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Assessment of relative phosphorus limitation of *Trichodesmium* spp. in the North Pacific, North Atlantic, and the north coast of Australia

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Abstract

Trichodesmium spp. is a colonial diazotrophic cyanobacterium that occurs in the oligotrophic tropics and subtropics. Because of its ability to fix atmospheric N_2, it is likely to be growth limited by P or Fe, and it has been hypothesized that limitation differs among different ocean basins. Two assays used as indices of P limitation or stress in *Trichodesmium* spp. are uptake of $^{33}PO_4^{3-}$ to determine maximal P uptake (V_{max}) and hydrolysis of P from methylumbelliferone phosphate to estimate alkaline phosphatase activity (APA). The kinetics of PO_4^{3-} uptake were determined for *Trichodesmium* spp. colonies in the North Pacific, North Atlantic, and in waters north of Australia, whereas APA was determined in the North Pacific and North Atlantic. *Trichodesmium* spp. V_{max} was significantly greater (~fourfold or more) in the North Atlantic compared with the North Pacific and waters north of Australia when normalized to both chlorophyll *a* content and number of trichomes per colony. APA in the North Atlantic was also greater than in the North Pacific. The half-saturation constant for PO_4^{3-} uptake (K_s) was not significantly different among the three locations. These data indicate that *Trichodesmium* spp. is more strongly P limited in the North Atlantic compared with the North Pacific or waters along the north coast of Australia. We suggest that the *Trichodesmium* spp. communities in the North Pacific and waters north of Australia are primarily Fe rather than P stressed and that these differences reflect differing relative inputs and availability of two major controlling variables for diazotrophy, P and Fe, in these geographically divergent areas.

N_2 fixation is now recognized as an important source of new N in the tropical oligotrophic ocean and comparable with the diffusive flux of nitrate from below the photic zone in those systems (Capone et al. 2005). However, where the upward flux of nitrate co-occurs with carbon dioxide (CO_2) at Redfield proportions, that nitrate cannot locally support a net flux of CO_2 from atmosphere to ocean (Eppley and Peterson 1979; Michaels et al. 2001). Atmospheric input of N or in situ N_2 fixation are additions of N without concurrent CO_2 and are required to promote positive C sequestration over a nitrate-supported baseline. N_2 fixation in the ocean is thus inextricably linked to the C cycle and possibly even climate change (Michaels et al. 2001). Understanding what controls N_2 fixation in the open ocean is a vital part of understanding C cycling in the past, present, and future ocean.

N has long been considered the primary limiting nutrient throughout the open ocean (Thomas 1966). Diazotrophs avoid this limitation by exploiting the largest N reservoir on the planet, N_2 gas, through the process of N_2 fixation. However, there is no analog of this process for P. This led to the conclusion that P is the ultimate limiting nutrient of marine primary production, as any N deficiencies are made up by N_2 fixation (Redfield 1958). The assumption that follows is that marine N_2 fixation is a P-limited process. Alternatively, Fe may constrain diazotroph growth, as diazotrophs have a higher Fe requirement than other phytoplankton (Raven 1988; Kustka et al. 2003*b*), and Fe concentrations are generally low in the upper ocean (Wu et al. 2001). This study focuses on the role of P in limiting open-ocean diazotrophs.

The colony-forming, diazotrophic cyanobacterium *Trichodesmium* spp. is cosmopolitan in tropical and subtropical waters and contributes a substantial amount of new N to the areas where it occurs (Capone et al. 2005). Thus, the study of this one organism is important to our understanding of open-ocean N_2 fixation. Knowledge of *Trichodesmium* spp. susceptibility to P limitation or stress in different ocean basins will improve our understanding of its ability to contribute new N to the open ocean.

Common indices used to diagnose P stress in phytoplankton are PO_4^{3-} uptake kinetics and the activity of alkaline phosphatase—an enzyme that cleaves PO_4^{3-} from organic P molecules. Many algal species respond to P stress by altering PO_4^{3-} uptake kinetics and alkaline phosphatase activity (APA, Donald et al. 1997), and these measures can be used to infer relative P limitation. Recently, both maximal PO_4^{3-} uptake (V_{max}, Fu et al. 2005) and APA (Mulholland et al. 2002) were shown to increase with P depletion in *Trichodesmium* IMS 101 cultures. On the basis of these culture studies, both V_{max} and APA can be used to compare relative P limitation or stress in field populations of *Trichodesmium* spp.

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We undertook our study in three distinct regions predicted to have different susceptibility to P limitation: the subtropical North Pacific where both P and Fe limitation are hypothesized (Karl et al. 1997; Wu et al. 2000), the tropical North Atlantic where P limitation of diazotrophs has been shown (Sañudo-Wilhelmy et al. 2001), and the north coast of Australia, where P concentrations are generally higher than the two other areas. PO_4^{3-} uptake kinetics and APA measurements were made to assess the P stress of *Trichodesmium* spp. colonies in these diverse locations. An additional set of experiments was carried out to assess the diel periodicity and light dependence of *Trichodesmium* spp. PO_4^{3-} uptake in the field.

