

## Cross-basin comparison of phosphorus stress and nitrogen fixation in *Trichodesmium*

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### Abstract

We investigated the phosphorus (P) status and N<sub>2</sub> fixation rates of *Trichodesmium* populations from the North Pacific, western South Pacific, and western North Atlantic. Colonies of *Trichodesmium* were collected and analyzed for endogenous alkaline phosphatase (AP) activity using enzyme-labeled fluorescence (ELF) and for nitrogenase activity using acetylene reduction. AP hydrolyzes dissolved inorganic phosphate (DIP) from dissolved organic phosphorus and is active in *Trichodesmium* colonies experiencing P stress. Across multiple stations in the subtropical North and South Pacific, there was low to moderate ELF labeling in *Trichodesmium*, although labeling was present in other taxa. In contrast, *Trichodesmium* ELF labeling in the North Atlantic ranged from low to high. Low ELF labeling corresponded with high DIP concentrations while high ELF labeling occurred only at North Atlantic stations with DIP concentrations  $\leq 40$  nmol L<sup>-1</sup>, indicating that *Trichodesmium* was not experiencing dramatic P stress in the Pacific Ocean while P stress was evident in the western North Atlantic. However, nitrogenase activity was significantly higher in the P-stressed western North Atlantic than in the Pacific Ocean (0.40–1.30 compared to 0.01–0.46 nmol C<sub>2</sub>H<sub>4</sub> h<sup>-1</sup> colony<sup>-1</sup>). These data underscore the differential basin-level importance of P availability to *Trichodesmium* and suggest that factors other than P are constraining their N<sub>2</sub> fixation rates in the Pacific.

Fixed nitrogen (N) is considered to be the proximal limiting nutrient in ocean ecosystems. For a system in steady-state, new production is the excess production available for export and is a function of new N entering the system and stimulating production (Eppley and Peterson 1979). New N comes from eddy-diffusion processes, seasonal deep mixing, atmospheric deposition, lateral advection, and N<sub>2</sub> fixation by diazotrophs (Dugdale and Goering 1967). Upwelled water from the deep ocean contains both elevated dissolved inorganic nitrogen (DIN) and dissolved inorganic carbon (DIC), so export production from upwelled DIN does not generally result in a significant net removal of DIC from surface water. However, DIN from N<sub>2</sub> fixation is not related to the DIC supply from deep water and can drive uptake of CO<sub>2</sub> and export production (Hood et al. 2000). In global marine models, the presence of N<sub>2</sub>-fixers increased primary production by diatoms and small phytoplankton through the excretion of dissolved organic nitrogen (DON) in N-limited areas (Moore et al. 2004). In the subtropical Atlantic, model results show that N<sub>2</sub> fixation increases DIN, phytoplankton biomass, primary production, and export flux (Coles et al. 2004).

The cyanobacterium *Trichodesmium* is an important source of new N in tropical and subtropical oceans (Capone et al. 1997). In the North Atlantic, N<sub>2</sub> fixation rates of *Trichodesmium* can equal or exceed the vertical flux of nitrate into surface waters (Capone et al. 2005), while in the North Pacific, N<sub>2</sub> fixation is the source of up to half of the new N (Karl et al. 1997). Global ecosystem models use

parameters from *Trichodesmium* to model N<sub>2</sub> fixation (Coles et al. 2004; Moore et al. 2004; Moore and Doney 2007). Elucidating the factors limiting the growth and N<sub>2</sub> fixation rates of *Trichodesmium* is vital to understanding the N cycle and its link to the carbon cycle.

N<sub>2</sub> fixation by *Trichodesmium* can be limited by a variety of factors including light, temperature, mixed-layer depth, dissolved oxygen, and nutrients such as phosphorus (P) and iron (Fe) (Wu et al. 2000; Moore et al. 2004). Like N, new P can reach the surface by mixing, upwelling from deep waters, lateral advection, or deposition; however, there is no process analogous to N<sub>2</sub> fixation to act as a source for bioavailable P (Karl 2002). Nitrogenase, the enzyme responsible for N<sub>2</sub> fixation, requires multiple Fe atoms per molecule. Sources of Fe to open-ocean environments are dust deposition, advection from continental margins, and upwelling (Fung et al. 2000). Due to these obligate requirements and low oceanic concentrations, P and Fe bioavailability may influence the growth and N<sub>2</sub> fixation rates of *Trichodesmium* field populations (Berman-Frank et al. 2001; Sañudo-Wilhelmy et al. 2001). In the central Atlantic, N<sub>2</sub> fixation positively correlated to the P content of *Trichodesmium* (Sañudo-Wilhelmy et al. 2001) while in the eastern tropical Atlantic, N<sub>2</sub> fixation was co-limited by P and Fe (Mills et al. 2004). In the North Pacific Subtropical Gyre (NPSG), P supply may be the ultimate control on new and export production at Sta. ALOHA (Karl et al. 1997), but Fe rather than P is likely to limit N<sub>2</sub> fixation (Wu et al. 2000).

Although subtropical gyres of both the North Pacific and the North Atlantic are oligotrophic, the NPSG has higher dissolved inorganic phosphate (DIP) concentrations:

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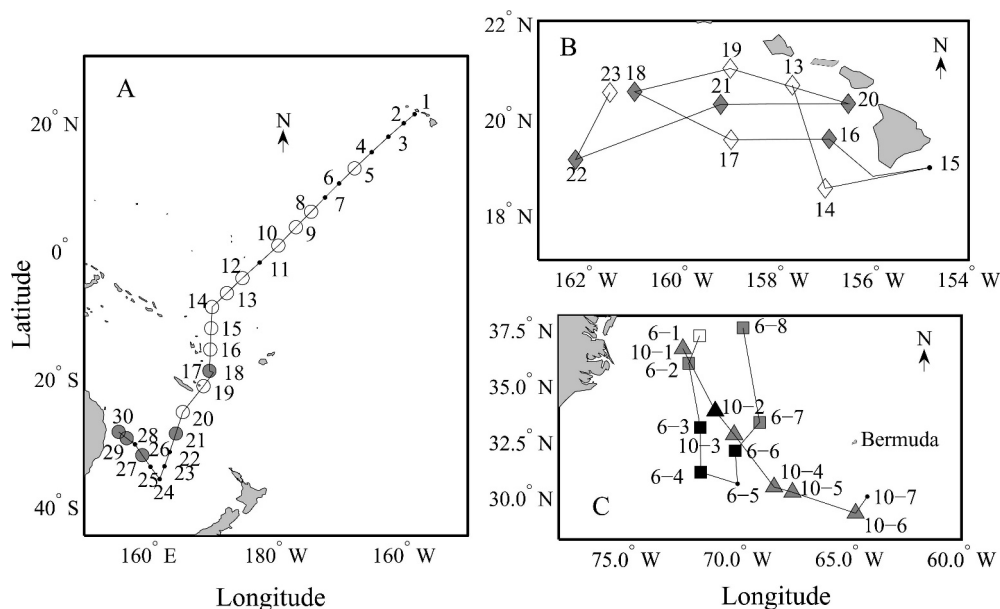


Fig. 1. Maps of cruise tracks: (A) KM0701 (circles) during January and February 2007, across the west Pacific warm pool; (B) MP09 (diamonds) during August 2003, in the North Pacific; (C) EN355 (squares) during June 2001, and EN361 (triangles) during October 2001, in the western North Atlantic. The highest level of ELF labeling for all colony types at each station is indicated by color: black symbols = '+ +', gray symbols = '+ -', open symbols = '- -', small dots = no ELF data.

3.0–191.3 nmol L<sup>-1</sup> in the NPSG compared to 0.2–1.0 nmol L<sup>-1</sup> in the North Atlantic (Hawaii Ocean Time-series [HOT], [http://hahana.soest.hawaii.edu/hot/hot\\_jgofs.html](http://hahana.soest.hawaii.edu/hot/hot_jgofs.html); Wu et al. 2000). Dissolved organic and particulate pools of P are also higher in the NPSG than the North Atlantic (Ammerman et al. 2003). In the western South Pacific, DIP concentrations ranged from 80 nmol L<sup>-1</sup> to 218 nmol L<sup>-1</sup> in oceanic regions (Campbell et al. 2005) and from <30 nmol L<sup>-1</sup> to 63 nmol L<sup>-1</sup> near New Caledonia (Van Den Broeck et al. 2004). P limitation may be related to inputs of Fe (Wu et al. 2000). The NPSG has lower aeolian Fe input than the subtropical North Atlantic: 0.08–0.16 μmol Fe m<sup>-2</sup> d<sup>-1</sup> compared to 0.2–0.8 μmol Fe m<sup>-2</sup> d<sup>-1</sup>, respectively (Wu et al. 2000). Open-ocean dissolved-Fe concentrations in the upper 100 m averaged 720 pmol L<sup>-1</sup> in the North Atlantic, 200 pmol L<sup>-1</sup> in the North Pacific, and 110 pmol L<sup>-1</sup> in the Equatorial Pacific (Moore and Braucher 2008). During a cruise in the western South Pacific, open-ocean dissolved-Fe concentrations ranged from <60 pmol L<sup>-1</sup> to 760 pmol L<sup>-1</sup> while coastal dissolved-Fe concentrations ranged from <60 pmol L<sup>-1</sup> to 1000 pmol L<sup>-1</sup> (Campbell et al. 2005). In addition to low concentrations, bioavailability of Fe and P can limit abundance and activity of *Trichodesmium*, and differences in the availability of these nutrients across ocean basins create a variety of limitation scenarios.

