# Nitrogen fixation by *Trichodesmium* spp. and unicellular diazotrophs in the North Pacific Subtropical Gyre

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[1] Nitrogen ($N_2$) fixation is an important process that fuels export production in the North Pacific Ocean, as evidenced by seasonally low $\delta^{15}N$ of sinking organic nitrogen (N) at the Hawaii Ocean Time series station. However, relatively few direct measurements of $N_2$ fixation exist across the North Pacific. On two cruises there in fall 2002 and summer 2003, the abundance and $N_2$ fixation rate of *Trichodesmium* spp. and *Richelia*, as well as bulk water samples, were measured. *Trichodesmium* spp. were only detected in the area near the Hawaiian Islands, in similar densities on both cruises. Despite similar densities, the areal $N_2$ fixation rate of *Trichodesmium* spp. in fall 2002 was nearly four times greater than in summer 2003 at stations proximal to the Hawaiian Islands. In the central North Pacific Gyre far from the Hawaiian Islands, where *Trichodesmium* spp. was not present, whole water $N_2$ fixation rates were relatively high (~100 $\mu$mol N $m^{-2}$ $d^{-1}$). Presumably unicellular diazotrophs were responsible for activity there. Our studies show a geographical variation in the dominant diazotroph in the North Pacific Subtropical Gyre in the summer with *Trichodesmium* being dominant around the Hawaiian Islands, *Richelia* associated with diatoms to be found in high numbers to the south of the islands while unicellular diazotrophs dominated to the west, away from the islands and evidence from the literature suggests iron may play a role.

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## 1. Introduction

[2] The export of organic matter from the surface to the deep ocean in oligotrophic waters is dependent on the supply of new nutrients, especially nitrate, to the euphotic zone [*Eppley and Peterson*, 1979]. Vertical eddy diffusion of nitrate was previously thought to be the most important new nitrogen (N) source [*Eppley and Peterson*, 1979]. However, recent studies have shown dinitrogen ($N_2$) fixation, the process by which a limited number of organisms can access the large but biologically unavailable pool of $N_2$ gas by reducing it with the nitrogenase enzyme [*Postgate*, 1998], can be of equal importance as a new nitrogen source in oligotrophic waters of the North Atlantic [*Capone et al.*, 2005] and *Karl et al.* [1997] found that $N_2$ fixation can fuel up to half the new production at the Hawaii Ocean Time series (HOT) site. Considering that carbon dioxide ($CO_2$) is, on average, brought up in Redfield proportion with nitrate from below the thermocline, $N_2$ fixation, in addition to atmospheric deposition and N introduced by rivers, are the only sources of truly new N that can promote a net sequestration of $CO_2$ from the atmosphere to deeper waters [*Eppley and Peterson*, 1979]. This effect may be indirect, through the introduction of new N then available to the food web, or in some cases direct, as has been observed for diatom-diazotrophic associations (DDAs) [*Subramaniam et al.*, 2008]. Thus, it is important to quantify this source of N to oligotrophic oceans and understand what factors contribute to differences seen on spatial and temporal scales.

[3] The North Pacific Subtropical Gyre (NPSG) is a highly oligotrophic, stratified system that has been well studied by the ongoing Hawaii Ocean Times-series program. Decadal scale patterns that have been discerned include the shift in the dominant primary producers from eukaryotes to smaller, photosynthetic prokaryotes [*Karl et al.*, 2001], a decrease in the inventory of soluble reactive phosphorus in the euphotic zone [*Karl and Tien*, 1997] and a concurrent increase in $N_2$ fixing cyanobacteria [*Karl et al.*, 1997]. Geochemical estimates show that about half of particulate N export at station ALOHA is supported by N coming from $N_2$ fixation [*Dore et al.*, 2002] and recent work

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by *Grabowski et al.* [2008] report rates ranging from 20 to 307 $\mu$mol $m^{-2}d^{-1}$.

[4] One important diazotroph found in tropical and subtropical waters is *Trichodesmium* spp. *Trichodesmium* spp. is a colony forming diazotrophic cyanobacterium that is cosmopolitan in warm, oligotrophic waters [*Capone et al.*, 1997]. Global estimates show that $N_2$ fixation by this genus could account for up to 80 Tg N $yr^{-1}$, about half of current geochemical estimates of total $N_2$ fixation (ranging from 100 to 200 Tg N $yr^{-1}$) [*Carpenter and Capone*, 2008]. Clearly, quantification of $N_2$ fixation by *Trichodesmium* spp. is important in deriving global and local estimates of $N_2$ fixation. The occurrence of *Trichodesmium* spp. in the NPSG has been reported in the past [*Letelier and Karl*, 1996; *Mague et al.*, 1977]. However, it was found predominantly as free trichomes in the NPSG [*Letelier and Karl*, 1996], rather than as the colonies that are most prevalent in some other locations such as the Sargasso Sea [*Carpenter et al.*, 2004].

[5] While *Trichodesmium* spp. is an important contributor to marine $N_2$ fixation, it still cannot account for all geochemically estimated $N_2$ fixation. Another important contributor is *Richelia*, a symbiont associated with diatoms such as *Hemiaulus* spp. and *Rhizosolenia* spp. These diatom diazotroph associations (DDAs) have been commonly noted in the NPSG [*Church et al.*, 2008; *Scharek et al.*, 1999; *Venrick*, 1974; *Villareal and Carpenter*, 1989]. $N_2$ fixation by unicellular cyanobacteria in the 3–10 $\mu$m phytoplankton size fraction has also been identified in these waters [*Zehr et al.*, 2001] and areal rates of $N_2$ fixation in this fraction can equal or exceed those measured for *Trichodesmium* spp. [*Montoya et al.*, 2004].

[6] We undertook two cruises as part of the Marine Nitrogen fixation and Tropospheric Responses to Aeolian Inputs (MANTRA) Biocomplexity project to quantify the dominant diazotroph and $N_2$ fixation rates and study their geographical distribution in the NPSG. We measured *Trichodesmium* spp. and *Richelia* abundance, $N_2$ fixation by these organisms as well as bulk $N_2$ fixation measurements. At station lacking larger diazotrophs, these measurements could be attributed to unicellular diazotrophs.

## 2. Methods

### 2.1. Sample Collection

[7] Samples were collected on two cruises in the NPSG. Measurements were carried out in September and October of 2002 aboard the R/V *Kilo Moana* (MP6) and in July and August 2003 aboard the R/V *Roger Revelle* (MP9). Samples were largely collected around the Hawaiian Islands, but on leg 1 of MP9, a transect to 175 °E allowed collection of samples and rate measurements across a wide area of the NPSG.

### 2.2. *Trichodesmium* spp. and *Richelia* Counts

[8] *Trichodesmium* spp. counts were done using the method previously described by *Carpenter et al.* [2004]. Briefly, the entirety of a 10 L Niskin bottle with water collected from depths corresponding to 100%, 55%, 28%, 10% and 1% of surface light (light depths) was drained onto a 47 mm, 10 $\mu$m polycarbonate filter placed in a swinex filter holder and attached to the spout of the Niskin bottle. Epifluorescent microscopy was used to enumerate *Trichodesmium* spp. colonies, free filaments and the number of *Richelia* heterocysts on the filters, either on board, or preserved in 0.4% paraformaldehyde and frozen until counted in the lab. The number of *Trichodesmium* spp. trichomes per colony was determined at each station by placing 10 representative colonies into a vial with filtered seawater and shaking to disaggregate the colonies. This sample was then filtered onto a 10 $\mu$m Nuclepore filter and the trichomes counted under a microscope. The trichome abundance was then calculated by multiplying the colony abundance by the number of trichomes per colony. Samples for trichome counts were taken all the way to 175°E and back during MP9, but no *Trichodesmium* spp. was detected by this method west of the station at 161°W. For the purposes of comparisons between the two cruises, we excluded the observations west of 167 °W from our averages (Data Set S1).[1]

### 2.3. $N_2$ Fixation Measurements

[9] *Trichodesmium* spp. samples were collected by towing a 1 m, 202 $\mu$m mesh net (0.5–1 knot) at a depth of 15–20 m or by towing a small 64 $\mu$m mesh net at the surface. Previous work had demonstrated that there are no systematic differences in activity for samples collected by hand in situ and by towing as described [*Carpenter et al.*, 1987]. Colonies were isolated with a plastic transfer loop and placed in a filtered seawater rinse before picking them into incubation bottles. To collect free trichomes, the diluted plankton tow was allowed to briefly sit while the trichomes rose to the surface and clumped together. The aggregation could then be gently removed with a plastic loop or wide bore plastic pipette and placed into filtered seawater to make a slurry, diluted to a specific volume, and used for experiments. Samples were checked under a dissecting microscope for presence of other organisms before use and a subsample saved for trichome density determination.