Materials and methods

Study sites—Data for this study were collected on three separate research cruises (Fig. 1). The first cruise was in November 1999 aboard the RV *Maurice Ewing* off the northern coast of Australia, where *Trichodesmium erythraeum* was the predominant species (E. J. Carpenter pers. comm.). The second cruise was from April to May 2003 in the tropical North Atlantic, in and around the Amazon River plume, aboard the RV *Seward Johnson*. *Trichodesmium* spp. is routinely found along the edges of this plume, presumably because nutrients are slightly enriched compared with the oceanic waters farther from the plume (A. Subramanium pers. comm.). The third cruise took place from July to August 2003 in the subtropical North Pacific, specifically the area south of the Hawaiian Islands, on the RV *Roger Revelle*. *Trichodesmium thebautii* was the dominant species on the North Atlantic and Pacific cruises (E. J. Carpenter pers. comm.). $^{33}PO_4^{3-}$ uptake was measured on all three cruises. APA measurements from the Pacific and Atlantic cruises are presented in this study, whereas APA measurements from north of Australia were previously presented in Mulholland et al. (2002). Because we encountered a different predominant *Trichodesmium* species in Australia, we assume here that there are not species-specific differences in PO_4^{3-} uptake and APA. Fu et al. (2005) showed that uptake kinetics for PO_4^{3-} in two different clonal isolates of *Trichodesmium erythraeum* were the same when size was considered, but differences between species for these parameters have yet to be studied. To account for differences in colonies between the three sites, kinetic and APA data are normalized to both colony chlorophyll content and trichome number (E. J. Carpenter pers. comm.). Additionally, because *Trichodesmium* spp. colonies support a community of heterotrophic bacteria, cyanobacteria, and other phytoplankton (O'Neil and Roman 1992), kinetic and APA measurements will include contributions from these organisms. It is possible that these organisms play a role in nutrient acquisition and cycling within the *Trichodesmium* spp. colony. However, the total volume of bacteria, diatoms, and dinoflagellates found on colonies collected in the North Atlantic was less than 6% of the volume of the colonies themselves (calculated from data found in Sheridan et al. [2002] and assuming average diameters of 0.5, 10, and 10 μm of bacteria, diatoms, and dinoflagellates, respectively). We believe that a *Trichodesmium* spp. biovolume of nearly 95% supports the assumption we make here that the kinetic and APA responses are primarily due to *Trichodesmium* spp.

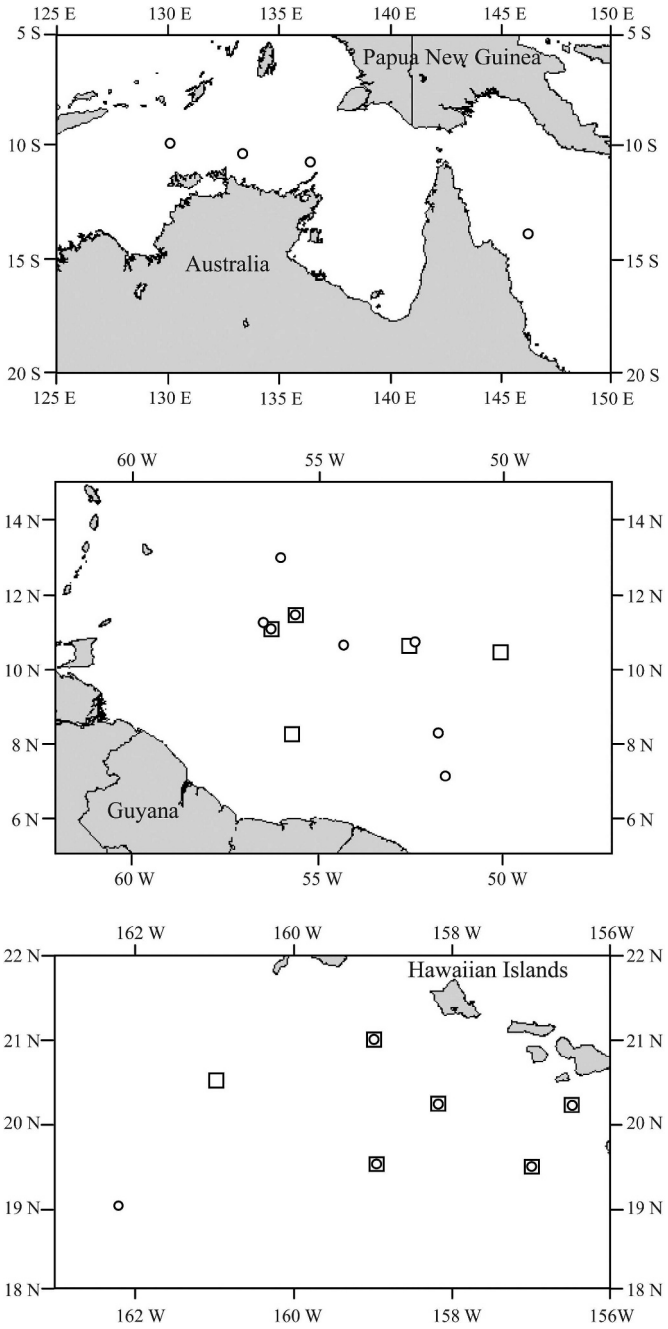

Fig. 1. Location of stations where V_{max} (circles) and APA (squares) were measured on the three cruises in this study.

