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Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*

Ilana Berman-Frank

Environmental Biophysics and Molecular Ecology Program, Institute of Marine and Coastal Sciences, Rutgers University, 71 Dudley Road, New Brunswick, New Jersey 08901

Jay T. Cullen

Institute of Marine and Coastal Sciences, Rutgers University, 71 Dudley Road, New Brunswick, New Jersey 08901

Yeala Shaked

Department of Earth Sciences, Hebrew University, Givat Ram, Jerusalem, Israel

Robert M. Sherrell

Institute of Marine and Coastal Sciences and the Department of Geology, Rutgers University, 71 Dudley Road, New Brunswick, New Jersey 08901

Paul G. Falkowski

Environmental Biophysics and Molecular Ecology Program, Institute of Marine and Coastal Sciences and the Department of Geology, Rutgers University, 71 Dudley Road, New Brunswick, New Jersey 08901

Abstract

Iron availability is suggested to be a primary factor limiting nitrogen fixation in the oceans. This hypothesis is principally based on cost–benefit analyses of iron quotas in the dominant nitrogen-fixing cyanobacteria, *Trichodesmium* spp., in the contemporary oceans. Although previous studies with *Trichodesmium* have indicated that iron availability enhanced nitrogen fixation and photosynthesis, no clear relationship has been reported between cellular iron quotas and nitrogen fixation. We re-examined the proposed link between iron availability and nitrogen fixation in laboratory isolates and natural populations collected from coastal waters north of Australia. In laboratory cultures grown under iron-limiting conditions, we measured a decline in cellular iron quotas, photochemical quantum yields, the relative abundance of photosystem I to photosystem II reaction centers, and rates of nitrogen fixation. Nitrogen fixation displayed a critical threshold of the dissolved sum of total inorganic Fe species ($[Fe']$) of ca. $\log[Fe'] = -9.7$. Field populations of *Trichodesmium*, collected during bloom conditions, showed high iron quotas consistent with high nitrogen fixation rates. Using seasonal maps of aeolian iron fluxes and model-derived maps of surface water total dissolved Fe, we calculated the potential of nitrogen fixation by *Trichodesmium* in the global ocean. Our results suggest that in 75% of the global ocean, iron availability limits nitrogen fixation by this organism. Given present trends in the hydrological cycle, we suggest that iron fluxes will be even more limiting in the coming century.

Nitrogen fixation by planktonic prokaryotes is a major source of new nitrogen for the oceans (Capone et al. 1997; Karl 2000). In all cyanobacteria, the enzyme responsible for this process, nitrogenase, consists of two proteins, an iron

protein (nitrogenase reductase) and an iron-molybdenum protein (dinitrogen reductase), which, together, contain 15 iron atoms for each heterodimeric protein molecule (Raven 1988). Because fixed inorganic nitrogen appears to be a major limiting element in the oceans and nitrogen fixers do not seem to be extremely abundant, it has been suggested that iron availability is critical in controlling rates of nitrogen fixation and, therefore, new production in the ocean (Rueter

¹ Corresponding author (irfrank@imcs.rutgers.edu).

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1988; Rueter et al. 1992; Falkowski 1997; Wu et al. 2000). There is, however, very little experimental evidence to support or refute this hypothesis.

In the contemporary ocean, a large fraction of nitrogen fixation is attributed to the filamentous, nonheterocystous cyanobacteria belonging to the genus *Trichodesmium*. Paerl et al. (1987) and Rueter (1988) proposed that natural populations of *Trichodesmium* spp. may be iron limited and that the addition of bioavailable Fe would therefore influence inputs of newly fixed nitrogen into surface waters. Their work demonstrated that photosynthesis, nitrogen fixation, and growth were enhanced when iron was added (Rueter 1988; Rueter et al. 1990; Paerl et al. 1994); however, they did not report cellular iron quotas for these experiments. Rueter et al. (1992) measured iron quotas in natural populations from the Caribbean and reported Fe:C ratios (assuming Redfield C:N ratios) that were about 10-fold higher than those measured in other oligotrophic phytoplankton, yet they did not find any relationship between nitrogen fixation rates and cellular iron quotas. In this paper, we re-examine the proposed link between iron availability, carbon-normalized iron quotas, and nitrogen fixation in a laboratory isolate (IMS101) and in natural *Trichodesmium* populations from shallow stations along the northern coast of Australia. Iron quotas were quantified using two independent methods (high-resolution, sector field inductively coupled plasma mass spectrometry [HR-ICP-MS] and ^{59}Fe uptake). Based on our experimental results, we use maps of aeolian iron fluxes to the world oceans (Gao et al. 2001) and a simple iron solubility model to estimate areas of the ocean that are conducive to nitrogen fixation by *Trichodesmium*.

Materials and methods

Culture growth conditions—Cultures of *Trichodesmium* IMS101 were grown at 26°C under a 12:12 h light:dark (LD) cycle at 85 μmol quanta m^{-2} s^{-1} supplied by VHO fluorescent tubes. Cultures were grown in either a modified Sigma seawater (catalog number S-9148, lot 29H8415) (background $\log[\text{Fe}'] = -10.8$ where $[\text{Fe}'] = \Sigma$ inorganic Fe species) amended with 0.5 μM phosphate and F/2 vitamins, or in YBCII medium (Chen et al. 1996). Fe (FeCl_3) was added at desired concentrations complexed with ethylenediaminetetraacetic acid (EDTA). In the first experiment, to compare Fe quotas measured by radiotracer and ICP-MS, Fe:EDTA was added in a constant ratio to achieve a range of total Fe (0.04 to 4 μM) with relatively little change in $\log[\text{Fe}']$ (-7.95 to -7.8). In subsequent experiments, varying amounts of total Fe were added along with a constant background of EDTA (20 μM), resulting in a range of $\log[\text{Fe}']$ from -7.8 to -10.8 . Fe concentrations are reported as the sum of total inorganic Fe species ($[\text{Fe}']$). Unless noted otherwise, measurements were made on three parallel cultures at each iron concentration, and cells were harvested for experiments during the exponential phase of growth. Growth rates were determined from linear regression analysis of the increase of logarithmic transformed C and N concentrations versus time in laboratory cultures.