[10] *Trichodesmium* spp. $N_2$ fixation measurements were carried out using the acetylene ($C_2H_2$) reduction method described by *Capone* [1993]. Ten colonies in 10 ml GF/F filtered seawater or 10 ml of a free trichome slurry was placed into acid cleaned 14 ml glass serum vials. Twenty $\mu$M ethylenediaminetetraacetate (EDTA) was added as it extends the life of the *Trichodesmium* spp. during incubation, allowing for the collection of more data points, but does not affect the initial acetylene reduction rate [*Burns et al.*, 2006]. Vials were sealed with silicone rubber stoppers and 1 ml of instrument grade $C_2H_2$, sparged through deionized water, was added. The production of ethylene ($C_2H_4$) in the headspace of the vial was monitored over time courses of 6–10 h by flame ionization detection gas chromatography. Triplicate vials were incubated at 100%, 55%, 28%, 10% and 1% of surface irradiance in on deck incubators with flowing surface seawater to control temperature. The $C_2H_2$ reduction rate was derived from the linear regression of incubation time versus $C_2H_4$ concentration in the vials. The $C_2H_2$ reduction rate was then converted to $N_2$ fixation rate using a conversion factor of 3 $C_2H_4$:1 $N_2$ [*Capone et al.*, 2005] then multiplied by 2 (as there are 2 ammonium molecules released from the splitting of $N_2$ gas).

[1] Auxiliary materials are available at ftp://ftp.agu.org/apend/jg/2010jg001513.

**Table 1.** Colony and Free Trichome Specific Acetylene Reduction Rates[a]

| Percent of Surface Irradiance | Average Depth (m) | $C_2H_2$ Reduction (nmol $col^{-1}$ $h^{-1}$ or pmol $trichome^{-1}$ $hr^{-1}$) | Min | Max | Number of Stations (n) |
|---|---|---|---|---|---|
| | | *MP6 Colonies* | | | |
| 100 | 0 | 0.43 (0.11) | 0.1 | 1.26 | 10 |
| 55 | 10.2 (0.5) | 0.34 (0.08) | 0 | 0.7 | 11 |
| 28 | 25.2 (0.9) | 0.39 (0.08) | 0.02 | 0.87 | 11 |
| 10 | 53.1 (2.8) | 0.22 (0.05) | 0.02 | 0.56 | 9 |
| 1 | 92.1 (4.3) | 0.14 (0.02) | 0.07 | 0.23 | 7 |
| | | *MP6 Free Trichomes* | | | |
| 100 | 0.6 (0.1) | 8.38 (1.08) | 2.38 | 15.68 | 9 |
| 55 | 10.0 (0.3) | 8.21 (1.31) | 1.57 | 16.30 | 9 |
| 28 | 26.1 (1.1) | 7.35 (0.86) | 2.28 | 12.63 | 9 |
| 10 | 56.4 (3.2) | 3.47 (0.51) | 1.37 | 7.32 | 9 |
| 1 | 98.3 (8.3) | 3.35 (0.61) | 0.91 | 5.81 | 8 |
| | | *MP9 Colonies* | | | |
| 100 | 0 | 0.20 (0.02) | 0.14 | 0.37 | 8 |
| 55 | 19.6 (2.1) | 0.18 (0.03) | 0.06 | 0.35 | 8 |
| 28 | 41.1 (3.0) | 0.11 (0.02) | 0.04 | 0.27 | 8 |
| 10 | 65.3 (4.3) | 0.03 | 0.02 | 0.05 | 5 |
| 1 | 99.8 (7.6) | 0.02 | 0.0 | 0.04 | 5 |

[a]Standard errors are shown in parentheses.

[11] To calculate areal rates of $N_2$ fixation by *Trichodesmium* spp., per colony or per trichome rates at each light level were multiplied by colony or trichome abundance at the same light depth and a trapezoidal integration was used to calculate $N_2$ fixation in a square meter of the water column. At some stations, low abundance of colonies in the net tows did not allow measurement of $N_2$ fixation at all light levels. In such cases, $N_2$ fixation was measured for as many light levels as possible (always concentrating on the higher light levels) and a cruise average ($C_2H_2$ reduction rate per colony; see Table 1) was used as the rate for the other light levels. Similarly, due to low biomass, we were not able to measure $N_2$ fixation rates of free trichomes at every station. On MP6, the cruise average for each light level for the 9 stations sampled was multiplied by the abundance of free trichomes. However, during MP9 we did not collect enough measurements of $N_2$ fixation by free trichomes to confidently apply this method, and therefore, *Trichodesmium* spp. $N_2$ fixation rates are based on colonies only. Estimates of free trichome $N_2$ fixation were made by multiplying free trichome densities by the per trichome $N_2$ fixation rate of colonies.

[12] $N_2$ fixation measurements were also carried out using tracer $^{15}N_2$ uptake on large water samples [*Montoya et al.*, 2004; *Montoya et al.*, 1996]. Duplicate samples were collected from depths corresponding to 100%, 55%, 28%, 10% and 1% of surface light using a CTD rosette with 10 L Niskin bottles attached. Water from the Niskin bottles was gently drained with a sampling tube into 4.5 L polycarbonate bottles. No prefilter was used. Air bubbles were removed and bottles were sealed with septa lined caps, followed by the addition of 3 ml of 99% $^{15}N_2$. Incubations were conducted in deck board incubators covered with blue screening to simulate the light depth of collection and flowing surface seawater. The first four light depths, where the large majority of the $N_2$ fixation occurred, fell above the thermocline and the in situ temperature at the sampling depth was within about 1°C of the surface water used to maintain the on deck incubators. Thus the difference between incubation and in situ temperatures were not likely to appreciably affect our calculations of areal rates of $N_2$ fixation.

[13] After 24 h incubation, the whole content of the bottle was filtered onto precombusted GF/F filters. Filters were dried and pelletized, then the $\delta^{15}N$ of the sample was analyzed on a VG IsoPrime mass spectrometer interfaced to a Eurovector elemental analyzer at the USC stable isotope facility. Standards were routinely analyzed during sample runs and included acetanilide for C and N elemental mass and ammonium sulfate and glycine for $\delta^{15}N$. Uptake of $^{15}N_2$ in the samples was calculated as described by *Montoya et al.* [1996]. Areal rates were calculated using trapezoidal depth integration. Multivariate cross correlation matrices were calculated using JMP software.