Herein we define nutrient stress as the exposure of a cell to low inorganic nutrient conditions that stimulates a response from the cell. Phytoplankton can launch a physiological response under conditions of P stress to ameliorate potential limitation. DIP is the most readily available form of phosphorus. The utilization of dissolved organic phosphorus

(DOP) can prevent limitation of *Trichodesmium* growth and N<sub>2</sub> fixation rates in low-DIP environments. However, accessing the DOP pool requires the cell to invest energetic, carbon, and nitrogen resources in enzymes such as alkaline phosphatase (AP). We define this as distinct from P limitation, which is the reduction of either growth or N<sub>2</sub> fixation rates as a result of persistent stress. AP hydrolyzes DIP from DOP and is commonly induced in phytoplankton species experiencing P stress (Stihl et al. 2001; Dyhrman et al. 2002). DOP is likely a significant source of P for *Trichodesmium* spp. (Sohm and Capone 2006; Mather et al. 2008), and the presence of AP activity can indicate *Trichodesmium* field populations responding to low levels of DIP (Stihl et al. 2001). AP activity can be measured using colorimetric and fluorometric assays or detected through molecular diagnostic tools. Enzyme-labeled fluorescence (ELF) is a commercially available, cell-specific molecular diagnostic tool that detects AP activity. The phosphatase substrate precipitates when cleaved by AP and fluoresces green under ultraviolet light (González-Gil et al. 1998). In the laboratory, P-depleted *Trichodesmium erythraeum* IMS101 batch cultures were ELF-labeled while replete cultures were not (Dyhrman et al. 2002). In shipboard incubations, addition of DIP to ELF-positive populations of *Trichodesmium* reduced ELF labeling within 48 h (Webb et al. 2007). We believe that ELF labeling of P-stressed *Trichodesmium* gives valuable insight into the in situ physiological status of the cells.

In this study we compare *Trichodesmium* P stress and nitrogenase activity among tropical and subtropical regions of the western North Atlantic, the North Pacific, and the western South Pacific (Fig. 1). We collected colonies of

*Trichodesmium* from four cruises and analyzed endogenous AP activity using ELF, measured nitrogenase activity using acetylene reduction, and sampled surface water nutrients to assess the P status of *Trichodesmium* populations. Our results show that populations in the western North Atlantic were more P stressed but had higher nitrogenase activities than populations in the North and South Pacific.

## Methods

**Hydrological context**—Samples were collected along a north–south transect of the Pacific Ocean as part of the Western Pacific Warm Pool cruise (KM0701) on the R/V *Kilo Moana* in January–February 2007 (Fig. 1A). As part of the Marine Nitrogen Transformation Biocomplexity Project, samples were taken from south of Hawaii during MP09 in August 2003, on the R/V *Revelle* (Fig. 1B). North Atlantic field samples were collected near the Bermuda Atlantic Time-series Station in June and October 2001, aboard the R/V *Endeavor* (EN355 and EN361, respectively; Fig. 1C). Temperature, salinity, pressure, and density data from each cruise were obtained from a conductivity–temperature–depth (CTD) system. Mixed-layer depths (MLD) were estimated from potential density profiles. Sea surface temperature (SST) and salinity were obtained from the ships' underway data.

**Collection of colonies**—A 0.5-m-diameter, 130.0- $\mu\text{m}$  mesh phytoplankton net with a 30-m line was hand-towed for 10–20 min. Tows for ELF were typically done in the morning while tows for nitrogenase activity were done around noon, the peak of  $\text{N}_2$  fixation in *Trichodesmium*, targeting local times of 11:00 h, 12:00 h, and 13:00 h. Specimens were immediately shaded and taken into an air-conditioned laboratory. Colonies of *Trichodesmium* spp. were collected with polyethylene bulb pipets or plastic bacteriological transfer loops (Fisher Scientific); separated by colony morphology into spherical 'puffs', fusiform 'tufts', and occasionally 'bowties'; and washed four times in sterilized seawater (0.2–0.4- $\mu\text{m}$ -filtered and microwaved to a rolling boil). The colonies were processed immediately for AP or nitrogenase activity. Stations with no data reported had too little *Trichodesmium* biomass to run the assays.

**Enzyme-labeled fluorescence (ELF)**—The presence of AP activity was determined using the ELF 97 Endogenous Phosphatase Detection Kit (Molecular Probes-Invitrogen) following the improved protocol described in Dyrhrman et al. (2002) for two colonies of each morphology. The slides were stored damp and in the dark at 4°C. ELF slides were viewed with epifluorescent microscopy using a 4, 6-diamidino-2-phenyl-indole (DAPI) long-pass filter set (360 nm). The entire area of the slide was examined. Digital pictures of *Trichodesmium* from MP09 and the Atlantic cruises were taken at 200 $\times$  using a Zeiss Axioplan2 microscope equipped with a Zeiss Axiocam digital camera and Openlab software (Improvision). Digital pictures from KM0701 were taken at 400 $\times$  using a Zeiss Axiostar microscope equipped with a MRC5 Axiocam and Axiovision software (Zeiss). Samples were rated '– –' for

'no labeling,' '+ –' for 'some labeling' if many filaments showed moderate labeling but were not wholly covered or if there was mixture of labeled and unlabeled filaments, and '+ +' for 'high labeling' if many filaments showed dense labeling and/or were wholly covered. For the North Atlantic samples, multiple slides were made for each colony type so one rating was given based on all the slides. Filament color was noted as 'normal' if the orange color of the phycoerythrin autofluorescence was visible or 'dim' if the filaments appeared abnormally dark, green or blue. Digital pictures of pennate diatoms associated with *Trichodesmium* colonies were taken at 400 $\times$  with the DAPI long-pass filter and a chlorophyll filter (660 nm) using AxioVision 4.2 software.

**Nutrient analysis**—For the North and South Pacific (KM0701) and the North Atlantic (EN355 and EN361), surface water was collected using either niskin bottles from a rosette or Go-Flo bottles, filtered through acid-cleaned 0.4- $\mu\text{m}$  polycarbonate filters, and frozen. Samples for KM0701 were sent to the College of Oceanic and Atmospheric Sciences, Oregon State University and DIP was analyzed using a Technicon AutoAnalyzer II by J. Jennings with a detection level of 6 nmol L<sup>-1</sup>. North Atlantic samples were sent to the Ocean Data Facility of the Scripps Institution of Oceanography (<http://sts.ucsd.edu/sts/chem>) for analysis by a Skalar San Plus autoanalyzer with a detection level of 3 nmol L<sup>-1</sup>. DIP concentrations for the North Pacific during MP09 were provided by K. Björkman (pers. comm.). Two-way ANOVA Tukey–Kramer tests were used to determine significant differences in DIP concentrations among ELF ratings and oceans for stations with both DIP concentrations and ELF samples ( $\alpha = 0.05$ ). DIP concentrations below detection level (BDL) were considered to be 0 in the calculations.