Sample collection—Colonies of *Trichodesmium* spp. were collected using either a 1-m, 202-μm mesh plankton net at a depth of 15–20 m or a 0.25-m, 64-μm plankton net towed at the surface. Colonies were picked out of the tow with plastic inoculating loops and placed in GF/F filtered seawater (FSW; nominal pore size 0.7 μm) to rinse. Nutrient contamination was guarded against by using

clean supplies, but trace metal clean techniques were not used in this study.

Chlorophyll a concentrations and trichome counts—*Trichodesmium* spp. chlorophyll *a* (Chl *a*) content was determined with triplicate samples by placing 10 colonies into 10 mL of acetone, allowing 24 h for extraction, then reading the sample on a fluorometer set to detect Chl *a* and comparing it with a known standard.

The number of trichomes per colony (col) was determined in duplicate by shaking apart 10 colonies in GF/F FSW to break them into single trichomes. Trichomes were filtered onto a polycarbonate filter, and the entire filter was counted under an epifluorescent microscope.

Results from diel PO_4^{3-} uptake measurements are reported per colony, as neither Chl *a* nor trichome counts were determined at every time point. Chl *a* and trichome counts are assumed to be the same in each assay of the light vs. dark experiment, and are thus also reported per colony.

^{33}P uptake—Five to 10 rinsed colonies were placed in 50 mL of FSW in 60-mL polycarbonate bottles with 18.5–231 kBq of $H_3{}^{33}PO_4$, added as 10–20 μL of a working stock solution. All incubations were run in triplicate. Samples were incubated in 50% of surface light in on-deck incubators for 60–90 min, as time-course experiments at the beginning of each cruise indicated that $^{33}PO_4^{3-}$ uptake was linear for about the first 90 min. Experiments comparing light and dark PO_4^{3-} uptake were performed by incubating *Trichodesmium* spp. colonies in 50% surface light and in dark bottles in parallel. To measure diel P uptake, samples were collected by net tow and incubated every 2–3 h over the course of a day, beginning before sunrise and ending after sunset. To generate kinetic curves, 0.025–0.5 μmol L^{-1} K_2HPO_4 was added to incubations. V_{max} and K_s were found by fitting the Michaelis–Menten equation to each data set with regression analysis in Sigmaplot. The kinetics of nutrient uptake are described by the equation $V = (V_{max}S)/(K_s + S)$, where V is the uptake rate at nutrient concentration S (the soluble reactive phosphorus [SRP] concentration of surface water plus the concentration of cold K_2HPO_4 added), V_{max} is the maximal, or saturated, uptake rate, and K_s is the nutrient concentration when uptake is one-half V_{max}. SRP concentration data were provided by K. Björkman (pers. comm.).

Alkaline phosphatase activity—APA in *Trichodesmium* spp. and bulk water was determined using the method described by Ammerman (1993), which uses an organic molecule, methylumbelliferone (MUF), with a PO_4^{3-} attached (MUF-P) as a substrate for alkaline phosphatase. Alkaline phosphatase cleaves the PO_4^{3-} moiety from MUF-P, causing it to fluoresce. Twenty-five to 40 rinsed *Trichodesmium* spp. colonies were placed into 250 mL of unfiltered, bucket-collected surface seawater with 100 nmol L^{-1} (Atlantic) and 200 nmol L^{-1} (Pacific) MUF-P added. Samples were incubated in flowing seawater at 50% ambient light to simulate in situ conditions. The activity of seawater alone was also measured, and its activity subtracted from the activity of *Trichodesmium* spp. plus seawater to obtain activity of *Trichodesmium* spp. alone. Experiments were conducted in this manner because previous experiments have shown that filtering seawater increases activity over unfiltered water, possibly due to cell breakage and release of the phosphatase enzyme.

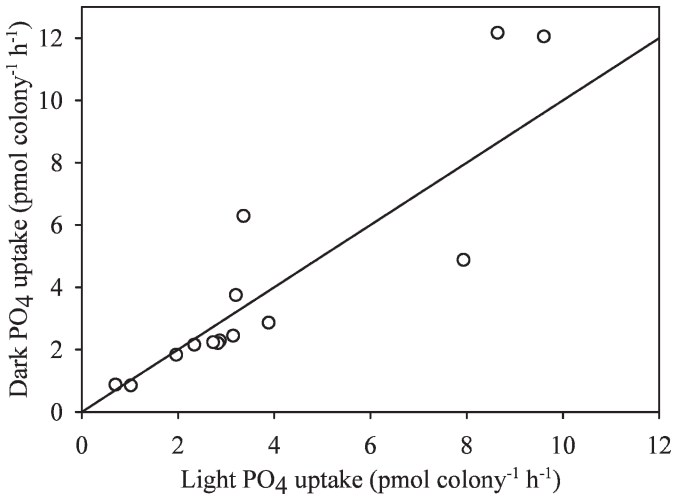

Fig. 2. PO_4^{3-} uptake in 50% of surface light vs. the dark for colonies collected in the north Atlantic. The 1 : 1 line is shown on the graph.