Field study—Field populations of *Trichodesmium erythraeum* and *Trichodesmium thiebautii* were collected off the northern coast of Australia between 21 October and 16 November 1999. Colonies and filaments were obtained with net tows at varying depths, hand-picked or filtered on acid-washed QMA filters, and then frozen at -20°C for transport and later analysis for Fe content (ICP-MS), organic carbon, and nitrogen. For measurements of the ratio between photosystem I and photosystem II (PSI:PSII), colonies and filaments were hand picked, resuspended in filtered seawater, placed in NMR quartz cuvettes, and immediately frozen in liquid nitrogen.

Determination of Fe quotas— ^{59}Fe incorporation: Intracellular and total cellular iron concentrations were measured in parallel bottles spiked with $^{59}\text{FeCl}_3$ (specific activity 30 mCi mg^{-1} , New England Nuclear). Cells were grown with the radiotracer to the end of exponential phase and were harvested on polycarbonate membrane filters (Nuclepore, 10 μm pore size). Total cellular iron was determined on triplicate filters rinsed thoroughly with filtered (0.2 μm) seawater. Intracellular iron was measured on triplicate filters rinsed first for 2 min with a Ti(III)EDTA-citrate reducing solution to dissolve iron hydroxides and remove iron adsorbed to the cell surface (Hudson and Morel 1989) then with Sigma seawater. Microscopic observations were used to check cell integrity after the Ti(III)EDTA-citrate treatment. ^{59}Fe retained on the filters was measured with a liquid scintillation counter (Beckman LS 6500). No corrections were made for filter blanks of media without cells because it accounted for <10% of sample activity. The ^{59}Fe activity in the cells was divided by the measured total activity of added radiolabel and multiplied by the total concentration of iron in the medium to give cellular iron concentrations (mol Fe ml^{-1} of medium filtered). Cellular carbon content and chlorophyll *a* (Chl *a*), determined simultaneously in parallel bottles, allowed an independent evaluation of Fe:C and Fe:Chl *a*.

ICP-MS measurements: Laboratory cultures and field populations were collected, stored, and analyzed for a suite of trace elements, including Fe, Al, and P, using established methodology (Cullen and Sherrell 1999) with minor modifications. Briefly, for filter-collected field and laboratory samples, subsamples (1.21 cm^2) of 47-mm quartz microfiber filters (Whatman QM-A) were placed in 15-ml screw-cap Teflon vials (Savillex), and the cells were digested completely by refluxing in 1,000 μl of 16 N HNO_3 on a hotplate at 120°C for 4 h. For field samples, 50 μl of 32 N HF was added to solubilize more refractory inorganic phases. Hand-picked *Trichodesmium* samples were first lyophilized and then resuspended in a 1 N HCl solution. Samples were homogenized by sonication, and aliquots were removed to Teflon vials where they were digested as previously described for filter samples. Digests of samples and filter blanks, and digest blanks (acid only), were analyzed for trace elements and P by magnetic sector HR-ICP-MS (Element, Finnigan-MAT) using a combination of internal and external standardization (Cullen, Field, and Sherrell unpubl. data). Quotas are reported as carbon-normalized ratios of Fe:C.

Nitrogenase activity—Nitrogen fixation rates for *Trichodesmium* were measured using the acetylene reduction method according to Capone (Capone 1993). Cultures (10–30 ml depending on biomass) were sealed in serum bottles, injected with acetylene (20% of headspace volume), and incubated for 2 h at growth irradiance and temperature. Ethylene production was measured on an SRI 310 gas chromatograph with a flame ionization detector and quantified relative to an ethylene standard. Results were normalized to carbon and chlorophyll. Tight circadian control results in a diel pattern of nitrogenase activity (Chen et al. 1996). We assumed that this pattern would be maintained also in Fe-depleted cultures. Sampling for nitrogenase activity was done at the midpoint in the light cycle when nitrogenase activity is maximal in our cultures.

Relative abundance of PSI and PSII—The fluorescence signature of PSI can be resolved from that of PSII at cryogenic temperatures. The ratio between PSI and PSII was estimated from 77K fluorescence emission spectra (excitation wavelength 435 nm) performed on cells that were concentrated on 10- μ m nitex filters, resuspended in media, placed in quartz cuvettes, and flash frozen in liquid nitrogen. The spectra were analyzed on an AB2 Aminco spectrofluorometer equipped with a liquid nitrogen Dewar. Measurements were made at 77K using a 2-nm band-pass. Emission peaks of PSII (685 and 695 nm) and of PSI (720–730 nm) were resolved, and the peak ratio was determined using PeakFit[™] Software (SPSS).

Variable fluorescence and photosynthetic parameters—We used a fast repetition rate fluorometer (FRRF), which measures fluorescence transients induced by a series of sub-saturating excitation pulses from a bank of blue-green LEDs to derive photosynthetic parameters (Kolber et al. 1998). The photochemical quantum yield (F_v/F_m) was determined from the initial, dark-adapted fluorescence (F_0) and the maximal fluorescence (F_m) when all PSII reaction centers are photochemically reduced [$F_v/F_m = (F_m - F_0)/F_m$]. The functional absorption cross section of PSII (σ_{PSII}), and changes in the kinetics of Q_A^- reoxidation (τ) were calculated from the saturation rates from F_0 to F_m .

Immunolocalization of nitrogenase-containing cells—Cultures grown at low ($\log[Fe'] = -10.8$) and high ($\log[Fe'] = -7.8$) iron concentration were probed for nitrogenase using a modification of the method of Lin et al. (1998). Our main modification consisted of conjugating the primary antibody directly to a fluorescent probe, thereby eliminating the requirement for a secondary antibody. Cells were fixed with 100% ethanol overnight, permeabilized in 5% dimethyl sulfoxide (DMSO) in phosphate buffer for 15 min at room temperature, and probed for 2 h with the Fe protein of nitrogenase conjugated to a fluorescent probe at a concentration of 20 μ g ml⁻¹ (Alexa-488, Molecular Probes). Samples were viewed on a Zeiss LSM410 confocal laser microscope at 488/528 nm excitation/emission. To obtain the percentage of cells labeled with nitrogenase, 1,000 cells were counted microscopically for each replicate culture.