**Nitrogenase activity**—Nitrogenase activity was assayed during KM0701 and the Atlantic cruises by the acetylene reduction assay described by Capone and Montoya (2001). Five to twenty colonies were placed in 20–30 mL of 0.2- $\mu\text{m}$ -filtered seawater in 75-mL square polycarbonate culturing bottles with silicone septa. Two to three replicate bottles were used for each incubation. Acetylene was produced on board by mixing 50 mL of double-distilled water with 15 g calcium carbide and stored in a bladder. Six mL of acetylene were injected into the bottles. The bottles were incubated in a Percival incubator at ambient surface seawater temperatures under soft white fluorescent bulbs at 50- $\mu\text{mol}$  quanta m<sup>-2</sup> s<sup>-1</sup> light. Zero times were targeted at 11:00 h, 12:00 h, and 13:00 h, local time. At times 0, 1 h, and 2 h after injection, duplicate 0.2–0.3-mL samples were removed from the headspace of the bottles and injected into a Shimadzu GC-8A gas chromatograph and integrated by a Shimadzu CR8A Chromatopac to measure the ethylene peaks. Standards of 9.1- $\mu\text{L}$  L<sup>-1</sup> ethylene were used to calibrate the peak heights. Ethylene formed was calculated according to Capone and Montoya (2001) with Bunsen coefficients calculated from surface salinity and temperature according to Breitbarth et al. (2004). Nitrogenase activity was calculated as the average

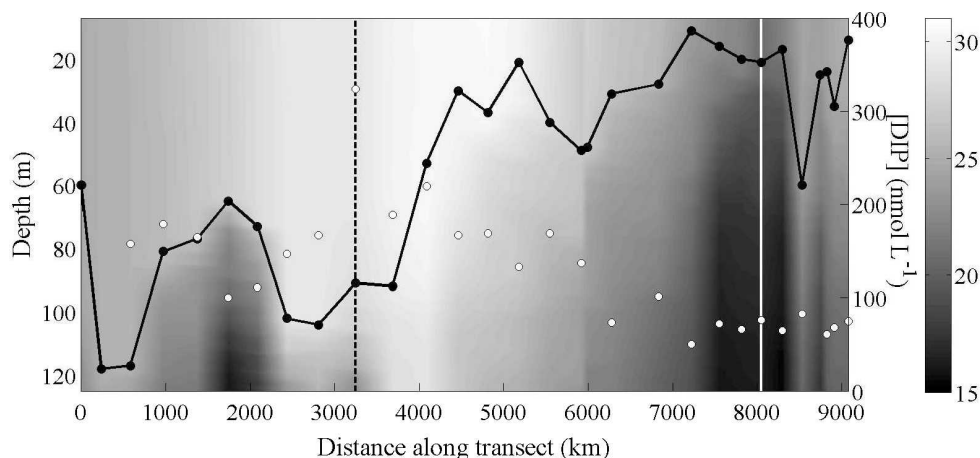


Fig. 2. Hydrographic conditions of KM0701. The grayscale pseudo-color plot is potential temperature ( $^{\circ}\text{C}$ ) as a function of depth (m) vs. distance along the transect (km) starting from Hawaii, U.S.A. The connected black circles are mixed-layer depth (m) while the white circles indicate DIP concentrations ( $\text{nmol L}^{-1}$ ). The vertical dashed black line represents the Equator and the vertical solid white line denotes a turning point in the transect.

rate of ethylene production per colony ( $\text{nmol C}_2\text{H}_4 \text{ h}^{-1} \text{ colony}^{-1}$ ). Acetylene reduction rates for MP09 were provided by D. Capone (pers. comm.). One-way ANOVA Tukey–Kramer tests were used to determine statistically different rates between colony morphologies and among the cruise means ( $\alpha = 0.05$ ).

## Results

**Physical conditions**—The North Atlantic upper ocean was stratified with late-spring SST ranging from  $23^{\circ}\text{C}$  to  $25^{\circ}\text{C}$  and MLD ranging from 4 m to 37 m during EN355 and autumn SST ranging from  $25^{\circ}\text{C}$  to  $28^{\circ}\text{C}$  and MLD ranging from 70 m in the northern part of the transect to 26 m in the south during EN361. During MP09, the summer North Pacific upper ocean was highly stratified with SST ranging from  $26.5^{\circ}\text{C}$  to  $27.5^{\circ}\text{C}$  and MLD ranging from 25 m to 58 m. KM0701 cut across the west Pacific warm pool, with early winter deep mixing up to 118 m in the north, early summer stratification as shallow as 11 m in the south, and a maximum SST of  $>30^{\circ}\text{C}$  just south of the Equator (Fig. 2). As the MLD shoaled in the southern end of the west Pacific transect, DIP concentrations decreased (Fig. 2).

**ELF**—North Atlantic samples consistently showed more ELF labeling than Pacific samples. A spatial representation of the maximum ELF labeling at each station is shown along the cruise tracks in Fig. 1. During the timeframe of this study, North and South Pacific *Trichodesmium* field samples did not show high ELF labeling. All samples were rated ‘+ –’ or ‘– –’, indicating that there was low to no AP activity (Table 1; Figs. 1A,B; 3A–H). Labeling of South Pacific *Trichodesmium* colonies was highest on the shelf near Australia at ‘+ –’ (Fig. 1A). In contrast, North Atlantic ELF samples ranged from ‘– –’ to ‘+ +’ (Table 1; Figs. 1C, 3I–P). Labeling of the two colony morphologies was not always at the same level, as seen at Sta. 16, 18, and

20 of MP09 (Table 1; Fig. 3A,B) and Sta. 14, 26, and 29 of KM0701 (Table 1; Fig. 3G,H). Labeling of colonies at the same station on different days could vary, as seen at MP09 Sta. 17 and 22 (Table 1; Fig. 3C,D) and KM0701 Sta. 14 (Table 1). Two colonies on the same slide could also differ. For example, Sta. 7 and 8 from EN355 had some highly labeled filaments among unlabeled filaments, giving the slides a rating of ‘+ –’ (Fig. 3J–L). Although *Trichodesmium* colonies collected were identified by morphology, two to three different filament sizes were observed in over half of the samples (Fig. 3L). Color from the autofluorescence of phycoerythrin in the filaments varied, ranging from the usual orange color (Table 1; Fig. 3A,B, E–H) to a dim green or blue (Table 1; Fig. 3C,D,J).

In spite of efforts to wash the colonies for the ELF assay, a variety of microbes were found in association with *Trichodesmium* colonies including diatoms, heterotrophic bacteria, and the filamentous  $N_2$ -fixing cyanobacteria *Plectonema* sp. and *Calothrix* sp. (Fig. 4A–E). As in Dyhrman et al. (2002), some of these organisms were found to be ELF-labeled even when *Trichodesmium* was not, indicating AP activity in these other taxa: diatoms (North Pacific MP09-21, North Atlantic EN361-1; Fig. 4A–B), heterotrophic bacteria (over half of the stations on all four cruises; Fig. 4C), and *Plectonema* sp. (North Pacific MP09-19, North Atlantic EN361-1, 2, 3; Fig. 4D). *Calothrix* sp. was found in association with *Trichodesmium* near Hawaii during MP09 and was not ELF-labeled (Sta. 13, 17, 18, 19, 21, 23; Fig. 4E).

**DIP and ELF**—ELF labeling varied with ocean basin and corresponded negatively with DIP concentrations. North Atlantic stations had significantly lower DIP concentrations than the North and South Pacific stations in a two-way ANOVA ( $p = 0.0001$ ; Table 2). DIP concentrations ranged from BDL to  $25 \text{ nmol L}^{-1}$  in the North Atlantic,  $30\text{--}320 \text{ nmol L}^{-1}$  in the North Pacific, and  $50\text{--}220 \text{ nmol L}^{-1}$  in the South Pacific. Stacked histograms

Table 1. Data summary for stations with enzyme-labeled fluorescence (ELF) data. For stations with multiple colony morphologies, data are separated by '/'. NA = not available.