## 3. Results

### 3.1. *Trichodesmium* spp. Abundance

[14] *Trichodesmium* spp. was present both as aggregates (colonial form) and as free trichomes on both MP6 and MP9 cruises (Figure 1). *Trichodesmium* spp. abundances in colony form were very similar and not statistically significantly different ($p > 0.55$) between the two cruises for the stations sampled ($5.6 \pm 0.8$ trichomes $\times 10^6$ $m^{-2}$ during MP6 and $4.8 \pm 1.0$ trichomes $\times 10^6$ $m^{-2}$ during MP9) while free trichomes averaged $1.6 \pm 0.3$ trichomes $\times 10^6$ $m^{-2}$ during MP6, about twice the abundance of free trichomes during MP9. However, the average abundances of free trichomes from the two cruises were not significantly different from each other ($p > 0.06$). Across all stations, the percentage of total trichomes that were found in colonies averaged 70% on MP6 and 74% on MP9. The data ranged widely around these means, and the median values were 78% and 83% (Data Set S1). The abundance of *Trichodesmium* spp. increased in our samples toward the Hawaiian Islands on the first leg of MP9, from the

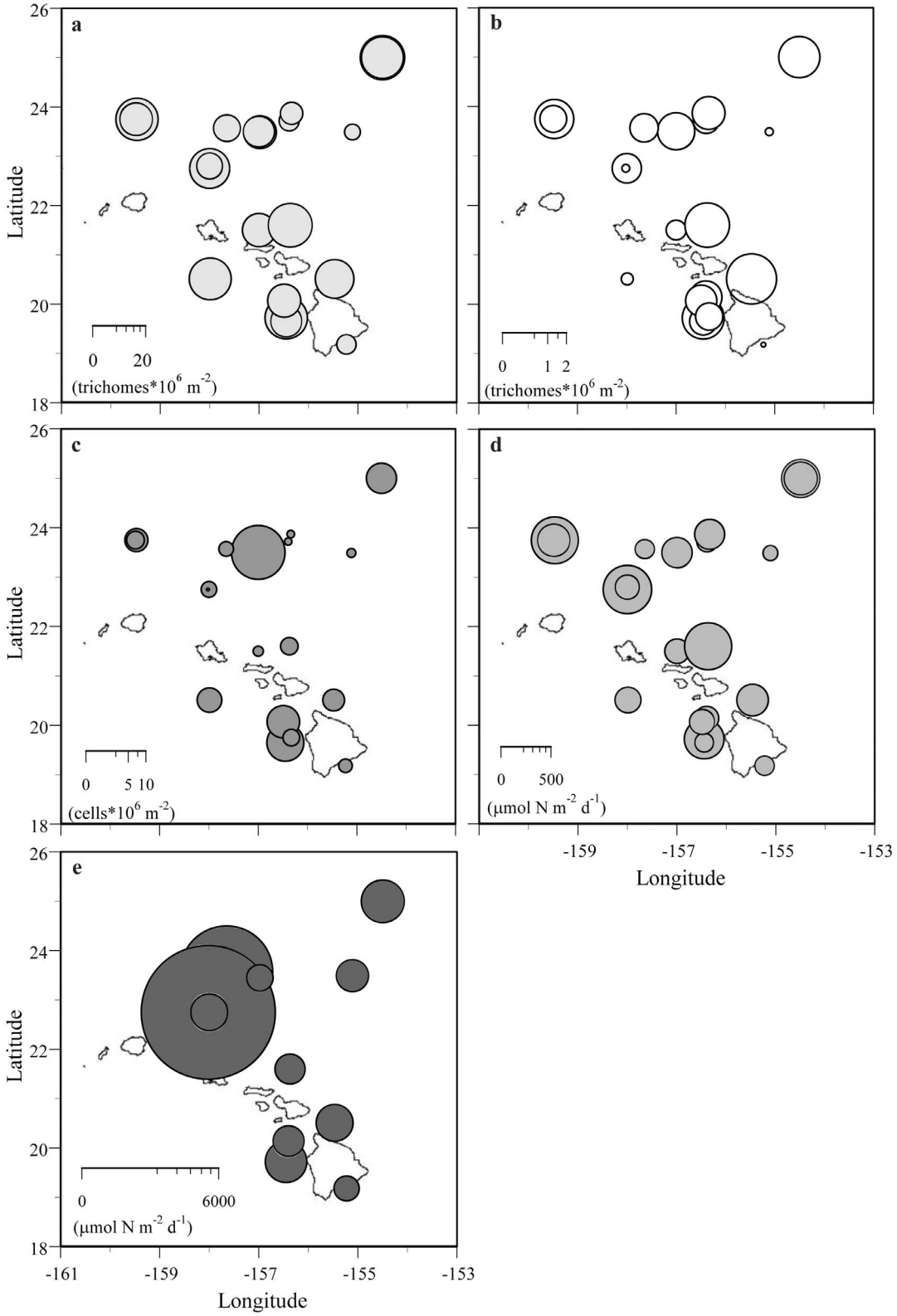


**Figure 1.** Depth integrated abundances (per $m^2$ of surface area) of (a) *Trichodesmium*, (b) *Katagnymene*, and (c) *Richelia* and rates of (d) *Trichodesmium* and (e) bulk water $N_2$ fixation during MANTRA Biocomplexity cruise MP6.

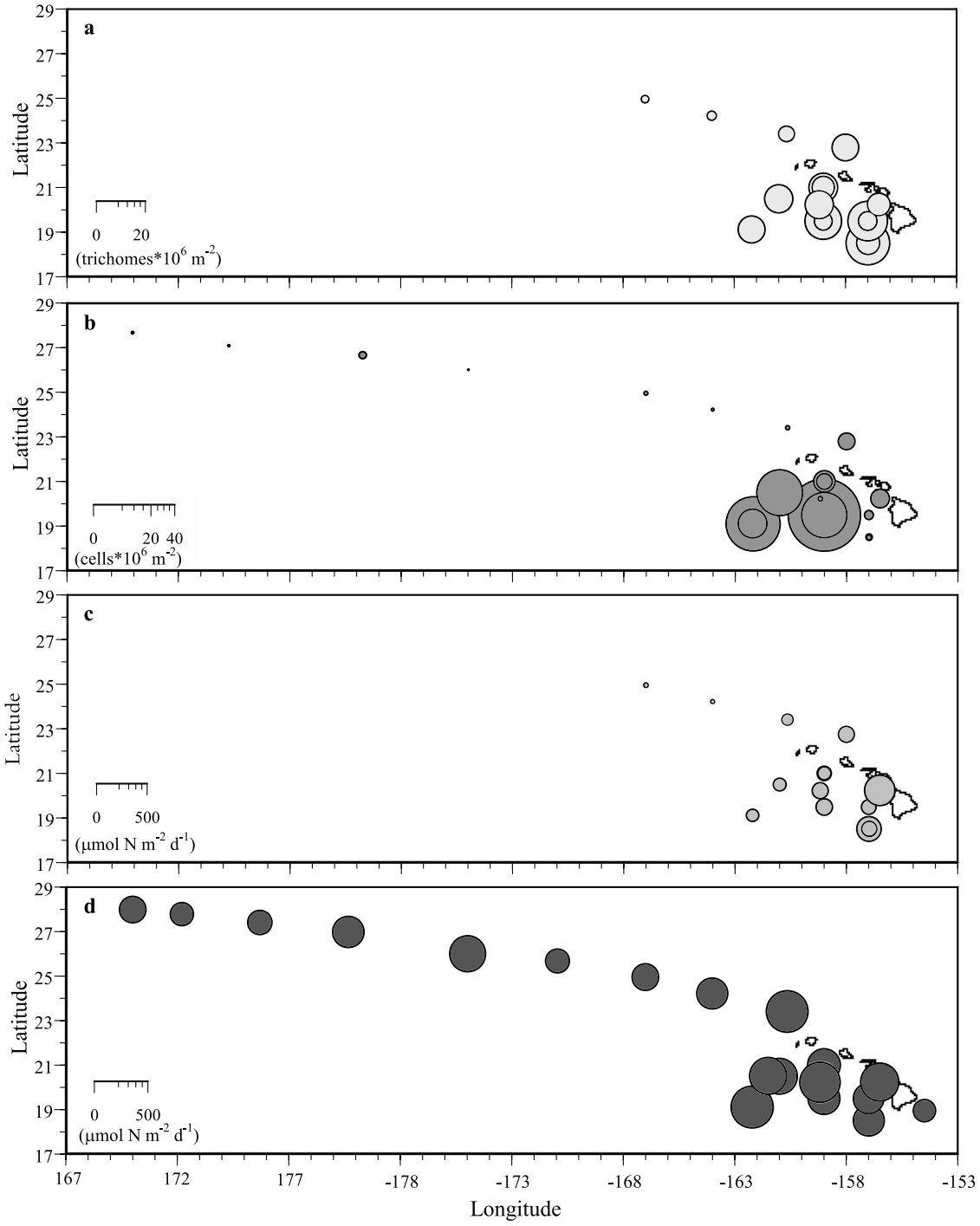


**Figure 2.** Depth integrated abundances (per $m^2$ of surface area) of (a) *Trichodesmium* and (b) *Richelia* and rates of (c) *Trichodesmium* and (d) bulk water $N_2$ fixation during MANTRA Biocomplexity cruise MP9.