Protein assays—Western immunoblotting was performed on iron-replete and iron-limited cultures harvested at the midpoint of the light cycle. Proteins were extracted according to Chen et al. (1998) and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 8–16% gradient gels (Gradipore iGels). After transfer to a polyvinylidene difluoride membrane (PVDF, Millipore), the proteins were challenged with antisera raised against the Fe protein of nitrogenase (generously provided by P. Ludden, University of Wisconsin) conjugated to IgG HRP and visualized using SuperSignal[®] chemiluminescent substrate for HRP detection (Pierce).

Results

The effects of iron limitation on growth, photosynthesis, the relative ratios of the photosynthetic reaction centers, cellular iron quotas, and nitrogen fixation in *Trichodesmium* IMS101 were measured in exponential-phase cultures grown under different iron regimes. The photochemical quantum yield of PSII, F_v/F_m , provided an early diagnostic of iron limitation, as shown for other taxa of phytoplankton (Green et al. 1991; Kolber et al. 1994; Behrenfeld and Kolber 1999; Boyd et al. 2000) (Fig. 1). In *Trichodesmium*, nitrogen fixation follows a circadian rhythm, occurring only during the light and peaking around the middle of the photoperiod. F_v/F_m , measured at the peak of nitrogenase activity, was 50% lower ($F_v/F_m = 0.25$) for iron-depleted cultures ($\log[Fe'] = -10.8$) compared with iron-replete cultures ($F_v/F_m = 0.54$, $\log[Fe'] = -7.8$) (Fig. 1A). The decline in F_v/F_m resulted from lower values of F_m , which is indicative of nonfunctional reaction centers. Both the functional absorption cross-section (σ_{PSII}) and the rate of Q_A^- reoxidation ($1/\tau$) showed a threshold rather than a progressive response to iron depletion, with significant changes observed only under the lowest iron concentrations (not shown).

The carbon-specific growth rates we obtained are within the range of reported growth rates of *Trichodesmium* IMS101 and of *T. erythraeum* (Mulholland and Capone 2000). Growth rates declined from 0.12 to 0.04 d⁻¹ (Fig. 1B) as Fe was reduced from $\log[Fe'] = -7.8$ to -10.8 with Chl : C changing approximately threefold over the three orders of magnitude increase in inorganic dissolved iron (Table 1).

Fe availability had little effect on the bulk C:N:P elemental composition of *Trichodesmium* IMS101 over the three orders of magnitude change in dissolved iron (Table 1). C:N values varied from 8.9 to 16. C:P ratios averaged 60 ± 5 across the low iron treatments and increased to 156 ± 25 for iron-replete conditions ($\log[Fe'] = -7.8$). This pattern was also observed for the N:P ratios, which were 4.8 to 6.7, and increased by almost twofold to 13 ± 3 when iron was not limiting ($\log[Fe'] = -7.8$).

Iron uptake rates correlated with iron-dependent growth rates (not shown) and were reflected in the carbon-normalized iron quotas. Iron quotas were measured by two independent methods: directly by sector field ICP-MS and indirectly by following the incorporation of ⁵⁹Fe into cells. Analysis by ICP-MS allows for the simultaneous determination of trace elements and P as a proxy for biomass. When

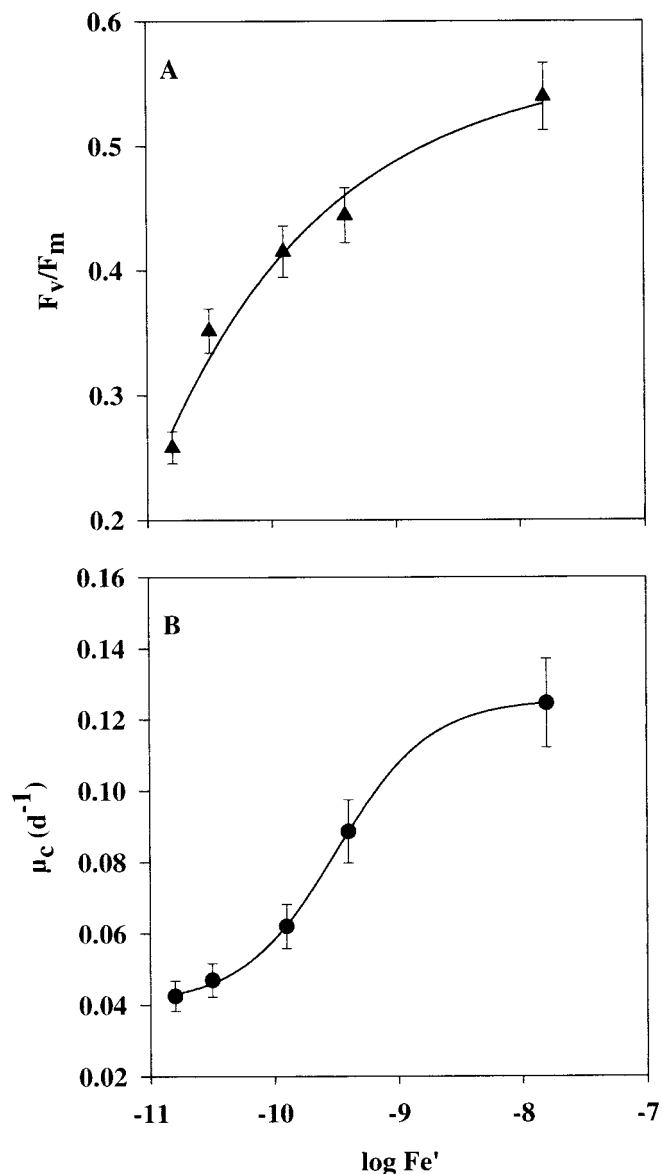


Fig. 1. The effect of iron availability on cultures of *Trichodesmium* IMS101 grown at 26°C, LD cycle of 12:12 h, and under 85 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ as measured for (A) photosynthetic quantum yields F_v/F_m and (B) carbon-specific growth rates. Error bars represent the standard deviation of averaged results from three replicate bottles.

combined with CHN analysis, this approach provides a comprehensive account of the elemental composition of *Trichodesmium*. Radioisotope-based estimates of cell quotas were partitioned (through Ti-citrate washes) into total versus intracellular. The total, carbon-normalized quotas we obtained from both methods are remarkably similar. The Fe:C ratio ranged from 7.1 ± 0.4 to $214 \pm 15 \mu\text{mol Fe mol}^{-1} \text{C}$ for cultures grown across a gradient of inorganic Fe (Tables 1, 2). Corresponding ratios measured by ICP-MS agreed well with the radiotracer method (Table 2). Removal of the surface-bound fraction in the ^{59}Fe experiments yielded intracellular Fe quotas that were 60–70% lower than total cellular Fe, with values of 3 ± 0.2 and $69 \pm 3 \mu\text{mol Fe mol}^{-1} \text{C}$ for $\log[Fe'] = -7.95$ and -7.8 , respectively (Table 2).