The increase in fluorescence in the incubations was measured every hour for 4 to 7 h by removing 3 mL of sample water and placing it into a glass test tube with 1 mL of 50 mmol L^{-1} borate buffer, pH 10.8. The buffer raises the pH above 10, where MUF becomes fluorescent. A Turner 10-AU fluorometer with a long WL oil lab filter kit was used to read the fluorescence. The slope of fluorescence vs. time is compared with a standard of MUF, made up at the same concentration as the substrate added. We report the turnover rate (h^{-1}) of MUF-P by calculating t = slope of fluorescence vs. time (fluorescence of the standard)$^{-1}$, then normalizing values to the biomass parameters Chl *a* content or trichome number. This removes any effect that higher substrate concentrations in the Pacific would have on the hydrolysis rate.

Results

Light vs. dark and diel P uptake—Unlike CO_2 and N_2 fixation in *Trichodesmium* spp., PO_4^{3-} uptake is not dependent on light or time of day. On any given day when light and dark PO_4^{3-} uptake were measured in the Atlantic, either of the treatments may have had higher uptake rates than the other (Fig. 2); however, a paired t-test of the entire data set shows there was no significant difference between the two ($p > 0.2$). When PO_4^{3-} uptake was measured over a diel cycle (three 1-d cycles determined), ambient uptake varied over the course of the day but no consistent pattern was observed (Fig. 3).

P uptake kinetics—Kinetic parameters of PO_4^{3-} uptake in *Trichodesmium* spp. were found for experiments where uptake was saturated or near saturated. There is fairly high variability in the data within the sample locations

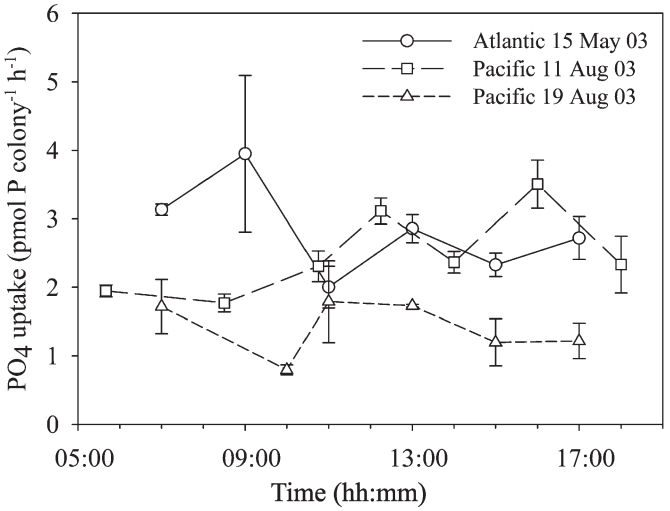

Fig. 3. Per-colony PO_4^{3-} uptake rate measured throughout the day (sunrise to sunset) in the north Atlantic and Pacific. Error bars show standard error of three replicates.

(Table 1); however, differences can be seen among locations. V_{max} in the tropical North Atlantic averaged 6.5 nmol P μg^{-1} Chl *a* h^{-1}, 5.4 and 3.8 times greater than the averages in the subtropical North Pacific and off the north coast of Australia, respectively (Table 2; Fig. 4). A two-tailed *t*-test showed that V_{max} in the North Atlantic was significantly different from both the North Pacific ($p = 0.008$) and north of Australia ($p = 0.016$), but there was no significant difference between the North Pacific and north of Australia ($p = 0.63$). When normalized to number of trichomes per colony, V_{max} is still significantly different when comparing the North Atlantic to the North Pacific (3.8-fold greater, $p = 0.029$), or to waters north of Australia (6.5-fold greater, $p = 0.013$) (Table 2) during the period of sampling.

Although differences were apparent in V_{max}, the half-saturation constant was not different among the three locations. K_s averaged 0.18 μmol L^{-1} in the Atlantic, 0.15 μmol L^{-1} in the Pacific, and 0.15 μmol L^{-1} north of Australia (Tables 1, 2). K_s was not significantly different for any of the comparisons ($p > 0.6$ for all comparisons).

