>North Pacific</td>
<td>1.9</td>
<td></td>
<td></td>
<td>Mague et al. (1977)</td>
</tr>
<tr>
<td>Bay of Bengal &amp; South China Sea</td>
<td>3–10</td>
<td>2.9</td>
<td></td>
<td>Saino (1977)</td>
</tr>
<tr>
<td>New Caledonia lagoon – S. Pacific</td>
<td>4.8–19.5</td>
<td>21–97</td>
<td></td>
<td>Mulholland et al. (unpublished data)</td>
</tr>
<tr>
<td><strong>Trichodesmium IMS101 (batch)</strong></td>
<td>1.7–9.8</td>
<td>5.6</td>
<td>46</td>
<td>Mulholland et al. (2004a)</td>
</tr>
<tr>
<td><strong>Trichodesmium IMS101 (continuous)</strong></td>
<td>3.0–22.2</td>
<td>11.4</td>
<td>74</td>
<td>Mulholland and Bernhardt (2005)</td>
</tr>
<tr>
<td><strong>Trichodesmium IMS101 (semi-continuous) – morning</strong></td>
<td>4.9–14.9</td>
<td>8.9</td>
<td>59.7</td>
<td>Hutchins et al. (2007)</td>
</tr>
<tr>
<td><strong>Trichodesmium IMS101 (semi-continuous) – afternoon</strong></td>
<td>14.3–45.6</td>
<td>23.5</td>
<td>81.6</td>
<td>Hutchins et al. (2007)</td>
</tr>
<tr>
<td><strong>Trichodesmium GBRTRLI101 – afternoon</strong></td>
<td>8.8–24.9</td>
<td>16.7</td>
<td>76.5</td>
<td>Hutchins et al. (2007)</td>
</tr>
<tr>
<td><strong>Other marine cyanobacteria:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizosolenia/Richelia association</td>
<td>9.3</td>
<td></td>
<td></td>
<td>Mague et al. (1974)</td>
</tr>
<tr>
<td>Mixed cyanobacteria –</td>
<td>3.8–20</td>
<td></td>
<td></td>
<td>Gallon et al. (2002)</td>
</tr>
<tr>
<td>Nodularia spumigena/Anabaena/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aphaniizomenon</td>
<td>4.7</td>
<td></td>
<td></td>
<td>Montoya et al. (1996)</td>
</tr>
</tbody>
</table>

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).

An interesting genomic finding is that Trichodesmium erythraeum is unusual among cyanobacteria in that it lacks any genes encoding known high-affinity carbon concentrat-
### Table 3. Paired comparisons of C and N$_2$ fixation.