Date	Station	Latitude	Longitude	Colony	ELF	Color
01 Jun 01	EN355-1	37°6'N	71°42'W	puff/tuft	- -/- -	dim/dim
02 Jun 01	EN355-2	35°52'N	71°43'W	puff	+ -	dim
03 Jun 01	EN355-3	33° 0'N	71°40'W	puff	++	dim
04 Jun 01	EN355-4	31°33'N	71°39'W	puff/tuft	+ +/+ -	dim/dim
05 Jun 01	EN355-5	30°29'N	70°0.0'W	puff/bowtie	NA/NA	NA/NA
07 Jun 01	EN355-6	32°2.4'N	70°19.51'W	NA	++	dim-normal
09 Jun 01	EN355-7	33°16.67'N	68°59.32'W	NA	+ -	dim-normal
11 Jun 01	EN355-8	37°27.32'N	69°45.28'W	NA	+ -	dim-normal
04 Oct 01	EN361-1	36°31.53'N	72°26.78'W	puff/tuft	+ -/+ -	dim-normal/normal
05 Oct 01	EN361-2	33°44.52'N	70°59.79'W	puff/tuft	- -/+ -	dim-normal/dim-normal
05 Oct 01	EN361-3	32°40.89'N	70°9.3'W	puff/tuft	+ -/+ -	dim-normal/dim-normal
06 Oct 01	EN361-4	30°20.35'N	68°22.25'W	puff/tuft/bowtie	+ -/+ -/+ -	dim-normal/dim-normal/dim-normal
06 Oct 01	EN361-5	30°6.42'N	67°32.88'W	puff/tuft	+ -/+ -	normal/dim-normal
07 Oct 01	EN361-6	29°10.21'N	64°43.528'W	puff/tuft	+ -/+ -	dim-normal/dim-normal
06 Aug 03	MP09-13	20°29.59'N	157°41.32'W	puff/tuft	- -	normal/normal
07 Aug 03	MP09-14	18°30.00'N	157°00.00'W	puff	- -	normal
10 Aug 03	MP09-16	19°32.49'N	156°55.74'W	puff/tuft	+ -/- -	normal/normal
11 Aug 03	MP09-16	19°30.55'N	156°55.74'W	puff/tuft	+ -/- -	normal/normal
12 Aug 03	MP09-17	19°31.23'N	158°58.75'W	puff/tuft	- -/- -	normal/normal
13 Aug 03	MP09-17	19°30.10'N	158°59.94'W	puff	+ -	normal
14 Aug 03	MP09-18	20°31.84'N	160°59.81'W	puff/tuft	- -/+ -	dim/dim
15 Aug 03	MP09-19	21°00.79'N	158°59.70'W	puff/tuft	- -	dim/normal
16 Aug 03	MP09-19	21°00.18'N	158°59.69'W	puff/tuft	- -/- -	dim/normal
17 Aug 03	MP09-20	20°16.09'N	156°31.72'W	puff/tuft	+ -/- -	normal/normal
18 Aug 03	MP09-21	20°15.61'N	159°11.21'W	puff/tuft	+ -/+ -	normal/dim
19 Aug 03	MP09-22	19°06.67'N	162°13.05'W	puff/tuft	+ -/+ -	normal/dim
20 Aug 03	MP09-22	19°06.29'N	162°13.60'W	puff/tuft	- -/- -	dim/dim
21 Aug 03	MP09-23	20°30.68'N	161°30.27'W	puff/tuft	- -/- -	normal/dim
07 Jan 07	KM0701-5	12°26.11'N	167°43.75'W	puff/tuft	- -/- -	normal/dim-normal
10 Jan 07	KM0701-8	5°38.86'N	174°32.18'W	tuft	- -	dim-normal
11 Jan 07	KM0701-9	3°14.40'N	176°53.04'W	puff	- -	normal
12 Jan 07	KM0701-10	0°22.02'N	179°38.60'W	tuft	- -	normal
15 Jan 07	KM0701-12	4°43.34'S	174°43.85'E	puff	- -	normal
16 Jan 07	KM0701-13	7°3.64'S	172°18.98'E	tuft	- -	normal
17 Jan 07	KM0701-14	9°15.03'S	169°59.96'E	puff/tuft/bowtie	- -/- -/- -	dim-normal/normal/normal
19 Jan 07	KM0701-14	9°15.01'S	170°0.01'E	puff/tuft	- -/+ -	normal/dim-normal
20 Jan 07	KM0701-15	12°34.55'S	169°51.55'E	tuft	- -	normal
21 Jan 07	KM0701-16	15°53.63'S	169°43.02'E	puff	- -	normal
22 Jan 07	KM0701-17	19°13.95'S	169°34.63'E	puff/tuft	+ -/+ -	dim-normal/normal
23 Jan 07	KM0701-19	21°37.42'S	168°39.49'E	puff/tuft	- -/- -	normal/normal
26 Jan 07	KM0701-20	25°40.30'S	165°25.03'E	NA/puff/tuft	- -/NA/NA	normal/NA/NA
27 Jan 07	KM0701-21	29°2.43'S	164°20.26'E	puff/tuft	- -/+ -	normal/normal
03 Feb 07	KM0701-26	32°25.29'S	159°5.33'E	puff/tuft	- -/+ -	normal/dim-normal
07 Feb 07	KM0701-28	30°15.51'S	157°18.19'E	puff/tuft	+ -/+ -	normal/dim-normal
08 Feb 07	KM0701-29	29°45.56'S	156°37.43'E	puff/tuft	- -/+ -	normal/normal
09 Feb 07	KM0701-30	28°45.68'S	155°22.16'E	puff/tuft	+ -/NA	normal/NA

of DIP concentrations were plotted for each ELF label rating (Fig. 5). *Trichodesmium* colonies with little or no ELF labeling were found in stations from all cruises, spanning the full range of DIP concentrations (Fig. 5A). Samples with moderate levels of labeling spanned a moderate range of DIP: BDL–169 nmol L<sup>-1</sup> (Fig. 5B). High levels of labeling were found only in samples from North Atlantic stations with DIP concentrations <50 nmol L<sup>-1</sup> (Fig. 5C). In a two-way ANOVA, DIP concentrations for stations with an ELF rating of ‘- -’

were greater than DIP concentrations for stations with an ELF rating of ‘+ -’ or ‘+ +’, significant at the 94% confidence level ( $p = 0.0554$ ; Table 2).

*Nitrogenase activity*—Nitrogenase activity varied significantly between the basins (Table 3; Fig. 6). In the P-stressed western North Atlantic, mean nitrogenase activities measured during EN355 and EN361 were significantly higher than rates in both the North and South Pacific ( $p = 5.35 \times 10^{-8}$ ):  $0.80 \pm 0.22$  nmol C<sub>2</sub>H<sub>4</sub> h<sup>-1</sup> colony<sup>-1</sup> and

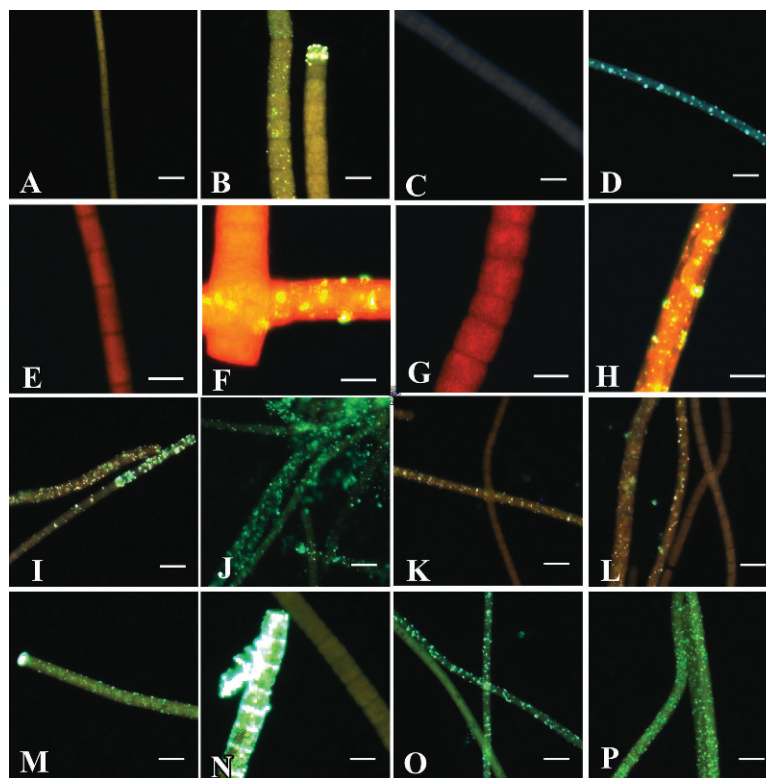


Fig. 3. Selected digital micrographs of ELF-assayed *Trichodesmium* from the four cruises: (A–D) MP09 during August 2003, in the North Pacific; (E–H) KM0701 during January and February 2007, across the west Pacific warm pool; and (I–L) EN355 during June 2001, and (M–P) EN361 during October 2001, in the western North Atlantic. White scale bars correspond to 10  $\mu\text{m}$ . (A) Sta. MP09-16, 10 August 2003, tuft, ‘– –’; (B) Sta. MP09-16, 10 August 2003, puff, ‘+ –’; (C) Sta. MP09-22, 20 August 2003, puff, ‘– –’; (D) Sta. MP09-22, 19 August 2003, tuft, ‘+ –’; (E) Sta. KM0701-14, 17 January 2007, puff, ‘– –’; (F) Sta. KM0701-17, 22 January 2007, puff, ‘+ –’; (G) Sta. KM0701-26, 03 February 2007, puff, ‘– –’; (H) Sta. KM0701-26, 03 February 2007, tuft, ‘+ –’; (I) Sta. 6-6, 07 June 2001, ‘+ +’; (J) Sta. 6-7, 09 June 2001, ‘+ –’; (K) Sta. 6-7, 09 June 2001, ‘+ –’; (L) Sta. 6-8, 11 June 2001, ‘+ –’; (M) Sta. 10-1, 04 October 2001, puff, ‘+ –’; (N) Sta. 10-2, 05 October 2001, tuft, ‘+ +’; (O) Sta. 10-3, 05 October 2001, puff, ‘+ –’; (P) Sta. 10-5, 06 October 2001, puff, ‘+ –’.