Alkaline phosphatase activity—APA was also quite variable at each location (Table 1), but was higher in the North Atlantic than the North Pacific, on average, during the time period sampled. The turnover rate of MUF-P was 0.16 μg Chl a^{-1} h^{-1} in the North Atlantic, 4.4 times greater than in the North Pacific. APA was 2.6 times greater in the North Atlantic than the North Pacific when normalizing to

Table 1. V_{max}, APA, and K_s at stations in the North Atlantic, North Pacific, and north of Australia. Values are shown normalized to both Chl *a* and number of trichomes per colony.

Date	V_{max} (nmol P μg^{-1} Chl *a* h^{-1})	APA (μg Chl a^{-1} h^{-1})	V_{max} (pmol P trichome^{-1} h^{-1})	APA ($\times 10^{-6}$ trichome^{-1} h^{-1})	K_s (μmol L^{-1})
Atlantic					
22 Apr 03	2.6	–	0.25	–	−0.16
24 Apr 03	4.5	–	0.068	–	0.093
25 Apr 03	4.0	–	0.16	–	0.059
26 Apr 03	–	0.70	–	38	–
27 Apr 03	6.0	0.052	0.57	4.9	0.38
30 Apr 03	13.8	–	1.1	–	0.19
01 May 03	10.8	0.045	0.55	2.3	0.20
03 May 03	–	0.074	–	6.0	–
10 May 03	6.1	–	0.69	–	0.093
11 May 03	3.9	–	0.54	–	0.24
12 May 03	–	0.10	–	4.1	–
13 May 03	–	0.052	–	5.2	–
15 May 03	–	0.090	–	7.9	–
Pacific					
10 Aug 03	0.64	0.083	0.055	7.1	0.070
10 Aug 03	0.93	–	0.079	–	0.19
12 Aug 03	0.33	0.012	0.095	3.6	0.093
14 Aug 03	–	0.017	–	1.1	–
15 Aug 03	2.8	0.037	0.32	2.9	0.22
17 Aug 03	2.6	0.0054	0.21	0.69	0.28
18 Aug 03	0.18	0.062	0.023	7.2	0.053
Australia					
18 Nov 99	4.0	–	0.14	–	0.33
19 Nov 99	4.0	–	0.19	–	0.26
20 Nov 99	0.76	–	0.056	–	0.050
21 Nov 99	0.22	–	0.018	–	0.009
24 Nov 99	0.44	–	0.030	–	0.175
26 Nov 99	0.73	–	0.028	–	0.057

Table 2. Summary of PO_4^{3-} uptake kinetics and APA, surface nutrient concentrations, and *Trichodesmium* spp. colony information in the North Atlantic, North Pacific, and north of Australia shown with standard error in parentheses. A two-way *t*-test was performed for all comparisons and it is noted where values are significantly different.

	Atlantic	Pacific	Australia
V_{max} (nmol P μg^{-1} Chl *a* h^{-1})	6.5 (1.4)*	1.2 (0.5)	1.7 (0.7)
V_{max} (pmol P trichome^{-1} h^{-1})	0.50 (0.12)†	0.13 (0.05)	0.078 (0.029)
K_s (μmol L^{-1})	0.18 (0.04)	0.15 (0.04)	0.15 (0.05)
APA (μg Chl a^{-1} h^{-1})	0.16 (0.09)	0.036 (0.013)	0.008 (0.003)‡
APA ($\times 10^{-6}$ trichome^{-1} h^{-1})	9.7 (4.7)	3.8 (1.2)	–
SRP (μmol L^{-1})§	0.045 (0.005)‖	0.036 (0.006)¶	0.11 (0.02)
dFe (nmol L^{-1})#	2.4 (0.4)**	0.35 (0.05)	0.37 (0.06)
SRP : dFe	18.8	51–360	297
trichomes col^{-1}††	149 (26)	103 (19)‡‡	204 (7)
Chl *a* col^{-1}††	0.0095 (0.0014)	0.013 (0.004)	0.012 (0.002)
N_2 fixation (nmol N col^{-1} d^{-1})‡‡	0.093 (0.013)	0.12 (0.02)	0.15 (0.03)

* Significantly different from V_{max} in the Pacific ($p=0.008$) and Australia ($p=0.016$).
† Significantly different from V_{max} in the Pacific ($p=0.029$) and Australia ($p=0.013$).
‡ Data recalculated from Mulholland et al. 2002.
§ Atlantic and Pacific data from K. Björkman and Australia data from D. G. Capone (unpubl.).
‖ Significantly different from SRP in the Pacific ($p=0.016$) and Australia ($p=0.002$).
¶ Significantly different from SRP north of Australia ($p=0.002$).
# Data from S. Sanudo-Wilhelmy (Atlantic, pers. comm.), Brown et al. (2005; Pacific, 160–150°W, 22–28°N), and A. Kustka (Australia, pers. comm.)
** Significantly different from dFe in the Pacific ($p=0.003$) and Australia ($p=0.003$).
†† Data from E. J. Carpenter (pers. comm.).
‡‡ At 55% light level. Atlantic data from Capone et al. (2005). Pacific and Australia data from D.G. Capone (unpubl.)

number of trichomes per colony. However, neither of these comparisons showed a significant difference ($p > 0.2$).