In *Trichodesmium* IMS101, Fe availability influenced the relative abundance of PSI and PSII reaction centers. Although there are three iron atoms associated with PSII (two in cytochrome b_{559} and one nonheme iron that coordinates the two core reaction center proteins (Falkowski and Raven 1997), PSI contains at least 12 iron atoms and is a major sink for iron in all oxygenic photoautotrophs. In the laboratory cultures, PSI:PSII peak area ratios decreased threefold from 1.3 ± 0.3 to 0.25 ± 0.06 as Fe was reduced from $\log[Fe'] = -7.8$ to -10.8 (Fig. 2A). The changes in PSI:PSII were highly correlated with Fe availability ($r^2 = 0.98$) (Fig. 2A), with nitrogen fixation rates ($r^2 = 0.94$) (Fig 2B), and with Fe:C (Fig 3A). PSI:PSII ratios were low for Fe:C of $<25 \mu\text{mol Fe mol}^{-1} \text{C}$. At Fe:C of 25 to 50 $\mu\text{mol Fe mol}^{-1} \text{C}$, PSI:PSII increased linearly and then plateaued at higher Fe:C, saturating at 50–190 $\mu\text{mol Fe mol}^{-1} \text{C}$ (Fig. 3A).

We calculated iron budgets for PSI, PSII, and cytochrome b_{6f} (Cyt b_{6f}) to obtain a measure of the amount of iron in the photosynthetic apparatus compared to the intracellular quotas we determined. We used measured values of $341 \pm 53 \mu\text{mol Chl } a \text{ mol}^{-1} \text{PSI}$ and $151 \pm 52 \mu\text{mol Chl } a \text{ mol}^{-1} \text{PSII}$ ($n = 12$) in iron-replete cells (Wyman and Falkowski unpubl. data), as well as our obtained Chl a :C. We assumed 12 atoms Fe per PSI, 3 atoms Fe per PSII, and 5 atoms Fe per Cyt b_{6f} (Raven et al. 1999). The abundance of Cyt b_{6f} was calculated assuming a ratio of 1.5 Cyt b_{6f} for each PSII reaction center (Falkowski and Raven 1997). Accordingly, in iron-replete cells, PSI accounted for ~20%, PSII for 11%, and Cyt b_{6f} for 27% of the total intracellular iron quotas. The sum of these values accounts for ~60% of all intracellular iron.

In the laboratory cultures, nitrogen fixation rates measured

Table 1. Chemical composition* of cultured *Trichodesmium* IMS101 grown with varying iron at 26°C and 85 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

	-10.78	-9.88	-9.4	-7.86	-7.8
Chl a :C ($\mu\text{g } \mu\text{mol}^{-1}$)	0.108	0.17	0.19	0.25	0.29
Fe:C ($\mu\text{mol mol}^{-1}$)	13 (9.9)	30 (2.7)	33 (1.8)	48 (2.2)	168 (23)
C:N (mol mol^{-1})	12 (1)	8.9 (1)	9 (0.3)	16 (2)	12 (4)
C:P (mol mol^{-1})	58 (11)	55 (2)	60 (15)	66 (13)	156 (25)
N:P (mol mol^{-1})	4.8 (1)	6.2 (1.5)	6.7 (0.9)	4.8 (1)	13 (3)
n	4	4	4	4	12

* Values are means (\pm SD) at varying $\log[Fe']$.

Table 2. Total and intracellular quotas (Ti-citrate washed) for *Trichodesmium* IMS101 as determined by ICP-MS and ^{59}Fe tracer experiments.

Method	<i>n</i>	Fe:C ($\mu\text{mol Fe mol}^{-1}\text{ C}$)*			
		-7.95	-7.86	-7.8	-7.79
Total quotas					
^{59}Fe	4	7.1 (0.4)	36.6 (2.6)	96.1 (4.8)	214 (15)
HR-ICP-MS	3	8.8 (1.3)	43.0 (8.5)	145.7 (20.8)	182.1 (18.2)
Intracellular quotas					
^{59}Fe	4	3.1 (0.15)	8.8 (1.22)	32.5 (3.25)	69.5 (3.48)

* Values are means (\pm SD) over varying $\log[\text{Fe}']$.

as acetylene reduction were dependent on iron availability and correlated with iron-dependent growth rates and with the Fe:C quotas (Figs. 3B, 4A,B). We derived the average ratio for acetylene reduced to nitrogen fixed assuming that all N assimilated by *Trichodesmium* originates from fixation and that the cells are in balanced growth. We used the relationship obtained between acetylene reduction and growth (slope of regression) and the ratio of Chl *a*:N to obtain an average ratio of 4.4 mol C_2H_2 reduced $\text{mol}^{-1}\text{ N}$.

Both nitrogen fixation and iron-dependent growth rates showed a similar relationship to ICP-MS-measured Fe:C (Fig. 3B,C). Low rates of both nitrogen fixation and growth occurred at Fe:C < 30. An Fe:C of 30 to 50 resulted in linear increases for both parameters, with saturated rates measured at Fe:C > 50 (Fig. 3B,C). The highest rates of nitrogen fixation were measured for cultures with the highest iron-dependent growth. When Fe was reduced from $\log[\text{Fe}'] = -7.8$ to -9.8 , the rates of ethylene produced decreased by 50%, falling from 2.84 ± 0.08 to 1.4 ± 0.3 nmol N μg^{-1} Chl *a* h^{-1} . Further reduction of Fe availability to $\log[\text{Fe}'] = -10.8$ led to an 85% decline in nitrogen fixation rates. The decline in nitrogen fixation rates corresponded to a 10-fold decrease in the Fe:C quotas and a threefold decrease in growth rates over the same range of Fe concentrations (Figs. 3B, 4A,B).