station at 167°W toward station ALOHA, just north of Oahu. *Trichodesmium* spp. was not detected west of 167°W.

[15] *Katagnymene* (recently renamed *Trichodesmium spiralis*) occurred in relatively high densities on MP6 with an average of 0.47 ± 0.07 trichomes × $10^6$ $m^{-2}$, but at much lower levels (0.09 ± 0.02 trichomes × $10^6$ $m^{-2}$) on MP9. In contrast, *Richelia* spp. associated with the diatoms *Hemiaulus* and *Rhizosolenia* occurred in average densities of 1.3 ± 0.4 × $10^6$ heterocysts $m^{-2}$ on MP6 while at much higher levels on MP9 (average 5.2 ± 2.1 cells × $10^6$ $m^{-2}$). Depth integrated abundances were in excess of $10^7$ heterocysts $m^{-2}$ at 4 stations on this cruise.

### 3.2. *Trichodesmium* spp. $N_2$ Fixation

[16] *Trichodesmium* spp. colonies and free trichomes exhibited high levels of nitrogenase activity, measured as $C_2H_2$ reduction, on both cruises (Figure 1 and 2 and Data Set S1). During MP6, $N_2$ fixation by colonies was greater

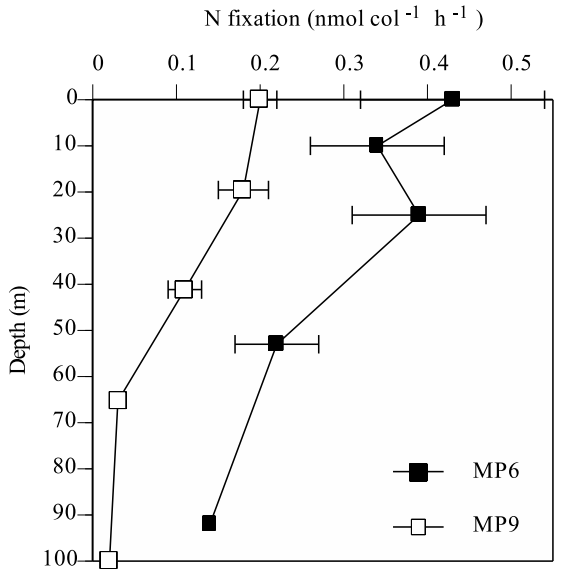


**Figure 3.** Depth distribution of colony specific rates of $N_2$ fixation from acetylene reduction measurements.

than by free trichomes. However, free trichomes fixed proportionally more N than colonies did when compared to their abundance (free trichomes were 23% of all *Trichodesmium* spp., but accounted for 31% of total $N_2$ fixation). This was probably due to the fact that the free trichomes on this cruise included a considerable number of *Katagnymene* with much larger trichomes than those contained in other *Trichodesmium* spp. colonies (data not shown). During MP9, only $N_2$ fixation by colonies was measured. While abundances of *Trichodesmium* spp. were nearly the same between MP6 and MP9, depth integrated $N_2$ fixation rates (colony plus free trichome for MP6 and colony only for MP9) were about five times greater, on average, in the fall (MP6) compared to the summer (MP9; 227 ± 26 $\mu$mol N $m^{-2}$ $d^{-1}$ versus 43 ± 12 $\mu$mol N $m^{-2}$ $d^{-1}$). If we add in an estimate for free trichome $N_2$ fixation on MP9 (based on per trichome $N_2$ fixation rates of colonies, multiplied by free trichome abundance) areal $N_2$ fixation rates only increase slightly, to 50 $\mu$mol N $m^{-2}$ $d^{-1}$. The difference in $N_2$ fixation rates between the two cruises can mostly be explained by the difference in colony normalized $C_2H_2$ reduction rates. The average per colony rate on MP6 was greater at all light depths than on MP9 (Table 1 and Figure 3). In addition, $C_2H_2$ reduction during MP9 was barely measurable at 10% and 1% of surface irradiance. Finally, water column integrated $N_2$ fixation rates by colonies of *Trichodesmium* spp. increased along a transect from unmeasurable (for lack of colonies) west of 167° W to 47 $\mu$mol N $m^{-2}$ $d^{-1}$ at station ALOHA during leg one of MP9 (Figure 2).

### 3.3. Bulk $N_2$ Fixation

[17] $N_2$ fixation was also measured using the uptake of $^{15}N_2$ into particulate matter in relatively large volumes (>4L) of water on the two cruises. In general, highest rates were at or near the surface and decreased at lower depths in the water column (Data Set S2). On MP6, two stations showed exceptionally high rates of activity. However, bulk $N_2$ fixation rates were comparable on the two cruises when excluding the two extremely high values.

[18] The integrated areal average from MP9, 183 $\mu$mol N $m^{-2}$ $d^{-1}$, exceeded the average from MP6, 143 $\mu$mol N $m^{-2}$ $d^{-1}$ (Data Set S1). This observation runs counter to the *Trichodesmium* spp. $N_2$ fixation rates measured with $C_2H_2$ reduction. It may suggest that, on the whole, organisms other than *Trichodesmium* spp. (perhaps unicellular diazotrophs) were responsible for a large part of the $N_2$ fixation during MP9 and that *Trichodesmium* spp. may have been responsible for much of the total $N_2$ fixation on MP6. Still, *Trichodesmium* spp. $C_2H_2$ reduction rates greatly exceeded bulk $^{15}N_2$ uptake on MP6. This could arise from a sampling bias against the large and patchy *Trichodesmium* spp. in the $^{15}N_2$ uptake assays. Alternatively, recent evidence suggests that $^{15}N_2$ uptake assays, which introduce the substrate as a bubble (as used here) rather than as a dissolved gas, may underestimate actual rates [*Mohr et al.*, 2010]. At the stations on leg 1 of MP9 where *Trichodesmium* spp. and *Richelia* were not detected in our counts, we attribute the $N_2$ fixation measurements to unicellular diazotrophs. At these remote central gyre stations, $N_2$ fixation averaged 137 $\mu$mol N $m^{-2}$ $d^{-1}$.

## 4. Discussion

### 4.1. *Trichodesmium* spp. Abundances

[19] The abundance of *Trichodesmium* spp. on the two cruises was about an order of magnitude lower than trichome abundances seen in the subtropical and tropical North Atlantic [*Carpenter et al.*, 2004; *Davis and McGillicuddy*, 2006]. Thus, *Trichodesmium* spp. does not appear to be as important in the central North Pacific as in the North Atlantic, and recent molecular evidence corroborates this finding [*Church et al.*, 2009; *Church et al.*, 2008; *Langlois et al.*, 2008]. It is not clear what factor or factors contribute to this pattern.