Discussion

Light vs. dark and diel P uptake—CO_2 and N_2 fixation are both reductive processes and therefore energetically demanding. Both processes are light dependent in *Trichodesmium* spp. and show diel patterns that are externally forced (Berman-Frank et al. 2001; Capone et al. 2005), and both processes are dependent on energy generated by photosynthesis. This does not appear to be true for PO_4^{3-}, which does not undergo redox transformation during uptake and assimilation. In natural populations of *Trichodesmium* spp., uptake of PO_4^{3-} is neither dependent on the presence of light nor time of day. PO_4^{3-} is found at very low levels in the oligotrophic environments where *Trichodesmium* spp. occurs. Hence, an adaptive strategy would be to assimilate PO_4^{3-} whenever and wherever it is encountered.

Studies with cultured cyanobacterial species and field populations have yielded similar results to those reported here. Fu et al. (2005) showed that PO_4^{3-} uptake in two different *T. erythraeum* strains grown at the same light level was the same when placed in light or dark. Additionally, neither *Phormidium laminosum* (Prieto et al. 1997), a freshwater cyanobacterium, nor *Synechococcus* WH7803 (Donald et al. 1997), a marine cyanobacterium, showed a difference in PO_4^{3-} uptake in the light or dark. PO_4^{3-} uptake in samples from the North Pacific subtropical gyre (NPSG) showed no diel rhythm (Björkman et al. 2000).

P uptake kinetics—Kinetic parameters of PO_4^{3-} uptake varied widely both within and among sites at the time of sampling. However, V_{max} was four times greater or more in the North Atlantic than in the North Pacific or north of Australia when normalized to either Chl *a* content and trichome number. Increased V_{max} is a common response in phytoplankton to P stress. Fu et al. (2005) reported that V_{max} of PO_4^{3-} uptake in a culture of *Trichodesmium (erythraeum)* IMS 101 was 0.68 pmol P trichome^{-1} h^{-1} in P-deplete conditions (assuming 50 cells trichome^{-1}) and 0.12 pmol P trichome^{-1} h^{-1} when P replete, strikingly similar to our values of 0.50 pmol P trichome^{-1} h^{-1} in the North Atlantic and 0.13 and 0.077 pmol P trichome^{-1} h^{-1} in the North Pacific and north of Australia, respectively. Other species of laboratory cultures show similar results. V_{max} can increase by an order of magnitude or more in cultures of diatoms (Perry 1976; Donald et al. 1997) and cyanobacteria (Donald et al. 1997; Ikeya et al. 1997). These results strongly support the conclusion that field populations of *Trichodesmium* spp. are P stressed in the North Atlantic, but not in the North Pacific or along the north coast of Australia, at least at the time of sampling. Data collected on two subsequent cruises in the western North Atlantic during spring and summer months show that elevated V_{max} (and thus P stress) is a persistent phenomenon in *Trichodesmium* spp. there (Sohm and Capone 2006; J. Sohm unpubl.).

It is important to note that there can be differences between *Trichodesmium* spp. isolates in V_{max} of PO_4^{3-} uptake, but that these are largely a function of size differences (Fu et al. 2005). The predominant species we encountered north of Australia was *T. erythraeum*, whereas *T. thiebautii* dominated in both the North Atlantic and Pacific. The typical diameter of a trichome of *T. erythraeum* is 20 μm, whereas *T. thiebautii* are more variable and can range from 6 to 20 μm (Siddiqui et al. 1992). Therefore, the trichomes from the north of Australia were likely larger than trichomes from the other two locations. Despite their