The change in nitrogenase activity was reflected in $\sim 50\%$ decrease in the fraction of cells containing nitrogenase, as enumerated from fluorescent immunolocalization of nitrogenase. In the iron-depleted ($\log[\text{Fe}'] = -10.8$) cultures, $11 \pm 5\%$ of cells were labeled with nitrogenase, whereas in iron-replete cultures ($\log[\text{Fe}'] = -7.8$), $20 \pm 8\%$ of the cells were labeled (paired *t*-test, $P = 0.02$) (Fig. 5). Western blots of nitrogenase reductase also revealed higher levels of protein in the Fe-replete cultures (Fig. 5 insert). Our results reveal clearly that iron availability influences both the synthesis and expression of nitrogenase in *Trichodesmium* IMS101.

Fe content in field populations of Trichodesmium—The carbon-normalized Fe quotas of field populations of *Trichodesmium* collected off northern Australia are summarized in Table 3. Duplicate measurements were performed for digests of filtered and hand-picked *Trichodesmium* samples. Samples with Al:C ratios $> 300 \mu\text{mol mol}^{-1}$, known to be typical of marine biogenic material (Collier and Edmond 1984; Moran and Moore 1988), were deemed “contaminated” with terrigenous inorganic material and excluded from the analysis. The Fe:C ratios varied from 36 to $740 \mu\text{mol Fe mol}^{-1}\text{ C}$, averaging 450 ± 242 ($n = 11$). Assuming that the fraction of intracellular Fe for field samples is similar to that for

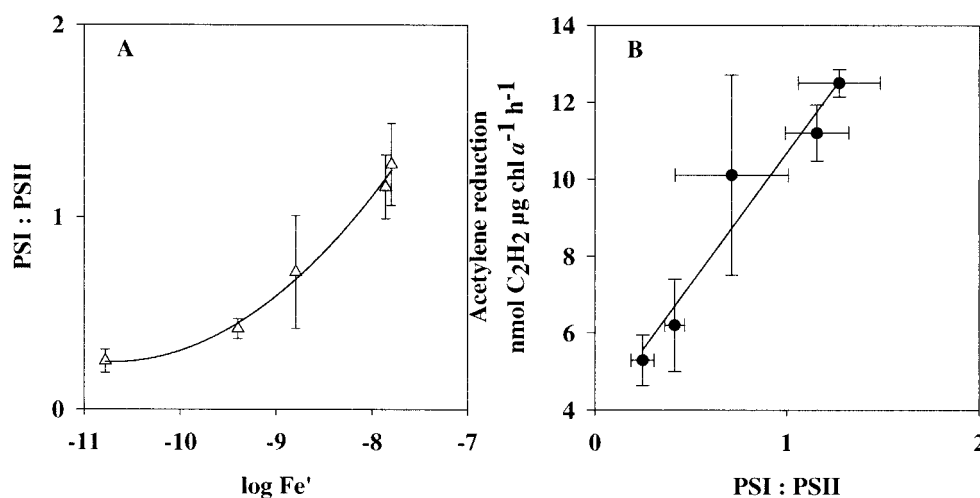


Fig. 2. (A) The influence of iron availability on the relative abundance of the photosynthetic units (PSI:PSII) as determined from emission spectra at 77K. (B) PSI:PSII as a function of nitrogen fixation rates.

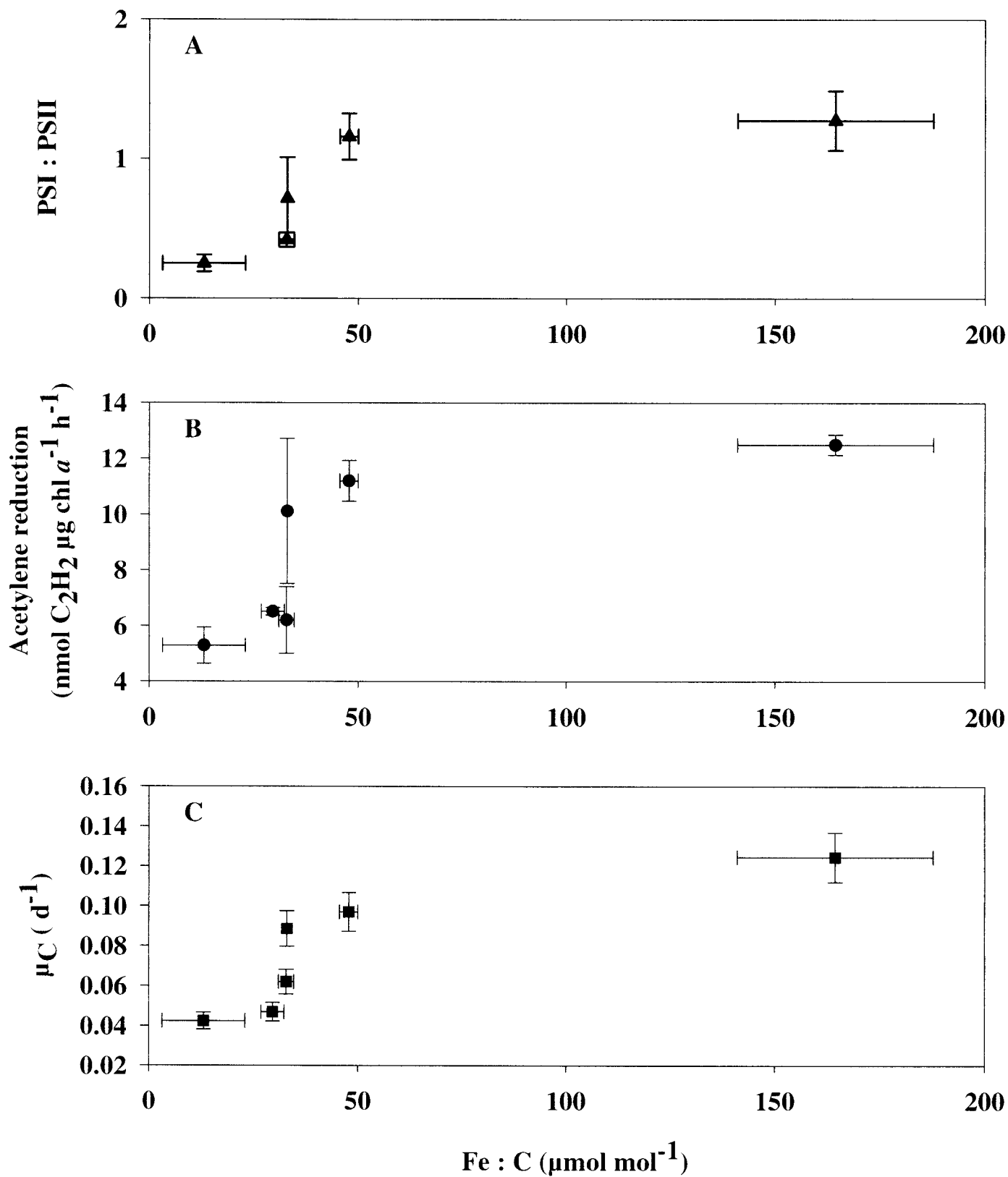


Fig. 3. (A) PSI:PSII, (B) nitrogen fixation rates as measured by acetylene reduction, and (C) carbon-specific growth rates as a function of cellular iron quotas measured by ICP-MS.