<table>
<thead>
<tr>
<th>Location</th>
<th>Range</th>
<th>Average</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trichodesmium:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gulf of Mexico</td>
<td>5.4–42.7</td>
<td>13.1</td>
<td>Mulholland et al. (2006)</td>
</tr>
<tr>
<td>New Caledonia (lagoon) – morning</td>
<td>3.7–51.3</td>
<td></td>
<td>Mulholland et al. (unpublished data)</td>
</tr>
<tr>
<td>North Atlantic (latitudinal gradient) – Aug</td>
<td>11.0–30.6</td>
<td>20.1</td>
<td>Mulholland et al. (unpublished data)</td>
</tr>
<tr>
<td>North Atlantic (latitudinal gradient) – March</td>
<td>5.2–22.4</td>
<td>12.8</td>
<td>Mulholland et al. (unpublished data)</td>
</tr>
<tr>
<td>North Pacific</td>
<td>1.2–2.1</td>
<td></td>
<td>Mague et al. (1977)</td>
</tr>
<tr>
<td>Sargasso Sea</td>
<td></td>
<td>16</td>
<td>Carpenter and Price (1977)</td>
</tr>
<tr>
<td>BATS (puffs)</td>
<td>13–437</td>
<td>128</td>
<td>Orcutt et al. (2001)</td>
</tr>
<tr>
<td>BATS (tufts)</td>
<td>15–703</td>
<td>198</td>
<td>Orcutt et al. (2001)</td>
</tr>
<tr>
<td>N. Atlantic (May–June 1994)</td>
<td>47.1</td>
<td></td>
<td>Carpenter et al. (2004); Capone et al. (2005)</td>
</tr>
<tr>
<td>N. Atlantic (April 1996)</td>
<td>37.7</td>
<td></td>
<td>Carpenter et al. (2004); Capone et al. (2005)</td>
</tr>
<tr>
<td>N. Atlantic (October 1996)</td>
<td>24.6</td>
<td></td>
<td>Carpenter et al. (2004); Capone et al. (2005)</td>
</tr>
<tr>
<td>Indian Ocean (Tanzania)</td>
<td>20</td>
<td></td>
<td>Lugomela et al. (2002)</td>
</tr>
<tr>
<td><em>Trichodesmium</em> IMS101 (batch)</td>
<td>4.6–132.5</td>
<td>28</td>
<td>Mulholland and Capone (2001)$^2$</td>
</tr>
<tr>
<td><em>Trichodesmium</em> IMS101 (batch)</td>
<td>6.5–15.2</td>
<td>9.6</td>
<td>Mulholland and Capone (2001)$^3$</td>
</tr>
<tr>
<td><em>Trichodesmium</em> IMS101 (continuous)</td>
<td>13.4–20.0</td>
<td></td>
<td>Mulholland and Bernhardt (2005)$^4$</td>
</tr>
<tr>
<td><em>Trichodesmium</em> IMS101 (semi-continuous) – morning</td>
<td>3.2–10.0</td>
<td>5.4</td>
<td>Hutchins et al. (2007)</td>
</tr>
<tr>
<td><em>Trichodesmium</em> IMS101 (semi-continuous) – afternoon</td>
<td></td>
<td></td>
<td>Hutchins et al. (2007)</td>
</tr>
<tr>
<td><em>Trichodesmium</em> GBRTRLI101</td>
<td>2.0–12.4</td>
<td>6.5</td>
<td>Hutchins et al. (2007)</td>
</tr>
<tr>
<td><strong>Other marine cyanobacteria:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hemiaulus/Richelia</em> association</td>
<td>12.5</td>
<td></td>
<td>Carpenter et al. (1999)$^5$</td>
</tr>
<tr>
<td>Mixed cyanobacteria – <em>Nodularia spumigena</em> / <em>Anabaena/Aphanizomenon</em></td>
<td>17.6 (0–7 m)</td>
<td>5.1 (7–14 m)</td>
<td>Gallon et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>1.5 (14–21 m)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$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$_2$ fixation 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$^{-1}$

(Mahaffey et al., 2005; Mulholland et al., 2006), any fraction of new production from diazotrophy that is exported (e.g., Eppley and Peterson, 1979) 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* spp. can fix N$_2$ at high rates, thereby introducing new nitrogen into nutrient impoverished areas of the tropical and subtropical ocean. Because *Trichodesmium* also release fixed N as bioavailable dissolved organic N (DON) (Glibert and Bronk, 1994; Capone et al., 1994), and ammonium (Mulholland and Capone, 2001; Mulholland et al., 2004a), they may route recently fixed N through a recycling loop. Elevated NH$_4^+$ and/or DON concentrations have been observed in and around *Trichodesmium* blooms in the Arabian Sea (Devassy et al., 1978, 1979), Pacific Ocean (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, accumulation of dissolved organic matter (C or N) is not always observed or predicted, even in areas such as the Sargasso Sea, near the Bermuda Atlantic Time-series Study site (BATS) (Hansell and Carlson, 2001; Hood et al., 2001; Knapp et al., 2005), where N$_2$ fixation is suspected to be high based on geochemical arguments.
(Michaels et al., 1996; Gruber and Sarmiento, 1997). 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 by organisms co-occurring in the water column, as has been observed in the Gulf of Mexico (Mulholland et al., 2004b, 2006). In the Baltic Sea, blooms of diatrophic cyanobacteria (Nodularia and Aphanizomenon) were shown to contribute N to picoplankton production (Ohlendieck et al., 2000).

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. Ohlendieck et al. (2000) demonstrated that direct release of N was important during the early stages of cyanobacterial blooms while release due to lysing and decay was more important during later blooms stages. *Trichodesmium* biomass may be degraded via bacterial activity and extracellular enzymes thereby rendering large organic compounds into smaller utilizable compounds (Sieburth and Conover, 1965; 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 were released per g of *Trichodesmium*. These authors also suggested that these release rates would have been much higher if DOP, urea and amino acids had been measured.