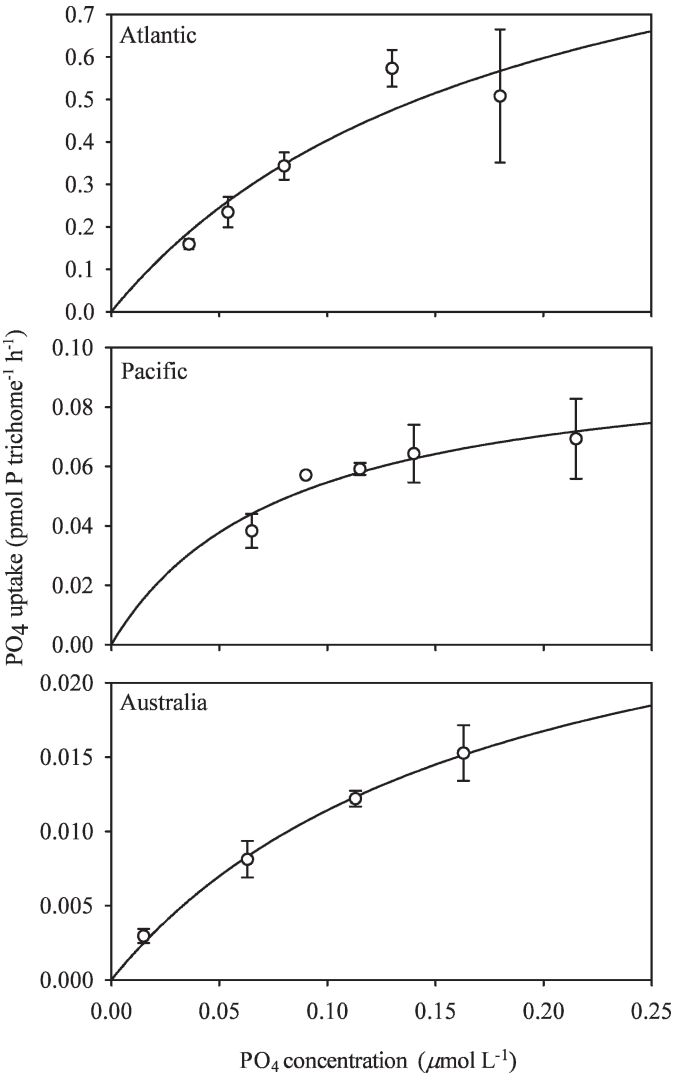

Fig. 4. Examples of PO_4^{3-} uptake kinetic curves from the North Atlantic, North Pacific, and waters north of Australia. Note that the scales on the *y*-axes are different. Error bars show standard error of three replicates.

size, trichome-normalized values in waters north of Australia were toward the lower end of values observed among the three sites, which would suggest that *Trichodesmium* spp. from these waters may be even less stressed than the data suggest. *Trichodesmium* spp. collected from the North Pacific and North Atlantic were dominated by the same species and so are more directly comparable. Thus, the species differences between *Trichodesmium* spp. from Australia and the North Atlantic and Pacific should not affect the overall conclusions of our research.

Another common kinetic response to P stress is a decrease in the half-saturation constant caused by the induction of a second, higher-affinity PO_4^{3-} transport system (Donald et al. 1997). However, not all species show this response. K_s decreased by an order of magnitude in two species of *Synechococcus* grown under P limitation (Donald et al. 1997; Ikeya et al. 1997), but was nearly the same in cultures of *Thalassiosira weisflogii* and *Emiliania huxleyi* grown in P-deplete and -replete conditions (Donald et al. 1997; Riegman et al. 2000). *T. erythraeum* cultures showed no difference in K_s under P-replete and -deplete conditions (Fu et al. 2005). K_s was not statistically significantly different among the three locations in our study either—locations that showed differential P stress in *Trichodesmium* spp. This suggests that *Trichodesmium* spp. does not possess a second, high-affinity PO_4^{3-} transporter that is induced by P stress.

Alkaline phosphatase activity—APA is a commonly used assay to assess P stress. The results from this study indicate that *Trichodesmium* colonies in the North Atlantic are more P stressed than colonies found in the North Pacific at the time of sampling. When combining these data with the maximal uptake rates found on the same cruises, the argument for P deficiency of *Trichodesmium* spp. in the North Atlantic compared with the North Pacific or north of Australia is even stronger. However, it does appear that V_{max} is a more sensitive indicator of P stress, as the difference in V_{max} between the North Atlantic and North Pacific was greater than the difference in APA. Other studies of APA in the Atlantic also suggest P stress there. Large areas of the Atlantic show P stress, as measured with APA, in bulk water samples (Vidal et al. 2003) and *Trichodesmium* spp. APA measured in the western tropical Atlantic near the Caribbean Sea is extremely high compared with Australia (two orders of magnitude; Mulholland et al. 2002), indicating *Trichodesmium* spp. in the Caribbean Sea may be even more stressed for P than in the areas in this study.

Nutrient limitation: Comparison with other studies—The results of this study indicate that P is an important nutrient controlling the distribution and potentially the activity (although not directly tested in this study) of *Trichodesmium* spp. in the North Atlantic, but not in the North Pacific or north of Australia. This agrees with other published assessments of open-ocean nutrient limitation. Krauk et al. (2006) used particulate N-to-P ratios of *Trichodesmium* spp. as a relative measure of P stress: higher N : P ratios in culture were related to SRP depletion. In the field, N : P ratios averaged 60 in the western North Atlantic, but 40 in the North Pacific and 22 north of Australia, showing that western North Atlantic *Trichodesmium* spp. was relatively more P stressed. (The North Pacific and Australia data were collected on the same cruises as in this study.) *Trichodesmium* spp. P quotas in the North Atlantic also positively correlate to N_2 fixation rates (Sañudo-Wilhelmy et al. 2001). Additionally, enzyme-labeled fluorescence of *Trichodesmium* spp. alkaline phosphatase shows that alkaline phosphatase is active in the North Atlantic (Dyhrman et al. 2002) but almost undetectable in the North Pacific (A. Hynes pers. comm.).