$0.79 \pm 0.14 \text{ nmol C}_2\text{H}_4 \text{ h}^{-1} \text{ colony}^{-1}$  compared to  $0.26 \pm 0.03 \text{ nmol C}_2\text{H}_4 \text{ h}^{-1} \text{ colony}^{-1}$  (D. Capone pers. comm.) and  $0.09 \pm 0.02 \text{ nmol C}_2\text{H}_4 \text{ h}^{-1} \text{ colony}^{-1}$ , respectively (Fig. 6). For stations with nitrogenase activity measurements for both puff and tuft colonies, rates were generally greater for tuft colonies than puff colonies although the rates were not significantly different between colony morphs according to one-way ANOVA Tukey–Kramer tests ( $p \geq 0.1355$ ). There were two exceptions to this pattern. At Sta. KM0701-14, puff nitrogenase activity was greater than that of tufts but not significantly ( $p = 0.2379$ ), and at Sta. KM0701-30 near Australia, the rate for tuft colonies was significantly greater than that of puff colonies ( $p = 0.0022$ ).

## Discussion

*Trichodesmium* is a source of new N in tropical and subtropical oceans around the world, but the factors limiting N<sub>2</sub> fixation in *Trichodesmium* are not completely understood. The South Pacific in particular is an under-

sampled region (Campbell et al. 2005). Our study investigated P stress and N<sub>2</sub> fixation in *Trichodesmium* in three major ocean regions: the western North Atlantic, the North Pacific, and the western South Pacific. Our results show patterns of both higher P stress and higher N<sub>2</sub> fixation rates in the western North Atlantic than in the Pacific.

During the 2001 western North Atlantic cruises, colonies of *Trichodesmium* showed more frequent and more complete ELF labeling than the Pacific samples in this study. This difference in ELF labeling is a qualitative indication of higher AP activity in the North Atlantic. The samples near Australia also showed more labeling than other regions of the South Pacific. In this region the MLD was shallower and the DIP concentrations were lower. *Trichodesmium* thrives in warm, stratified waters, and blooms are found in oligotrophic waters where the SST  $\geq 25^\circ\text{C}$  (Karl et al. 2002). However, supply of P to surface waters can be cut off by warming and stratification of surface layers. ELF is not quantitative, so finding a direct parametric correlation with DIP concentrations is not

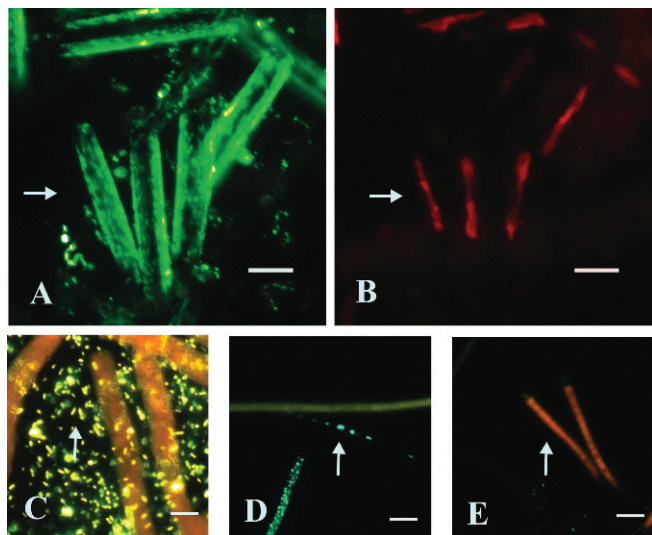


Fig. 4. Organisms associated with *Trichodesmium* colonies. White arrows indicate the organisms and white scale bars correspond to 10  $\mu\text{m}$ . (A) ELF-labeled pennate diatoms (Sta. MP09-17); (B) the same pennate diatoms in (A) viewed under a chlorophyll filter; (C) ELF-labeled heterotrophic bacteria (Sta. MP09-16, 11 Aug 03, puff); (D) ELF-labeled cyanobacterium *Plectonema* sp. (Sta. MP09-19, 15 Aug 03, puff); (E)  $\text{N}_2$ -fixing cyanobacterium *Calothrix* sp. (Sta. MP09-23, 21 Aug 03, tuft).

possible. However, by examining the range of DIP concentrations associated with the different levels of ELF label, it is apparent that higher labeling is associated with lower DIP concentrations. This is in contrast to Webb et al. (2007), where they found no significant relationship between ELF labeling and DIP during a transect in the western Equatorial to western South Atlantic. In our study, we had more data points of ELF and a broader range of DIP than Webb et al. (2007; 5–320  $\text{nmol L}^{-1}$  compared to 10–120  $\text{nmol L}^{-1}$ ), and this breadth of data may have enabled us to find a significant relationship between DIP and ELF labeling. Also in contrast to Webb et al. (2007), we did not see consistent differences in labeling between puff and tuft colonies. In a study of the North and South Atlantic subtropical gyres, surface-water column AP activity was higher and DIP was lower in the North than the South Atlantic (Mather et al. 2008). AP activity in the NPSG measured during cruise MP09 was 4.4 times lower than AP activity in the North Atlantic (Sohm et al. 2008), which is consistent with our ELF-based observations. The difference in AP activity observed by ELF labeling and hydrolytic rates among these regions suggests that the

Table 2. Two-way ANOVA on dissolved inorganic phosphate concentrations among enzyme-labeled fluorescence (ELF) ratings and ocean basins.

Factor	<i>F</i>	<i>p</i>	Groupings
ELF rating	3.04	0.0554	'++' and '+-'; '- -'
Ocean basin	11.55	0.0001	North Atlantic; North and South Pacific

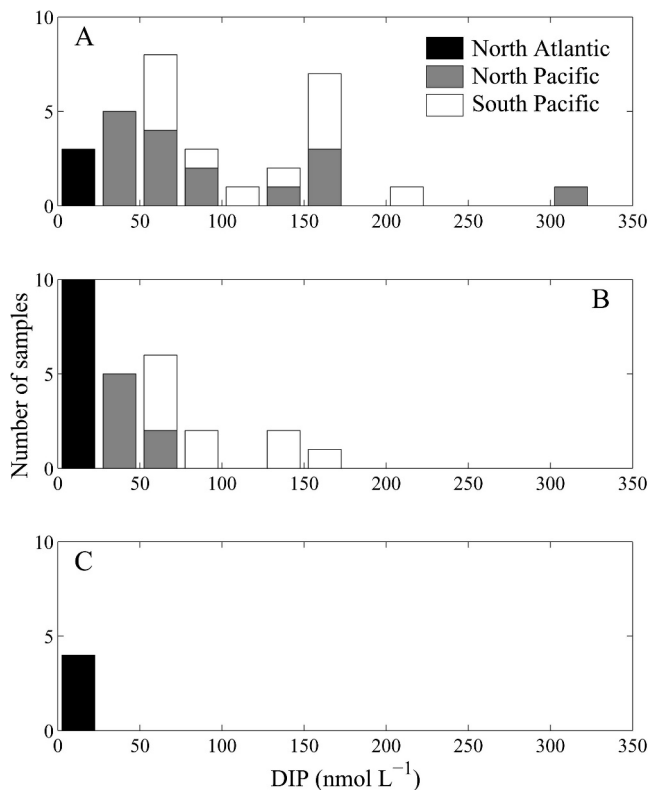


Fig. 5. Histogram of binned surface DIP concentrations ( $\text{nmol L}^{-1}$ ) separated by ELF label ratings: (A) '– –', (B) '+ –', (C) '+ +'. Colors represent ocean basin: North Atlantic (black), North Pacific (gray), and South Pacific (white). Bin width is 25  $\text{nmol L}^{-1}$ .

Atlantic populations were generally more P-stressed than those in the Pacific.