[20] *Trichodesmium* spp. is also known to occur in other areas of the North Pacific, the South China Sea (SCS) and in the Kuroshio [*Carpenter*, 1983]. Reports of *Trichodesmium* spp. abundance show an increase in density from 0.44 × $10^6$ trichomes $m^{-2}$ in the spring up to about 5.3 × $10^6$ trichomes $m^{-2}$ in the SCS in the summer and fall [*Chen et al.*, 2003], and abundance in the Kuroshio is about ten times greater than in the SCS. These abundances are similar to the abundances reported in our study, suggesting that seasonal $N_2$ fixation rates in the western Pacific Ocean may be as high as near the Hawaiian Islands. In contrast, recent studies in the western part of the NPSG, along 149°E and 155°E have reported very low abundances of *Trichodesmium* [*Kitajima et al.*, 2009; *Shiozaki et al.*, 2009].

[21] In some locations around the globe where *Trichodesmium* spp. has been enumerated, trichomes are often found predominantly in aggregates or colonies [*Carpenter et al.*, 2004, and references therein]. During our two cruises in the North Pacific, free trichomes were more important than reported elsewhere, accounting for 20–30% of the total, suggesting that some factor in this region of the North Pacific either promotes free trichomes or prevents colony formation. A three year study of *Trichodesmium* spp. abundance at station ALOHA reported that free trichomes were about 80% of the total [*Letelier and Karl*, 1996] and a

**Table 2.** Estimates of $N_2$ Fixation in the North Pacific by Both Direct and Geochemical Means

| Location | $N_2$ Fixation ($\mu$mol N m$^2$ d$^{-1}$) | Sample Type | Method | Study |
|---|---|---|---|---|
| | | *Direct* | | |
| NPSG | 33 | *Trichodesmium* | $C_2H_2$ red | *Mague et al.* [1977] |
| NPSG | 134 | *Trichodesmium* | $C_2H_2$ red | *Gunderson et al.* [1976] |
| 22°N, 158°W | 140 | *Trichodesmium* | abundance with assumed $N_2$ fixation rate | *Karl et al.* [1997] |
| 22°N, 158°W | 85 | *Trichodesmium* | $C_2H_2$ red | *Karl et al.* [1997] |
| NPSG | 169 | *Trichodesmium* | $C_2H_2$ red | this study |
| NPSG | 55 | *Trichodesmium* | $C_2H_2$ red | this study |
| Hawaii-Cal | 520 | <10 $\mu$m fraction | $^{15}N_2$ uptake | *Montoya et al.* [2004] |
| 22°N, 158°W | 11–103 | <10 $\mu$m fraction | $^{15}N_2$ uptake | *Montoya et al.* [2004] |
| 22°N, 158°W | 25–125 | bulk water | $^{15}N_2$ uptake | *Dore et al.* [2002] |
| NPTG, 155°E | 12–152 | bulk water | $^{15}N_2$ uptake | *Shiozaki et al.* [2009] |
| NPTG, 0 | 18–358 | bulk water | $^{15}N_2$ uptake | *Bonnet et al.* [2009] |
| 22°N, 158°W | 39–307 | bulk water | $^{15}N_2$ uptake | *Church et al.* [2009] |
| NPSG | 110 | bulk water | $^{15}N_2$ uptake | this study |
| NPSG | 174 | bulk water | $^{15}N_2$ uptake | this study |
| | | *Geochemical* | | |
| 22°N, 158°W | 100–400 | | modeled from $\delta^{15}N$ export at 150m | *Dore et al.* [2002] |
| 22°N, 158°W | 93 | | N:P mass balance | *Karl et al.* [1997] |
| North Pacific 30°N–30°S | 107 | | N* budget | *Deutsch et al.* [2001] |
| NPSG | 219 | | OM mass balance | *Abell et al.* [2000] |

recent study in the eastern North Atlantic also noted a general preponderance of free trichomes [*González Taboada et al.*, 2010].

### 4.2. Comparison to Direct Measurements

[22] The density of direct measurements of *Trichodesmium* spp. $N_2$ fixation rates in the North Pacific are relatively low (Table 2), at least when compared to the North Atlantic Ocean. *Capone et al.* [1997] calculated water column $N_2$ fixation from two studies in the NPSG to be 33 $\mu$mol N m$^{-2}$ d$^{-1}$ (subtropical [*Mague et al.*, 1977]) and 134 $\mu$mol N m$^{-2}$ d$^{-1}$ (tropical [*Gunderson et al.*, 1976]). *Karl et al.* [1997] used biomass abundance and an assumed cell specific $N_2$ fixation rate to estimate $N_2$ fixation from the ALOHA site to be about 140 $\mu$mol N m$^{-2}$ d$^{-1}$ and from the $C_2H_2$ reduction method to be 85 $\mu$mol N m$^{-2}$ d$^{-1}$. These are the only *Trichodesmium* spp. specific, water column integrated $N_2$ fixation rates in the NPSG that we are aware of, and they are remarkably similar to each other and the rates presented in this study. Areal $N_2$ fixation by *Trichodesmium* in the South China Sea was estimated at 126 $\mu$mol N m$^{-2}$ d$^{-1}$ [*Saino*, 1977] as calculated by *Capone et al.* [1997].

[23] $N_2$ fixation rates have also been measured over a wide area of the North Pacific using $^{15}N_2$ uptake on large water samples. On a transect from Hawaii to Southern California, $N_2$ fixation by unicellular diazotrophic cyanobacteria in the <10 $\mu$m fraction averaged 520 $\mu$mol N m$^{-2}$ d$^{-1}$, while multiple samplings at station ALOHA ranged from 11 to 103 $\mu$mol N m$^{-2}$ d$^{-1}$ [*Montoya et al.*, 2004]. *Dore et al.* [2002] measured $N_2$ fixation on whole water samples at station ALOHA over a one year period and found that it ranged from about 25–125 $\mu$mol N m$^{-2}$ d$^{-1}$; the highest rate occurred in July and was linked to the >10 $\mu$m fraction, which includes *Trichodesmium* spp. For the stations east of 161°W, our bulk rates of 143 $\mu$mol N m$^{-2}$ d$^{-1}$ during MP6 and 216 $\mu$mol N m$^{-2}$ d$^{-1}$ during MP9 fall within the range of these other studies. These studies suggest that *Trichodesmium* spp. is an important contributor to water column $N_2$ fixation in the area near Hawaii, but that $N_2$ fixation by unicellular diazotrophs can at times far exceed that by *Trichodesmium* spp. in the region of the Islands. $N_2$ fixation is predominantly associated with the <10 $\mu$m fraction in the region west of Hawaii [*Montoya et al.*, 2004]. *Bonnet et al.* [2009] recently reported on a transect along the equator from 140°W to 145°E. Through most of the transect, rates were low and dominated by the <10$\mu$m size fraction. *Trichodesmium* spp. became important at the extreme western end of the transect, near Papua New Guinea.

[24] Several recent studies have reported rates of nitrogen fixation on bulk water and in the small size fraction in meridional transects in the western Pacific southeast of Japan. Using a high sensitivity acetylene reduction assay, *Kitajima et al.* [2009] reported rates of 1 to 9 nmol N L$^{-1}$d$^{-1}$ in the summer along 149°E and from 5 to 20 nmol N L$^{-1}$d$^{-1}$ during the winter cruise along 155°E. The <10$\mu$m fraction accounted for most of the activity.

[25] In total, these studies suggest that $N_2$ fixation by unicellular diazotrophs dominate the open ocean areas of the North Pacific, while organisms in the large size fraction (*Trichodesmium* and *Richelia*) are important in coastal areas and near islands, although from these studies it is impossible to say whether or not there is seasonality to this overall pattern. Across the North Pacific, the $N_2$ fixation rates measured in small diazotrophs are consistently similar to the average reported here (137 $\mu$mol N m$^{-2}$ d$^{-1}$). Potential factors contributing to the dominance of unicellular diazotrophs in much of the Pacific will be discussed below.