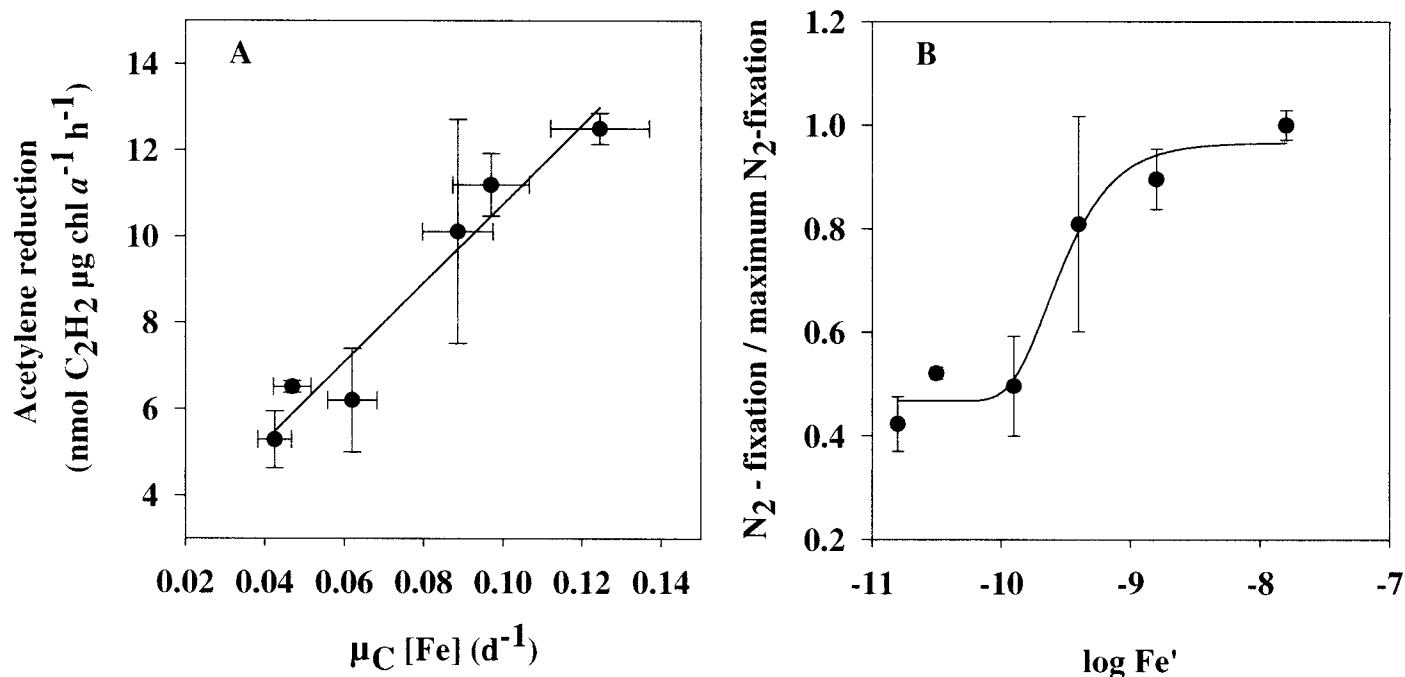


Fig. 4. (A) Nitrogen fixation rates as a function of iron-dependent carbon specific growth in *Trichodesmium* IMS101. (B) Calculated potential of nitrogen fixation (nitrogen fixation: maximum nitrogen fixation) as a function of iron availability in *Trichodesmium* IMS101.

laboratory cultures, then Fe:C should be reduced by about 65%, yielding Fe:C ranging from 23 to 480, with an average of 158 $\mu\text{mol Fe mol}^{-1}\text{C}$ (Table 2).

Discussion

This is the first study to provide a quantitative relationship between iron-dependent growth rates, Fe quotas, and nitrogen fixation for cultures of *Trichodesmium* IMS101 (Figs. 3, 4). To model the potential global contribution to nitrogen fixation by *Trichodesmium* in the oceans, we then applied the relationships between Fe quotas, nitrogen fixation rates and PSI:PSII, and growth.

There is little information on the elemental composition of *Trichodesmium* in the peer-reviewed literature. A recent study on cultures of *Trichodesmium* IMS101 reports that C:N ranges from 11 to 5 over the growth period (Mulholland and Capone 2001). The reported range of C:N for natural populations from the North Pacific and the North Atlantic is 4.7 to 7.1 (Mague et al. 1977; McCarthy and Carpenter 1979; Lewis et al. 1988; Karl et al. 1992). Later work from the North Pacific (Letelier and Karl 1998) measured C:N:P of sinking versus positively buoyant colonies and reports C:N of 5.68 to 7.32 and N:P of 34 to 44.8 for all data. Karl et al. (1992) report C:N:P of 142:20:1 and 891:125:1 for populations collected before and during a large *Trichodesmium* bloom. We have not observed such abnormally high C:P ratios. N:P ratios likely reflect cell growth under phosphorus-replete conditions in our cultures, compared with phosphorus-depleted North Pacific waters (Karl et al. 1992). Our data suggest that, as in diatoms (Green et al. 1991), changes in C:N:P are not correlated with iron avail-

ability (Tables 1, 3); hence, C:N:P ratios cannot be used to infer iron availability or quotas.