In addition to AP activity, indicators used to determine P stress or potential limitation include DIP concentrations, maximum uptake rate of  $\text{PO}_4^{3-}$  ( $V_{max}$ ), cellular N:P ratios, and turnover times of DIP. Results from these metrics used in the tropical and subtropical Atlantic and Pacific Oceans are consistent with our observations of AP activity. The critical concentration of DIP for *Trichodesmium* growth has been determined to be 9  $\text{nmol L}^{-1}$  (Moutin et al. 2005). Although there is some overlap, Atlantic DIP concentrations are typically lower than this critical value while Pacific concentrations are typically higher (Table 3; Wu et al. 2000). The  $V_{max}$  of  $\text{PO}_4^{3-}$  uptake by *Trichodesmium* colonies measured during a cruise in the North Atlantic was four times more than that of the North Pacific during cruise MP09 (Sohm et al. 2008). Concentrations of DIP in the Sargasso Sea region of the Atlantic are one to two orders of magnitude lower than those in the subtropical North Pacific (Wu et al. 2000). Molar ratios of cellular N:P may indicate nutrient limitation and/or storage. The reference Redfield ratio of N:P is 16. The P limitation of growth in *Trichodesmium* begins at N:P of 40–50 (Kustka et al. 2003), and in P-restricted cultures, N:P can reach  $\geq 90$  (White et al. 2006). *Trichodesmium* N:P ratios averaged  $40.1 \pm 2.53$  during the North Pacific MP09 cruise while they ranged from 60 in the west to 30 in the

Table 3. Comparison of the North Pacific, western South Pacific, and western North Atlantic (an overview of parameters in each basin, not a comprehensive regional survey).

Measurement	N. Pacific†	Refs*	S. Pacific†	Refs*	N. Atlantic†	Refs*
Dissolved inorganic phosphate (nmol L <sup>-1</sup> )						
Transect	100–324 <sup>a</sup> ; 26–108 <sup>b</sup>	1; 2	131–219 <sup>a</sup> ; 61–103 <sup>a,c</sup>	1; 1	BDL–25	1
Published values	3.0–191.3 <sup>d</sup>	3	80–218	4	0.2–1.0	5
Enzyme-labeled fluorescence	–, +, –	1	–, +, –	1	–, +, –, ++	1
Alkaline phosphatase activity (nmol MUF-P hydrolyzed (μg Chl a) <sup>-1</sup> h <sup>-1</sup> )	0.01–2.09 <sup>b,e</sup>	6	0.65–13.1 <sup>f</sup>	7	30–240 <sup>g</sup> ; 3.1–10.3	7; 8
<i>Trichodesmium</i> N:P	17–93; 34–40	9; 10	11–47 <sup>f</sup>	10	22–103; 60	9; 10
Surface nitrogenase activity (nmol C <sub>2</sub> H <sub>2</sub> colony <sup>-1</sup> h <sup>-1</sup> )	0.14–0.46 <sup>b</sup>	11	0.01–0.25	1	0.40–1.30	1
N <sub>2</sub> fixation, areal depth-integrated estimated from acetylene reduction (mmol N m <sup>-2</sup> yr <sup>-1</sup> )	31 ± 18	12			87.2 ± 13.9 <sup>g,h</sup>	13

\* 1 = This study; 2 = K. Björkman pers. comm.; 3 = Hawaii Ocean Time-series; 4 = Campbell et al. (2005); 5 = Wu et al. (2000); 6 = J. Sohm pers. comm.; 7 = Mulholland et al. (2002); 8 = Sohm and Capone (2006); 9 = White et al. (2006); 10 = Krauk et al. (2006); 11 = D. Capone pers. comm.; 12 = Karl et al. (1997); 13 = Capone et al. (2005).

† <sup>a</sup> = KM0701 data; <sup>b</sup> = MP09 data; <sup>c</sup> = observations east of Australia; <sup>d</sup> = HOT low-level phosphate data, December 1988–December 2004, 0–50 m, calculated assuming water density of 1.025 kg L<sup>-1</sup>; <sup>e</sup> = data not normalized to (MUF-P) as reported in Sohm et al. (2008); <sup>f</sup> = observations north of Australia; <sup>g</sup> = observations in western North Atlantic near the Caribbean; <sup>h</sup> = calculated from reported average areal depth integrated rate of 239 ± 38 μmol N m<sup>-2</sup> d<sup>-1</sup>.

east in the subtropical North Atlantic in 2000, indicating more P limitation in the western North Atlantic (Krauk et al. 2006). Turnover time is calculated as the inventory of a nutrient divided by its uptake rate and can be considered as the period it would take to deplete a nutrient if input was halted (Ammerman et al. 2003). Short turnover times of DIP ( $\tau_{DIP}$ ) result from low inventory and/or high uptake rates and can indicate low DIP availability. At the Climax station in the North Pacific,  $\tau_{DIP}$  was 48 h in the summer (Björkman et al. 2000). In contrast, Sargasso Sea  $\tau_{DIP}$  was measured to be around 9 h (Cotner et al. 1997). Turnover times of DIP in the South Pacific near New Caledonia varied widely and decreased from 468 h in the winter to 4 h

in the early autumn, indicating P depletion after the stratification of the water column in the summer (Van Den Broeck et al. 2004). These P stress indicators are consistent with higher potential for P limitation in the subtropical western North Atlantic than the subtropical North and South Pacific with a region of summer P limitation in the South Pacific near New Caledonia.

Colonies used in the ELF assay were chosen in an attempt to represent the community, but a number of issues arise because of the small sample size. Field populations of *Trichodesmium* have a range of characteristics including nutrient history, species, and degree of senescence that might not be reflected in two colonies per morphology per

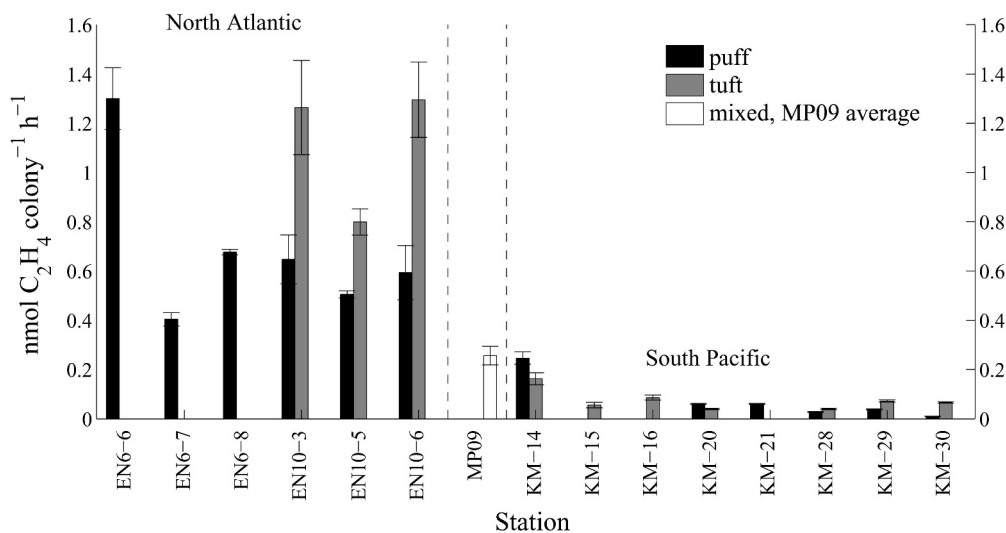


Fig. 6. Bar plot of nitrogenase activity (nmol C<sub>2</sub>H<sub>4</sub> produced colony<sup>-1</sup> h<sup>-1</sup>) for each station. Error bars show standard error of the mean. Data are separated by puffs (black), tufts (gray), or mixed puffs and tufts (white). The North Pacific is represented by the average rates for MP09 Sta. 14-22 (D. Capone pers. comm.). Ocean basins are separated by vertical dotted lines.

station. ELF labeling varies within a sample, a colony, or even along a filament. Although colonies of the same morphology were targeted, filaments observed on the ELF slides were from a variety of *Trichodesmium* species based on cell width and length. Samples also displayed a range of phycobiliprotein autofluorescence. The dim filaments observed may be due to senescent colonies, bleaching, or fading. In some cases, labeling on a slide was higher on dim filaments than normal filaments, which may indicate elevated AP activity in senescent colonies or in heterotrophic bacteria associated with these colonies in some cases. Although the colonies picked for the ELF assay may not capture the diversity of field populations, they do give useful snapshots of the state of the community.

A variety of organisms including bacteria, fungi, diatoms, dinoflagellates, chrysophytes, ciliates, amoebae, hydroids, and crustacean larvae can be enriched by 2–5 orders of magnitude in association with *Trichodesmium* colonies (Sheridan et al. 2002). Hydrolysis rates measured on *Trichodesmium* colonies may reflect the activity of the whole community rather than *Trichodesmium*-specific activity (Dyhrman et al. 2002; Webb et al. 2007). The activity of AP from associated organisms such as pennate diatoms, heterotrophic bacteria, and *Plectonema* may result in an indirect source of DIP that can be utilized by *Trichodesmium*. The presence of AP in these microorganisms may also result in the overestimation of *Trichodesmium* AP activity measured through bulk assays. With ELF, AP activity can be visually localized to specific cells.