### 4.3. Comparison to Geochemical Estimates

[26] A number of geochemically based estimates of $N_2$ fixation have been reported. At station ALOHA, an N:P mass balance yielded a $N_2$ fixation estimate of 93 $\mu$mol N m$^{-2}$ d$^{-1}$

**Table 3.** Estimates of Vertical Eddy Diffusivity and $NO_3$ Flux in the NPSG[a]

| Location | Method | $NO_3^-$ Gradient (mmol $m^{-4}$) | $K_z \times 10^5$ ($m^2$ $s^{-1}$) | N Flux ($\mu$mol N $m^{-2}$ $d^{-1}$) | Study |
|---|---|---|---|---|---|
| Tropical North Pacific | DGM | 0.15 | 0.5–5.1 | 180 | *Anderson* [1978] |
| East Pacific | PND | na | 8–40 | | *Eppley and Peterson* [1979] |
| ETNP | PND | na | - | 380–1760 | *King and Devol* [1979] |
| Central North Pacific | DGM | 0.08 | 1–3.6 | 800 | *Platt et al.* [1984] |
| North Pacific | DMG | 0.035–0.05 | 0.2, 1.2 | 52–528 | *McCarthy and Carpenter* [1983] |
| 22°N, 158°W | DGM | 0.03–0.07 | 3.7 | 112–259 | *Karl et al.* [1992] |
| 22°N, 158°W | export[b] | - | 0.5–4 | | *Christian et al.* [1997] |
| 10°N, 150°W | ADCP[c] | 1 | 0.03 | 30 | *Carr et al.* [1995] |
| 22°N, 158°W | APEX floats | - | - | 241 | *Johnson et al.* [2010] |
| NPSG (MP6) | DGM | 0.5–4[d] | 0.112 ± 0.005 | 48–388 | this study |
| NPSG (MP9) | DGM | 0.5–4[d] | 0.119 ± 0.011 | 51–411 | this study |

[a]DGM, depth gradient model; PND, photosynthetic N demand; na, not applicable.
[b]Comparing export and nutrient profiles, assuming steady state.
[c]Measured microstructure temp and shear with ADCP and freefalling vertical profiler.
[d]Kz range from *Christian et al.* [1997] used.

[*Karl et al.*, 1997]. $N_2$ fixation was also estimated there by measuring the $\delta^{15}N$ of sinking particulate N (PN) at 150m and using a 2 end-member mixing model with $N_2$ fixation (−1‰) and nitrate diffusing from below the thermocline (6.5‰) as the two sources of N to exported PN. This method yielded estimates ranging from 100 to 400 $\mu$mol N $m^{-2}$ $d^{-1}$ over the study period from May 2000 to July 2001 [*Dore et al.*, 2002]. The same technique applied to yearly data at station ALOHA gave estimates from 85 to 230 $\mu$mol N $m^{-2}$ $d^{-1}$ over the period from 1990 to 2000, with no consistent pattern of increase or decrease over time [*Dore et al.*, 2002]. *Deutsch et al.* [2001] used a mass balance approach in the Pacific Ocean to create a N budget for the basin. The basin scale $N_2$ fixation rate was estimated from the difference between the source and sink values and calculated to be 59 ± 14 Tg N $y^{-1}$ for the basin. Averaging this rate over the area from 30°N to 30°S gives an areal rate of 107 $\mu$mol N $m^{-2}$ $d^{-1}$. A mass balance of total organic P and N in the NPSG gave an estimate of 219 $\mu$mol N $m^{-2}$ $d^{-1}$ [*Abell et al.*, 2000]. These estimates are all of the same order of magnitude as our instantaneous calculated rates from *Trichodesmium* spp. However, it is important to note that *Trichodesmium* spp. is not ubiquitous and subject to seasonal changes, while geochemical estimates are integrated over longer time scales.

### 4.4. Comparison to Vertical Nitrate Flux

[27] New nitrogen as nitrate is also delivered to the euphotic zone through vertical eddy diffusive flux from the nitricline. Estimates of eddy diffusive $NO_3^-$ flux in the North Pacific are highly variable and range from 52 to 1760 $\mu$mol N $m^{-2}$ $d^{-1}$ (Table 3). Vertical $NO_3^-$ flux for each of the cruises in this study was calculated from the average $NO_3^-$ gradient and the range of the diapycnal eddy diffusivity coefficient ($K_z$) reported by *Christian et al.* [1997]. The estimates of diffusive $NO_3^-$ flux were very similar between MP6 and MP9, ranging from ~50 to 400 $\mu$mol N $m^{-2}$ $d^{-1}$. "Event driven" vertical fluxes of $NO_3^-$ may also contribute another 240 $\mu$mol N $m^{-2}$ $d^{-1}$ or more when averaged over the year [*Johnson et al.*, 2010]. While the eddy diffusivity coefficient is not well constrained and the frequency of short-term $NO_3^-$ injection events may vary from year to year, the $N_2$ fixation rates generated in this study are within the range of each of these new N sources, suggesting that, in the stratified period when it occurs, $N_2$ fixation can be as important a source of new N in the NPSG as the diffusive flux of $NO_3^-$ or "event driven" vertical fluxes. In the winter months, the mixed layer descends to the top of the nitracline (Hawaii Ocean Time series Data Organization and Graphical site, http://hahana.soest.hawaii.edu/hot/hot-dogs/) and deep convective mixing would be the most important N source.

[28] In their transect along 155°E, *Shiozaki et al.* [2009] reported that nitrogen fixation could account for up to 37% of new production (sum of nitrogen fixation and nitrate assimilation) at a station in the subtropical gyre at 24°N.

### 4.5. Potential Factors Affecting Diazotroph Abundance and $N_2$ Fixation

[29] Geographic variability, seasonality, and interannual variability could all have an effect on $N_2$ fixation in the NPSG. The surface chlorophyll values (Figure 4c) derived from monthly averages over an 8° square area north of the islands (24–22°N, 154–158°W) are always higher than the average monthly values south of the islands (20–18°N, 156–160°W). The satellite derived chlorophyll concentrations from the north and south boxes also demonstrate the strong seasonal and interannual variability in this region (Figure 4). The MP6 cruise coincided with the annual maximum in chlorophyll concentrations while the MP9 cruise coincided with the summer minimum and July 2003 appears to have one of the lowest chlorophyll concentrations recorded in that region from 2002 to present. Additionally, the $\delta^{15}N$ of sinking particulate N at station ALOHA ranged from 1 to 2.5‰ in 2002 and 2.5–4.5‰ in 2003, suggesting the $N_2$ fixation was a much greater contributor to export in 2002 than 2003 (Hawaii Ocean Time series Data Organization and Graphical site: http://hahana.soest.hawaii.edu/hot/hot-dogs/).