Other studies support the idea of P limitation in the North Atlantic. Water column nutrient data (Wu et al. 2000) and modeling efforts (Moore et al. 2004) both suggest that N_2 fixers should be P limited in the North Atlantic and Fe limited in the North Pacific. However, declining SRP

stocks in the North Pacific over the past decades, along with an increase in N_2-fixing organisms, led researchers to suggest that these organisms were depleting P and driving the system to P limitation (Karl et al. 1997). Our results do not support this hypothesis for *Trichodesmium* spp., a dominant N_2 fixer. Measures of P stress are low in the NPSG compared with the North Atlantic. Moreover, our results were obtained during July and August when limitation might be expected to be maximal because of seasonally low SRP inventories driven by increased production in the summer months (Karl et al. 1996). Others have recently reported that the unicellular cyanobacterium *Prochlorococcus* appears to be N rather than P limited in the NPSG (Van Mooy and Devol 2008). Taken together, these results suggest that a factor or factors other than P limit diazotrophs and perhaps the community as a whole in the NPSG.

Finally, we can speculate on the mechanisms leading to observed patterns in these three systems. Presumably, *Trichodesmium* spp. found north of Australia was not P deficient because measurable and often high concentrations of SRP were found there (0.27 μmol L^{-1} at one site), perhaps due to the generally shallow depths (<100 m) and proximity to the coastline of many of the stations on this cruise. Experimental Fe additions to colonies collected north of Australia suggest Fe is limiting to *Trichodesmium* spp. in this location at least some of the time (Kustka et al. 2003*a*).

Dissolved Fe concentrations measured on the Australia cruise were similar to reported values in the North Pacific near Hawaii and much lower than dissolved Fe concentrations on our North Atlantic cruise (Table 2). Dissolved Fe data collected in a similar area of the North Atlantic were lower than the average reported here, ~0.6–0.8 nmol L^{-1} (Bergquist et al. 2007); however, the high dissolved Fe (dFe) values in the North Atlantic waters sampled during this study appear to originate from the Amazon River plume (S. Sañudo-Wilhelmy pers. comm.), and thus it is appropriate to use these cocollected values for comparison, rather than data from other cruises and times. In contrast to dFe values, SRP concentrations in the North Pacific were statistically the same as those found in the North Atlantic, yet V_{max} and APA were much higher in the North Atlantic. When examining the SRP : dFe ratios for the three locations, surface waters of the North Atlantic had the lowest ratio by far (Table 2); these waters were enriched in Fe compared with P. Similar to the hypothesis proposed by Wu et al. (2000), we posit that the abundance of Fe in the North Atlantic drives *Trichodesmium* spp. to P deficiency and possibly limitation there, whereas the scarcity of Fe in the North Pacific and north of Australia renders *Trichodesmium* spp. P sufficient there. Interestingly, colony-specific rates of N_2 fixation were similar among the three locations (Table 2), suggesting that deficiency of different nutrients does not necessarily affect fixation rates differently. However, the response to changes in nutrient delivery that may occur in the future would be very different among the different locations. Because the supply of Fe and P to the open ocean occurs by different methods, the potential exists for future changes in one without the other. Episodic delivery of Fe to the open ocean occurs through dust storms, whereas P is supplied to the open ocean though upward diffusion of deep water and access to the dissolved organic P pool (an important source for *Trichodesmium* spp.; Sohm and Capone 2006). On the basis of the data collected here, increased dust deposition would have little effect in the North Atlantic where P stress is high, but could potentially have an effect on N_2 fixation in the Pacific. Therefore, the hypothesized differential nutrient limitation of *Trichodesmium* spp. in the areas where it occurs (e.g., Fe limitation in the North Pacific), and the response to nutrient inputs, is a topic requiring further study.

The globally significant diazotroph *Trichodesmium* spp. responds to P stress by altering its ability to acquire P from both organic and inorganic sources. Maximal uptake of PO_4^{3-} increases greatly under P limitation of *Trichodesmium* spp., as does APA. However, laboratory data (Fu et al. 2005), along with the data collected in this study, show that the half-saturation constant (K_s) is not reduced in *Trichodesmium* spp., as occurs in many other phytoplankton species, suggesting it does not induce a second, high-affinity PO_4^{3-} transporter under P stress. V_{max} and APA can thus be used as a diagnostic indicator of P deficiency or stress, whereas K_s can not. V_{max} and APA data collected in three different locations show that *Trichodesmium* spp. in the Atlantic is P limited relative to the Pacific and north of Australia at the time of sampling and suggest that this may be an enduring pattern. Other studies suggest that P is limiting in the Atlantic because of the abundance of Fe delivered in Saharan dust (or, alternatively, from rivers; Tovar-Sanchez et al. 2006, S. Sañudo-Wilhelmy pers. comm.) and is not limiting in the Pacific because Fe is in such short supply (Wu et al. 2001). Waters north of Australia were likely not P limited because SRP was about 2.5 times greater than in the Atlantic and Pacific sites. Our data provide direct diagnostic evidence that there are different primary limiting factors for *Trichodesmium* spp. in the tropics of the North Atlantic compared with the North Pacific, and underscore the need to delineate the divergent nutrient dynamics that lead to this condition.

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