In addition to AP measurements,  $N_2$  fixation rates attributed to *Trichodesmium* may be influenced by the diazotrophs tentatively identified as *Plectonema* sp. and *Calothrix* sp. found in close association with *Trichodesmium* colonies. Both of these cyanobacteria occupy a wide variety of habitats, including marine. The long, thin filaments of nonheterocystous *Plectonema* are found in *Trichodesmium* colonies around the world (Siddiqui et al. 1992). *Calothrix* is identified by its terminal heterocyst and tapering trichome (Waterbury 2006), and is found in the upper intertidal zone (Whitton and Potts 1982) and as an epiphyte of the pelagic diatom *Chaetoceros* (Foster and Zehr 2006). Although *Plectonema* is known to fix  $N_2$  only in lowered oxygen tension (Rippka et al. 1979), both *Plectonema* and *Calothrix* may be contributing to  $N_2$  fixation rates measured from *Trichodesmium* colonies.

During the four cruises analyzed in this study, *Trichodesmium* colony-based  $N_2$  fixation rates were significantly higher in the subtropical North Atlantic than in the tropical and subtropical North and South Pacific. North Atlantic values averaged  $0.80 \pm 0.12$  nmol  $C_2H_4$   $h^{-1}$  colony $^{-1}$  while Pacific values averaged  $0.13 \pm 0.02$  nmol  $C_2H_4$   $h^{-1}$  colony $^{-1}$ . As in Webb et al. (2007), tuft colonies generally had higher per colony rates than puffs; however, the difference between colony morphologies was not significant in our study with one exception. For comparison, literature values of  $N_2$  fixation rates measured by acetylene reduction averaged  $87 \pm 14$  mmol N  $m^{-2}$   $yr^{-1}$  in the western North Atlantic from 1994 to 2003 ( $1.6 \times 10^{12}$  mol N  $yr^{-1}$  over  $17.8 \times 10^6$  km $^2$ ; Capone et al. 2005), while the North Pacific averaged  $31 \pm 18$  mmol N  $m^{-2}$   $yr^{-1}$  from 1990 to

1992 (Karl et al. 1997). Biogeochemical estimates of the annual input of new N by  $N_2$  fixation were  $2.0 \times 10^{12}$  mol N  $yr^{-1}$ , or 72 mmol N  $m^{-2}$   $yr^{-1}$  in the subtropical North Atlantic (Gruber and Sarmiento 1997) and  $4 \pm 1 \times 10^{12}$  mol N  $yr^{-1}$ , or  $39 \pm 9$  mmol N  $m^{-2}$   $yr^{-1}$  in the tropical and subtropical Pacific (Deutsch et al. 2001). In a global biogeochemical elemental cycling ocean model,  $N_2$  fixation rates averaged 18 mmol N  $m^{-2}$   $yr^{-1}$  in the tropical and subtropical Atlantic and 11 mmol N  $m^{-2}$   $yr^{-1}$  in the tropical and subtropical Pacific (Moore and Doney 2007). Our nitrogenase activity measurements are in congruence with the pattern found in these estimates;  $N_2$  fixation rates are higher in the tropical and subtropical North Atlantic than in the tropical and subtropical Pacific.

The difference in  $N_2$  fixation rates between the Atlantic and Pacific Oceans may be due to disparate nutrient limitation regimes.  $N_2$  fixation in the western North Atlantic is more P-limited than Fe-limited (Wu et al. 2000; Sañudo-Wilhelmy et al. 2001; Moore et al. 2004). When *Trichodesmium* cells have met their Fe cell quota,  $N_2$  fixation correlates with cellular P quotas (Sañudo-Wilhelmy et al. 2001). In the western Equatorial and South Atlantic,  $N_2$  fixation in shipboard incubations was higher when P stress was relieved by the addition of DIP than in control or Fe addition incubations (Webb et al. 2007). *Trichodesmium* AP activity is higher in the North Atlantic than in the Pacific Ocean (Table 3; Mulholland et al. 2002; Sohm and Capone 2006; Sohm et al. 2008), and this difference is reflected by the visualization of AP activity through ELF (Table 3; Figs. 1, 3).

In contrast,  $N_2$  fixation in the North Pacific may be limited by factors other than P. The North Pacific receives less Fe-rich dust input than the North Atlantic (Fung et al. 2000), and Fe is necessary for *Trichodesmium* growth and the enzyme nitrogenase used in  $N_2$  fixation. In the South Pacific, regions near New Caledonia and Australia receive Fe from continental margins and Australian dust (Moore and Braucher 2008). Fe stimulates the growth of diazotrophs, drawing down P (Karl 2002; Mather et al. 2008), so Fe-rich areas such as the western North Atlantic and western South Pacific near Australia are more likely to be P-limited. In a coupled biogeochemistry ecosystem circulation model, diazotrophs were limited by P in the Atlantic and by Fe in the Pacific, and increasing Fe inputs in the model increased  $N_2$  fixation in the tropics and subtropics (Moore et al. 2004). These data suggest that  $N_2$  fixation rates of *Trichodesmium* in the western North Atlantic and in the South Pacific near Australia are limited by P while the Pacific subtropical gyres may be limited by Fe or co-limited by Fe and P.

Although P limitation may be alleviated by the use of DOP by Atlantic *Trichodesmium*, Fe limitation in Pacific populations cannot be alleviated by the use of an alternative pool (Webb et al. 2007; Mather et al. 2008). In a comparison of the North and South Atlantic, DIP released from AP activity in the P-stressed North Atlantic supported up to 30% of primary production and carbon fixation rates there were comparable to rates in the P-replete South Atlantic (Mather et al. 2008). Fe may be the proximal limiting nutrient according to Leibig's law while P

may be the ultimate limiting nutrient, controlling productivity over long timescales (Tyrrell 1999). Additional assessments of *Trichodesmium* P and Fe physiology in open-ocean gyres are necessary to elucidate nutrient controls on N<sub>2</sub> fixation, but our study supports previous observations that the North Atlantic is P-limited while the Pacific is likely to be Fe-limited. This study underscores the contrasting importance of P (and likely Fe) to the activity of the key diazotroph *Trichodesmium* in different ocean basins.