[30] In order to understand what might drive variability in diazotroph abundance and $N_2$ fixation, biological measurements for the two cruises (all data combined) were compared to environmental factors using multiple regression analysis. *Trichodesmium* spp. abundance was weakly positively correlated to mixed layer depth and the concentration of dissolved inorganic phosphorus (DIP) while $N_2$ fixation by *Trichodesmium* spp. was weakly negatively correlated to temperature and DIP (see Figure 5 for all comparisons and values). Bulk $N_2$ fixation was also weakly positively correlated to mixed layer depth. *Katagnymene* abundance was negatively correlated to temperature, while *Richelia* abun-

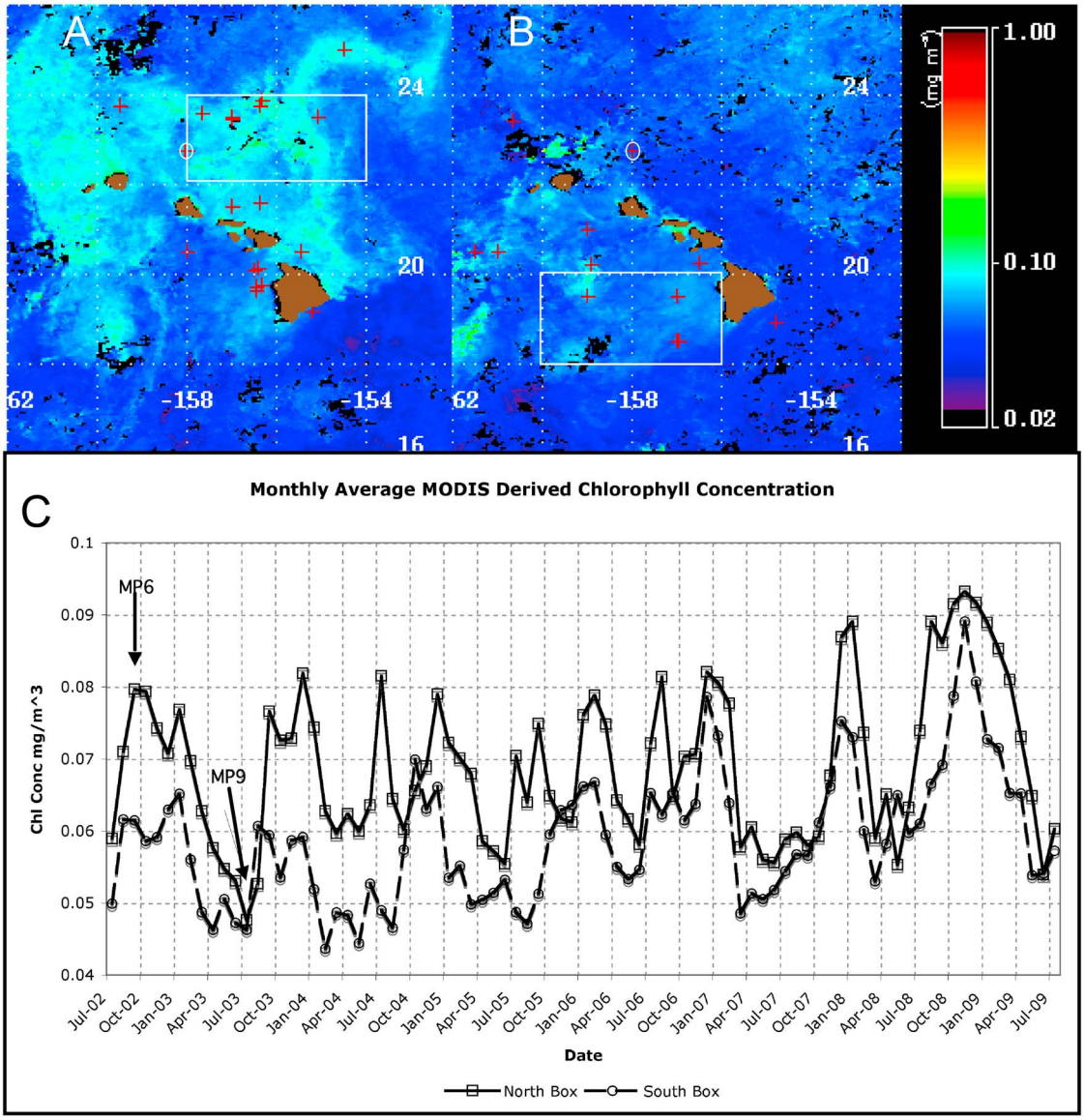


**Figure 4.** (a) Chlorophyll a concentration for September 2002 derived from MODIS Aqua satellite data. The red crosses indicate station locations occupied on MP6 and MP9. The location of station ALOHA is indicated by a white circle. The north box used for calculating the time series shown in Figure 4c is also shown. (b) Same as Figure 4a but for August 2003 with the south box indicated. (c) A time series of the monthly average chlorophyll in the north and south boxes indicated in Figures 4a and 4b from July 2002 to July 2009. The high value in October 2002 corresponding to MP6 and the low value in August 2003 corresponding to MP9 are indicated.

dance was positively correlated to temperature, the only biological factors to be significantly correlated to environmental factors ($p < 0.5$). These results show that it is difficult to determine the factors controlling $N_2$ fixation in the ocean in this manner, and this type of analysis likely suffers from two issues: (1) diazotroph abundance and $N_2$ fixation should be influenced by the conditions preceding the time of measurement, in addition to the conditions at the time of measurement, and (2) this data set may be too small on its own to tease out this information, given the scale of variability commonly encountered in the natural environment.

[31] Previous work on the factors controlling $N_2$ fixation in the NPSG suggest that $PO_4^{3-}$ is not a strong limiting factor [*Sohm et al.*, 2008; *Zehr et al.*, 2007]. However, bioassay experiments by *Grabowski et al.* [2008] with samples from Station Aloha yielded variable results with additions of $PO_4^{3-}$ and iron (Fe). Similarly variable results were obtained by J. A. Sohm and D. G. Capone (unpublished data) for $N_2$ fixation by *Trichodesmium* colonies amended with additions of $PO_4$ or Fe in this region. *Church et al.* [2009] have shown that mesoscale physical variability, in the form of sea surface height anomaly, can have a large impact on $N_2$ fixation rates during the lower nutrient summer months.

[32] Separate from the overall patterns of diazotrophy, the apparent shift in $N_2$ fixation by unicellular diazotrophs in the central gyre, to an increase in the contribution by

## Multivariate

### Correlations

| | SST | SSS | Wind | ML depth | Zeu | LLP | TotalTricho | Katagnymene | Richelia | NfixTricho | NfixBulk |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SST | 1.0000 | −0.5050 | −0.5524 | −0.0374 | 0.0236 | 0.2127 | 0.0664 | −0.3150 | 0.3993 | −0.2786 | −0.1356 |
| SSS | −0.5050 | 1.0000 | 0.3125 | 0.0116 | −0.3880 | −0.5254 | −0.0687 | 0.2203 | −0.0897 | 0.1995 | 0.0080 |
| Wind | −0.5524 | 0.3125 | 1.0000 | −0.0083 | 0.0575 | −0.0767 | −0.1928 | 0.2689 | −0.0383 | 0.2293 | −0.0697 |
| ML depth | −0.0374 | 0.0116 | −0.0083 | 1.0000 | 0.1090 | 0.4244 | 0.2704 | 0.1964 | −0.0845 | 0.1860 | 0.2414 |
| Zeu | 0.0236 | −0.3880 | 0.0575 | 0.1090 | 1.0000 | 0.5319 | −0.1233 | −0.2424 | −0.0825 | −0.1069 | −0.1528 |
| LLP | 0.2127 | −0.5254 | −0.0767 | 0.4244 | 0.5319 | 1.0000 | 0.2462 | −0.2461 | 0.1065 | −0.2272 | −0.0242 |
| TotalTricho | 0.0664 | −0.0687 | −0.1928 | 0.2704 | −0.1233 | 0.2462 | 1.0000 | 0.5801 | 0.1535 | 0.7150 | −0.1773 |
| Katagnymene | −0.3150 | 0.2203 | 0.2689 | 0.1964 | −0.2424 | −0.2461 | 0.5801 | 1.0000 | −0.0565 | 0.6830 | −0.0923 |
| Richelia | 0.3993 | −0.0897 | −0.0383 | −0.0845 | −0.0825 | 0.1065 | 0.1535 | −0.0565 | 1.0000 | −0.1804 | −0.0800 |
| NfixTricho | −0.2786 | 0.1995 | 0.2293 | 0.1860 | −0.1069 | −0.2272 | 0.7150 | 0.6830 | −0.1804 | 1.0000 | −0.2742 |
| NfixBulk | −0.1356 | 0.0080 | −0.0697 | 0.2414 | −0.1528 | −0.0242 | −0.1773 | −0.0923 | −0.0800 | −0.2742 | 1.0000 |

The correlations are estimated by Pairwise method.