In general, 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 thus making it impossible to measure isotopically-labeled products in short incubations. 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$_2$H$_2$) reduced to N$_2$ taken up have varied by at least an order of magnitude (Table 2). Because C$_2$H$_2$ 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 fixed 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$_2$ (Gallon et al., 2002; Mulholland et al., 2004a, 2006; Mulholland and Bernhardt, 2005). If this is the case, and the theoretical ratio, three, is assumed to be correct (although this itself is a subject of debate; see Mulholland et al., 2006, for a discussion of this assumption) in estimating N$_2$ fixation from C$_2$H$_2$ reduction, paired comparisons in which C$_2$H$_2$:N$_2$ reduction ratios of approximately 3:1 are observed, would indicate no N release while C$_2$H$_2$:N$_2$ reduction ratios of six would translate into a release rate of about 50%, and so on. Examining paired comparisons of C$_2$H$_2$ 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 (Mulholland et al., 2004a; Mulholland and Bernhardt, 2005) and among environments (Table 2 and references therein). 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$_2$H$_2$:N$_2$ reduction for unicells are available and so release by these organisms cannot be assessed 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 or whether they do so all the time. 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 released 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), and much of this may
have fueled production of co-occurring phytoplankton (Mulholland et al., 2004b). In diatoms, it has been suggested that nitrate uptake in excess of their nutritional requirements may act as a sink for electrons when there are transient imbalances between light energy harvesting and photosynthetic carbon assimilation (Lomas and Glibert, 1999; Lomas et al., 2000). These authors speculate that imbalances between these processes, and associated release of dissolved N, occur when cells shift from high to low temperature or low to high irradiance. 

**Trichodesmium** tend to occur in tropical and subtropical oligotrophic gyres where the water column and surface water temperatures are relatively stable. However, they are also known to form surface accumulations or “slicks” during which exposure irradiances are quite high and release of dissolved substances also high (e.g., Sieburth and Conover, 1965).

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$^{-1}$. These estimates agree well with the mortality rates calculated for **Trichodesmium** at BATS and in the equatorial Atlantic (2.1 to 2.5% d$^{-1}$; Hood et al., 2001, 2004).

### 5 C release

Dissolved organic N 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 et al. (1978) first quantified the extracellular release of photosynthesize in $^{14}$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 7% of the primary productivity, and, 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 compounds such as glycolate (Renström-Kellner et al., 1989) and amino acids (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) (Sellner, 1997).

Production of colored dissolved organic matter (CDOM) by **Trichodesmium** has 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 known to have and serve in photoprotection (Subramaniam et al., 1999).

Although globally we are interested in the fate of new production from diazotrophy as a means to export C, little has been done to quantify or characterize DOC release and its fate from **Trichodesmium** or other marine N$_2$ fixers. Robust isotopic and molecular tracers of diazotroph-derived DOC have yet to be identified. 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 (Sheridan et al., 2002). Despite the fact that colonies are rich microenvironments, there is a variety of evidence suggesting that **Trichodesmium** themselves go largely ungrazed (see...
below) 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* spp. (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 also 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* vary depending on morphology, 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 through *Trichodesmium*. Bryce- son and Fay (1981) first demonstrated that the trophic transfer of recently fixed N2 might be important in communities dominated by *Trichodesmium* and they subsequently demonstrated isotopic enrichment in non-*Trichodesmium* size fractions after incubation of *Trichodesmium* and natural marine communities with 15N2. They did not have control incubations to account for N2 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 (Bryce- son and Fay, 1981). Subsequently, the only other direct estimates of the trophic transfer of recently fixed N2 demonstrated that up to 11% of recently fixed N2 was transferred to non-N2 fixing cells in whole water samples even in short (2 h) incubations (Mulholland et al., 2004b). This suggests that *Trichodesmium* may support further productivity in the upper water column and the growth of co-occurring organisms, including heterotrophs, rather than a substantial direct particle sinking flux (Fig. 1).

Despite the idea that dissolved nutrients may be the primary route of trophic transfer of recently fixed N2, isotopically “light” zooplankton have been 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). In addition, low δ15N values have been reported in sediment trap material at both the Atlantic and Pacific time series sites (Carl et al., 1997, 2002; Knapp et al., 2005), indicating that recently fixed N2, which has an isotopic signature similar to atmospheric N, is being transferred to higher trophic levels and leaving the euphotic zone.