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#### References

- AMMERMAN, J. W., R. R. HOOD, D. A. CASE, AND J. B. COTNER. 2003. Phosphorus deficiency in the Atlantic: An emerging paradigm in oceanography. *EOS* **84**: 165–170.
- BERMAN-FRANK, I., J. T. CULLEN, Y. SHAKED, R. M. SHERRELL, AND P. G. FALKOWSKI. 2001. Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*. *Limnol. Oceanogr.* **46**: 1249–1260.
- BJÖRKMAN, K., A. L. THOMSON-BULLDIS, AND D. M. KARL. 2000. Phosphorus dynamics in the North Pacific subtropical gyre. *Aquat. Microb. Ecol.* **22**: 185–198.
- BREITBARTH, E., M. M. MILLS, G. FRIEDRICH, AND J. LAROCHE. 2004. The Bunsen gas solubility coefficient of ethylene as a function of temperature and salinity and its importance for nitrogen fixation assays. *Limnol. Oceanogr. Methods* **2**: 282–288.
- CAMPBELL, L., E. J. CARPENTER, J. P. MONTOYA, A. B. KUSTKA, AND D. G. CAPONE. 2005. Picoplankton community structure within and outside a *Trichodesmium* bloom in the southwestern Pacific Ocean. *Vie Milieu* **55**: 185–195.
- CAPONE, D. G., AND OTHERS. 2005. Nitrogen fixation by *Trichodesmium* spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean. *Glob. Biogeochem. Cycles* **19**: GB2024, doi:10.1029/2004GB002331.
- , AND J. P. MONTOYA. 2001. Nitrogen fixation and denitrification. In J. Paul [ed.], *Methods in marine microbiology*. *Methods Microbiol.* **30**, Academic.
- , J. ZEHR, H. PAERL, B. BERGMAN, AND E. J. CARPENTER. 1997. *Trichodesmium*: A globally significant marine cyanobacterium. *Science* **276**: 1221–1229.
- COLES, V. J., R. R. HOOD, M. PASCUAL, AND D. G. CAPONE. 2004. Modeling the impact of *Trichodesmium* and nitrogen fixation in the Atlantic Ocean. *J. Geophys. Res.* **109**: C06007, doi:10.1029/2002JC001754.
- COTNER, J. B., J. W. AMMERMAN, E. R. PEELE, AND E. BENTZEN. 1997. Phosphorus-limited bacterioplankton growth in the Sargasso Sea. *Aquat. Microb. Ecol.* **13**: 141–149.
- DEUTSCH, C., N. GRUBER, R. M. KEY, AND J. L. SARMIENTO. 2001. Denitrification and N<sub>2</sub> fixation in the Pacific Ocean. *Glob. Biogeochem. Cycles* **15**: 483–506.
- DUGDALE, R. C., AND J. J. GOERING. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. *Limnol. Oceanogr.* **12**: 196–206.
- DYHRMAN, S. T., E. A. WEBB, D. A. ANDERSON, J. W. MOFFETT, AND J. B. WATERBURY. 2002. Cell-specific detection of phosphorus stress in *Trichodesmium* from the Western North Atlantic. *Limnol. Oceanogr.* **47**: 1832–1836.
- EPPLEY, R. W., AND B. J. PETERSON. 1979. Particulate organic matter flux and planktonic new production in the deep ocean. *Nature* **282**: 677–680.
- FOSTER, R. A., AND J. P. ZEHR. 2006. Characterization of diatom–cyanobacteria symbioses on the basis of *nifH*, *hetR* and 16S rRNA sequences. *Environ. Microbiol.* **8**: 1913–1925, doi:10.1111/j.1462-2920.2006.01068.x.
- FUNG, I. Y., S. K. MEYN, I. TEGEN, S. C. DONEY, J. G. JOHN, AND J. K. B. BISHOP. 2000. Iron supply and demand in the upper ocean. *Glob. Biogeochem. Cycles* **14**: 281–295.
- GONZÁLEZ-GIL, S., B. A. KEAFER, R. V. M. JOVINE, A. AGUILERA, S. LU, AND D. M. ANDERSON. 1998. Detection and quantification of alkaline phosphatase in single cells of phosphorus-starved marine phytoplankton. *Mar. Ecol. Prog. Ser.* **164**: 21–35.
- GRUBER, N., AND J. SARMIENTO. 1997. Global patterns of marine nitrogen fixation and denitrification. *Glob. Biogeochem. Cycles* **11**: 235–266.
- HOOD, R. R., A. M. MICHAELS, AND D. G. CAPONE. 2000. Answers sought to the enigma of marine nitrogen fixation. *EOS Trans. Am. Geophys. Union* **81**: 133, 138–139.
- KARL, D., R. LETELIER, L. TUPAS, J. DORE, J. CHRISTIAN, AND D. HEBEL. 1997. The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. *Nature* **388**: 533–538.
- , AND OTHERS. 2002. Dinitrogen fixation in the world's oceans. *Biogeochem.* **57–58**: 47–98.
- KARL, D. M. 2002. Nutrient dynamics in the deep blue sea. *Trends Microbiol.* **10**: 410–418.
- KRAUK, J. M., T. A. VILLAREAL, J. A. SOHM, J. P. MONTOYA, AND D. G. CAPONE. 2006. Plasticity of N:P ratios in laboratory and field populations of *Trichodesmium* spp. *Aquat. Microb. Ecol.* **42**: 243–253.
- KUSTKA, A. B., S. A. SAÑUDO-WILHELMI, E. J. CARPENTER, D. CAPONE, J. BURNS, AND W. G. SUNDA. 2003. Iron requirements for dinitrogen- and ammonium-supported growth in cultures of *Trichodesmium* (IMS101): Comparison with nitrogen fixation rates and iron:carbon ratios of field populations. *Limnol. Oceanogr.* **48**: 1869–1884.
- MATHER, R. L., AND OTHERS. 2008. Phosphorus cycling in the North and South Atlantic Ocean subtropical gyre. *Nat. Geosci.* **1**: 439–443.
- MILLS, M., C. RIDAME, M. DAVEY, J. LA ROCHE, AND R. J. GEIDER. 2004. Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature* **429**: 292–294.

- MOORE, J. K., AND O. BRAUCHER. 2008. Sedimentary and mineral dust sources of dissolved iron to the world ocean. *Biogeosci.* **5**: 631–656.
- , AND S. C. DONEY. 2007. Iron availability limits the ocean nitrogen inventory stabilizing feedbacks between marine denitrification and nitrogen fixation. 2007. *Glob. Biogeochem. Cycles* **21**: GB2001, doi:10.1029/2006GB002762.
- , ———, AND K. LINDSAY. 2004. Upper ocean ecosystem dynamics and iron cycling in a global three-dimensional model. *Glob. Biogeochem. Cycles* **18**: GB4028, doi:10.1029/2004GB002220.
- MOUTIN, T., N. VAN DEN BROECK, B. BEKER, C. DUPOUY, P. RIMMELIN, AND A. LE BOUTELLER. 2005. Phosphate availability controls *Trichodesmium* spp. biomass in the SW Pacific Ocean. *Mar. Ecol. Prog. Ser.* **297**: 15–21.
- MULHOLLAND, M. R., S. FLOGE, E. J. CARPENTER, AND D. G. CAPONE. 2002. Phosphorus dynamics in cultures and natural populations of *Trichodesmium* spp. *Mar. Ecol. Prog. Ser.* **239**: 45–55.
- RIPPKA, R., J. DERUELLES, J. B. WATERBURY, M. HERDMAN, AND R. Y. STANIER. 1979. Generic assignments, strain histories, and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* **111**: 1–61.
- SAÑUDO-WILHELMY, S. A., AND OTHERS. 2001. Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean. *Nature* **411**: 66–69.
- SHERIDAN, C. C., D. K. STEINBERG, AND G. W. KLING. 2002. The microbial and metazoan community associated with colonies of *Trichodesmium* spp. : A quantitative survey. *J. Plankton Res.* **24**: 913–922.
- SIDDIQUI, P. J. A., B. BERGMAN, AND E. J. CARPENTER. 1992. Filamentous cyanobacterial associates of the marine planktonic cyanobacterium *Trichodesmium*. *Phycologia* **31**: 326–337.
- SOHM, J. A., AND D. G. CAPONE. 2006. Phosphorus dynamics of the tropical and subtropical north Atlantic: *Trichodesmium* spp. versus bulk plankton. *Mar. Ecol. Prog. Ser.* **317**: 21–28.
- , C. MAHAFFEY, AND D. G. CAPONE. 2008. Assessment of relative phosphorus limitation of *Trichodesmium* spp. in the North Pacific, North Atlantic, and the north coast of Australia. *Limnol. Oceanogr.* **53**: 2495–2502.
- STIHL, A., U. SOMMER, AND A. F. POST. 2001. Alkaline phosphatase activities among populations of the colony-forming diazotrophic cyanobacterium *Trichodesmium* spp. (Cyanobacteria) in the Red Sea. *J. Phycol.* **37**: 310–317.
- TYRRELL, T. 1999. The relative influences of nitrogen and phosphorus on oceanic primary production. *Nature* **400**: 525–531.
- VAN DEN BROECK, N., T. MOUTIN, M. RODIER, AND A. LE BOUTELLER. 2004. Season variations of phosphate availability in the SW Pacific Ocean near New Caledonia. *Mar. Ecol. Prog. Ser.* **268**: 1–12.
- WATERBURY, J. B. 2006. The cyanobacteria— isolation, purification and identification, *In* M. Dworkin, S. Falkow, E. Rosenberg, K. Schleifer and E. Stackebrandt [eds.], *The prokaryotes*, 3rd ed. Springer, doi:10.1007/0-387-30744-3-38.
- WEBB, E. A., R. WISNIEWSKI JAKUBA, J. W. MOFFETT, AND S. T. DYHRMAN. 2007. Molecular assessment of phosphorus and iron physiology in *Trichodesmium* populations from the western Central and western South Atlantic. *Limnol. Oceanogr.* **52**: 2221–2232.
- WHITE, A. E., Y. H. SPITZ, D. M. KARL, AND R. M. LETELIER. 2006. Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications. *Limnol. Oceanogr.* **51**: 1777–1790.
- WHITTON, B. A., AND M. POTTS. 1982. Marine littoral, *In* N. G. Carr and B. A. Whitton [eds.], *The biology of cyanobacteria*. Bot. Monogr. **19**, Univ. California Press.
- WU, J., W. SUNDA, E. A. BOYLE, AND D. M. KARL. 2000. Phosphate depletion in the western North Atlantic Ocean. *Science* **289**: 759–762.

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