### Scatterplot Matrix

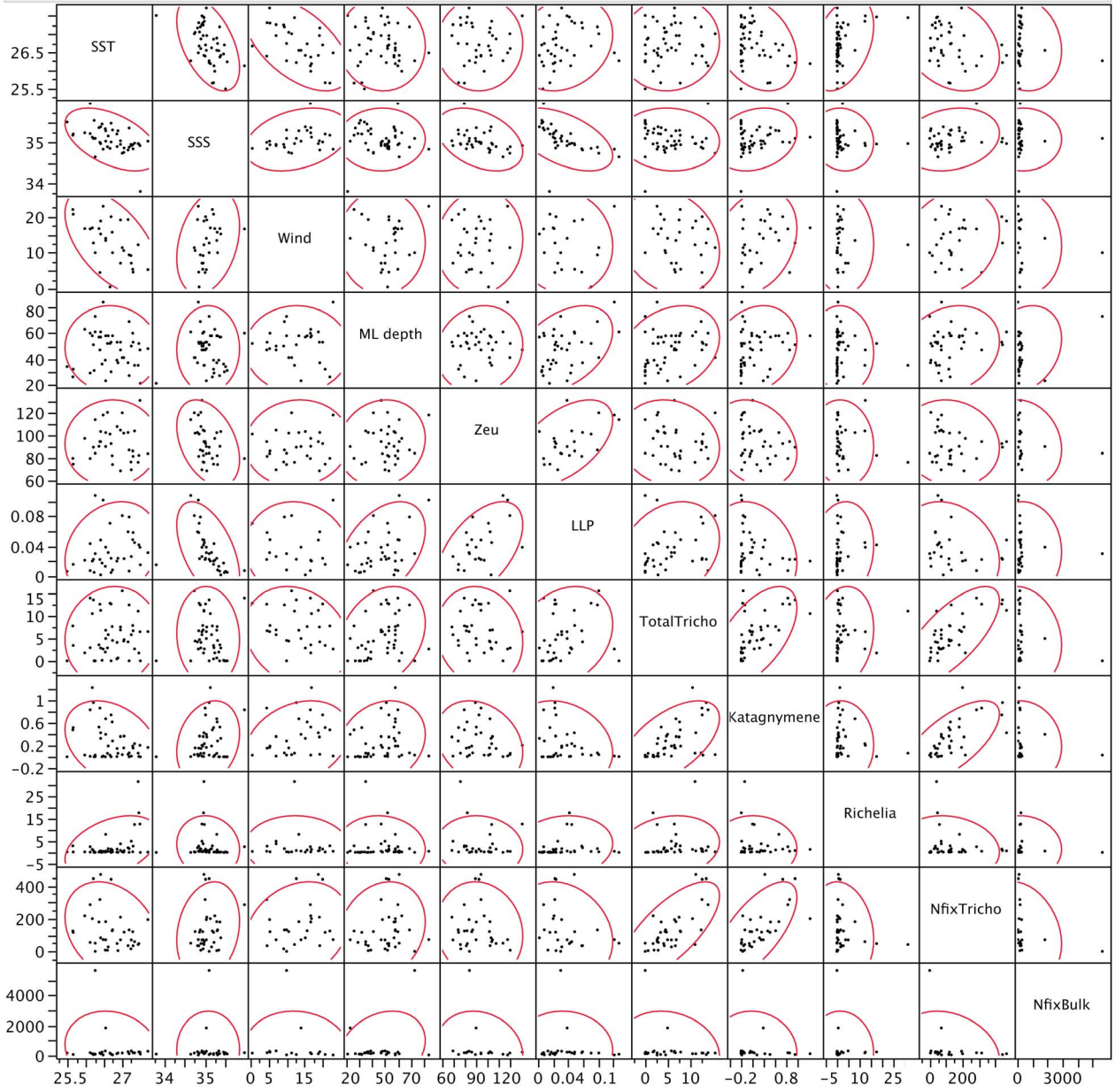


**Figure 5.** Pairwise regression analysis for the biological and environmental factors measured on this cruise. ML depth is mixed layer depth, Zeu is the depth of the euphotic zone, and LLP is low level phosphate.

*Trichodesmium* spp. near the Hawaiian Islands on leg 1 of MP9 is an intriguing pattern suggesting the presence of an important growth factor for *Trichodesmium* spp. near the islands. Although dissolved Fe (dFe) concentrations were not measured on MP9, dFe was measured in a wide area of the North Pacific on the 2002 Intergovernmental Oceanographic Commission cruise, including a large part of the NPSG. Surface water dFe was about 0.1 nM along a transect at 24°N from roughly 170°E to 165°W, then increased to ~0.6 nM in the area north of the Hawaiian Island chain [*Brown et al.*, 2005], a remarkably similar pattern to the presence of *Trichodesmium* spp. While the connection between *Trichodesmium* spp. and dFe is merely correlative, it may explain why *Trichodesmium* spp. was only found near the Hawaiian Islands. Despite the lack of *Trichodesmium* spp. in the central gyre, $N_2$ fixation was still found at appreciable rates there and is thus attributable to unicellular diazotrophs. We suggest, then, that Fe inputs may control the distribution and importance of *Trichodesmium* spp. and unicellular diazotrophs in the NPSG.

[33] Other factors could also be important in defining the distribution of diazotrophs. Temperature, salinity and mixed layer depth were not significantly different on leg 1 of MP9 at stations with *Trichodesmium* spp. compared to those without, while DIP was significantly lower at stations with *Trichodesmium* spp. present ($p = 0.013$). This seems counterintuitive, however, rather than being a cause of this difference, this may be an affect of the presence of *Trichodesmium* spp., as blooms can lead to the drawdown of DIP in the Pacific [*Hashihama et al.*, 2009].

[34] If the modes of transfer of newly fixed N into the food chain or export into the deep ocean are different between *Trichodesmium* spp. and unicellular diazotrophs, this distribution could have important biogeochemical consequences. The primary mode of N transfer from *Trichodesmium* spp. is thought to be through the release of dissolved forms [*Capone et al.*, 1994; *Glibert and Banahan*, 1988; *Mulholland et al.*, 2004], and *Trichodesmium* spp. has yet to be identified in sediment traps and has few grazers [*O'Neil et al.*, 1996]. Presently, no information is published on the ability of unicellular diazotrophs to release dissolved N, or the ability of zooplankton to consume them.

## 5. Conclusions

[35] $N_2$ fixation in the subtropical North Pacific Ocean is carried out by *Trichodesmium* spp., *Richelia* spp., and unicellular diazotrophs at rates comparable, but somewhat lower, those seen in the North Atlantic. Proximate to the Hawaiian Islands, *Trichodesmium* spp. was abundant and accounted for much of the $N_2$ fixation, however, over a large spatial area in the NPSG, *Trichodesmium* spp. and *Richelia* spp. was not present, or found at very low abundances, during our studies and $N_2$ fixation was thus carried out largely by unicellular diazotrophs, at a relatively high rate of ~100 $\mu$mol N $m^{-2}$ $d^{-1}$. We hypothesize that dFe is a controlling factor in the distribution of these different types of diazotrophs. Our studies show that the results reported by various investigators from work around the HOT site can be extended over a much larger geographical region in the summer. We found large diazotrophs such as *Trichodesmium* and *Richelia* to be the dominant diazotrophs in the vicinity of the Hawaiian Islands while unicellular diazotrophs seem to dominate to the regions west of the islands.

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