In contrast, Brandes et al. (1998) suggested that material derived from N2 fixation could also be remineralized in the upper water column and hypothesized that the input of isotopically light N from N2 fixation was responsible for a lightening of the isotopic nitrate signal in surface waters above the oxygen minimum zone in the Arabian Sea. Based on excess N2 gas concentrations, Devol (2007) and Devol et
al. (2006) have gone on to speculate that the particle rain from diazotrophic production fuels denitrification in the oxygen minimum zone there. These observations suggest that diazotrophic production can be remineralized in surface waters fueling microbial production and complicating interpretation of geochemical tracers such as stable isotope signatures and N*.

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). Bacterial associates also included 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 bacterial colonization of *Trichodesmium* aggregates.

In terms of their productivity, Nausch (1996) found thymidine incorporation to be enhanced in association with colonies of *Trichodesmium* relative to that of the water column and comparable to the enrichment found in marine snow. However, because colony-associated bacterial abundance was so much higher, when normalized per unit bacteria, thymidine incorporation associated with colonies appeared to be lower than that measured in the surrounding water. In the Gulf of Mexico, leucine incorporation 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 were 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 productivity: primary productivity ratio). The authors attribute this to N release and alleviation of competition between bacteria and phytoplankton for scarce NH$_4^+$.

High rates of amino acid oxidase activity (Mulholland et al., 1998; Gilbert 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 other organisms (e.g., phytoplankton and grazers) associated with colonies actively cycle nutrients. Rates of enzyme activity in and around colonies in these studies were higher than those observed in the surrounding water column reflecting either more active or more abundant microbial communities. 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, *Chaetoceros* populations increased, followed by a succession of cladocerans, dinoflagellates, green algae, 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* 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). Although direct evidence of trophic transfer from *Trichodesmium* to phytoplankton in nature is lacking, Brycecon 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. Further, the low $\delta^{15}$N observed in sediment trap material suggests that at least some N derived from diazotrophy is leaving the euphotic zone (Karl et al., 1997, 2002; Capone et al., 1998; Montoya et al., 2002; Knapp et al., 2005).

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* spp. are not grazed by many of the dominant zooplankton in marine ecosystems.
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 spp. flowed into grazers and because Macrosetella appears to have a higher C:N ratio than the Trichodesmium spp. 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 (e.g., Montoya et al., 2002). 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, 2007).

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 diazotrophy in the context of N and C dynamics in the ocean. Because many direct estimates of global N$_2$ fixation are based on highly spatially, temporally, and physiologically limited and 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. Further, because we know so little about the physiology of marine diazotrophs, it is difficult to model the contribution of new production from diazotrophy in the

Fig. 2. 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 an oligotrophic gyre with N$_2$ fixation and associated export of new diazotrophic production. Panel (C) represents an alternative fate for diazotrophic production wherein new production is respired in the euphotic zone or surface ocean, and results in no net drawdown or export of carbon or nitrogen from the euphotic zone (red). Redrawn and expanded from Hood et al. (2000).
present, past, or future ocean where conditions vary in space and time.

It is also difficult to determine the effect of N\textsubscript{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. 2; also see Sect. 6.3 above). For example, it was observed that along 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 does colony size affect colony-specific estimates of N and C fixation, but the degree of aggregation and size of colonies also affects the degree to which *Trichodesmium* is colonized by other organisms and thereby recycled via bacteria, grazers, and other enzymatically-mediated processes. 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). Better common metrics need to be employed to express rate measurements not only because *Trichodesmium* morphologies vary, but also because we now know there are a variety of non-*Trichodesmium* diazotrophs.

Finally, different diazotrophic groups may have different fates and we know even less about non-*Trichodesmium* marine N\textsubscript{2} fixers. Diatom/Richelia assemblages may be prone to gravitational settling while unicellular cyanobacteria may be more readily grazed. There are a variety of conflicting reports based on isotopic and geochemical tracers suggesting that diazotrophic growth fuels export production versus remineralization. The absence of robust direct estimates of both, hinder our ability to speculate regarding the fate of new N from diazotrophic growth.

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