In contrast to the bulk elemental composition, Fe:C quotas appear to be more directly linked to iron availability, growth rates, PSI:PSII, and nitrogen fixation (Figs. 2, 3). The high iron requirement for nitrogen fixation is reflected in generally higher Fe:C quotas of *Trichodesmium*, compared with other marine phytoplankton such as diatoms, whose Fe:C quotas range from 1 to 7 $\mu\text{mol mol}^{-1}$ (Johnson et al. 1997; Sunda 1997; Maldonado et al. 1999). The values we obtained for the total cellular quotas in laboratory cultures are generally lower than those reported by Rueter et al. (1992) from natural *Trichodesmium* populations in the Caribbean. Our highest values (180–214 $\mu\text{mol Fe mol}^{-1}\text{C}$) were measured in cultures grown with high total iron of $\log[\text{Fe}'] = -7.8$. Such an average iron concentration is at least an order of magnitude higher than values in the open ocean. Our laboratory results indicate that Fe:C in *Trichodesmium* may be more directly related to total Fe rather than to free ferric or $[\text{Fe}']$. We suggest that photoreduction of Fe:EDTA or the ability of *Trichodesmium* to reduce Fe:EDTA may increase the bioavailable pool of Fe to growing cells (Anderson and Morel 1982). Rueter et al. (1992) reported an average Fe:C of 326 ± 127 ($n = 13$). However, our field data approximates the published values with an average of 450 ± 242 $\mu\text{mol Fe mol}^{-1}\text{C}$ ($n = 11$) (Table 2). If the laboratory data are indicative, then the intracellular quotas of the field measurements should be reduced by ~65%, which would result in average values of 292 and 211 $\mu\text{mol Fe mol}^{-1}\text{C}$ for both our field data and Rueter's field data, respectively. These lower values are comparable to the highest quotas we obtained for cultures grown under iron-replete



Fig. 5. Trichomes of *Trichodesmium* IMS101 probed with the antibody for the iron protein of nitrogenase tagged with the fluorophore Alexa 488 and viewed under a confocal laser microscope. Photo shows clusters of cells within each trichome containing the nitrogen-fixing enzyme. Fluorescently labeled cells are counted as cells containing nitrogenase. (Insert) Western blot showing differences in the expression of the nitrogenase reductase for Fe-replete and Fe-limited cultures. Equal biomass was loaded on each lane.

conditions. Lack of data on dissolved or total iron from the Rueter et al. (1992) or our own cruise track precludes correlation of carbon-normalized iron quotas to dissolved iron concentrations. However, our samples were collected from mostly shallow stations (<200 m deep) off the northern coast of Australia, which may be exposed to episodic aeolian iron supplies.

Our iron budgets suggest that most of the intracellular iron in *Trichodesmium* is allocated to the photosynthetic apparatus. Laboratory studies have shown that iron deficiency in phytoplankton results in the synthesis of fewer photosynthetic reaction centers and less efficient light absorption and energy transfer (Green et al. 1991; Geider and La Roche 1994). The results from this study illustrate clearly that PSI:

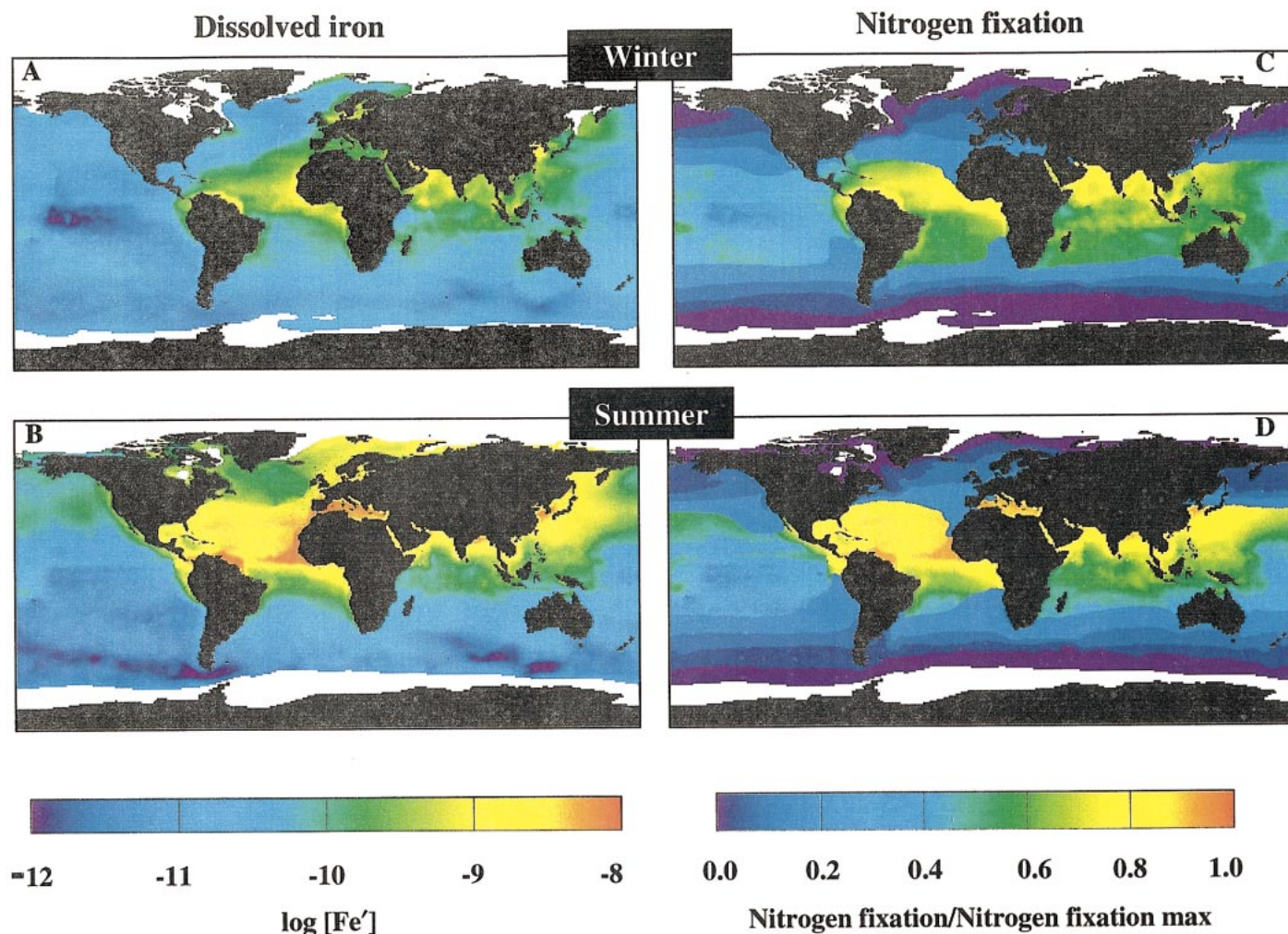


Fig. 6. Seasonal maps showing the potential for nitrogen fixation in the world oceans. Maps were generated by converting global dust iron fluxes to total dissolved iron and using the relationship shown in Fig. 4 between $\log[Fe']$ and nitrogen fixation: maximum nitrogen fixation. The following sources were used in the model inputs: mixed layer depth (modeled seasonal climatologies from monthly NCAR climatologies), sea surface temperature (Integrated Global Ocean Services Systems Bulletin [IGOSS]), ice cover (LDGO Atlas of Surface Marine Data). See text for detailed discussion.

PSII ratios in *Trichodesmium* are dependent on ambient iron concentrations, the depletion of which reduces the abundance and energy transfer through PSI relative to PSII. In filamentous nonheterocystous cyanobacteria, such as *Trichodesmium*, cyclic electron flow around PSI provides adenosinetriphosphate (ATP) for N_2 fixation, whereas linear electron flow through PSI reduces the inhibitory effects of oxygen generated in PSII through the Mehler reaction (Kana 1992, 1993). Thus, iron limitation of PSI will influence N_2 fixation both directly (via synthesis of PSI) and indirectly (by reduced ATP production). The tight correlation between PSI:PSII and nitrogen fixation rates is surprising because the relative abundance of photosynthetic units is highly influenced by other parameters such as irradiance. However, the significant correlations we obtained between cellular iron quotas, nitrogen fixation rates, and PSI:PSII may provide a good diagnostic of iron limitation in *Trichodesmium* and could possibly be used to monitor the iron nutritional status of other oceanic diazotrophs in the field.

The results of the laboratory study with *Trichodesmium* were used to estimate the degree of spatial and temporal coupling between aeolian Fe input and N_2 fixation capacity in the surface ocean. Global maps of seasonally averaged, total aeolian Fe deposition to the surface ocean were obtained from Gao et al. (2001). We modified these total aeolian Fe fluxes ($mg\ m^{-2}\ month^{-1}$) using a model of mean mixed layer depth and an Fe solubility of 1.2% (Spokes and Jickells 1996; Zhu et al. 1997) to yield total dissolvable Fe_{atm} fluxes ($nmol\ L^{-1}\ month^{-1}$). Steady state total dissolved Fe concentrations were calculated assuming

$$[Fe]_T = \frac{Fe_{atm}}{k_{sc}}, \quad (1)$$

where Fe_{atm} is the flux of dissolvable Fe ($nmol\ L^{-1}\ month^{-1}$), k_{sc} is the scavenging rate constant for Fe ($month^{-1}$), and $[Fe]_T$ is steady state Fe concentration ($nmol\ L^{-1}$). A k_{sc} of $0.8\ yr^{-1}$ was adopted after Archer and Johnson (2000). To account

Table 3. Chemical composition of field populations of *Trichodesmium* collected in shelf waters off northern Australia.

Study location				Composition ($\mu\text{mol mol}^{-1}$)			Species*
Latitude	Longitude	Date	Time (h)	Fe:C	C:N	C:P	
Filtered							
09°57.66'	131°8.96'	4 Nov 99	1030	745	7.25	165	<i>a + b</i>
09°57.66'	131°8.96'	4 Nov 99	1400	879	6.38	171	<i>b</i>
09°57.66'	131°8.96'	4 Nov 99	1530	850	6.16	201	<i>b</i>
11°49.8'	128°42.6'	5 Nov 99	800	673	5.41	137	<i>a + b</i>
12°0.9'	128°12.1'	5 Nov 99	1845	763	5.69	120	<i>a</i>
12°44.6'	127°32.5'	14 Nov 99	1300	923	5.87	132	<i>a</i>
Average				806	6.13	154	
SD				94	0.6	30	
Hand-picked							
10°45.9'	145°09.00'	30 Oct 99	900	657			<i>b</i>
10°45.9'	145°09.00'	30 Oct 99	1000	393			<i>b</i>
10°45.9'	145°08.80'	30 Oct 99	1400	674			<i>b</i>
11°49.8'	128°42.6'	5 Nov 99	800	383			<i>a</i>
11°47.2'	128°39.9'	5 Nov 99	1300	170			<i>a slick</i>
14°08.50'	123°05.90'	8 Nov 99	1900	36			<i>a slick</i>
14°46.5'	122°07.10'	12 Nov 99	1400	739			<i>a + b</i>
12°44.6'	127°32.5'	14 Nov 99	1300	707			<i>a + b</i>
12°44.6'	127°32.5'	14 Nov 99	1330	462			<i>a + b</i>
12°44.6'	127°32.5'	14 Nov 99	1330	548			<i>a + b</i>
12°44.6'	127°32.5'	14 Nov 99	1330	177			<i>a slick</i>
Average				450			
SD				242			
Rueter et al. 1992							
Average				326			
SD				127			
N				13			

* *a*, *Trichodesmium erythraeum*; *b*, *Trichodesmium thiebautii*.
slick: dense surface bloom.

for organic complexation of Fe (Rue and Bruland 1995), we assumed that an organic ligand [L] with $K' = 10^{21}$ was present at a concentration of 1.0 nM (Millero 1998). A numerical relationship between $[\text{Fe}]_T$ and $\log[\text{Fe}']$ was determined using the thermodynamic equilibrium software package MINEQL+.

$$\log[\text{Fe}'] = 0.4836 \ln[\text{Fe}]_T + 0.056 \quad (2)$$

Although our laboratory data suggest that *Trichodesmium* Fe content may reflect total dissolved Fe availability, in order to extrapolate from the laboratory cultures to field populations, we constructed our model in terms of $\log[\text{Fe}']$. Potential N_2 fixation rates were then calculated from a quadratic equation fit of Fig. 4B for each grid point.

$$\frac{N_{\text{fix}}}{N_{\text{fix max}}} = 0.84 - 0.19 \log[\text{Fe}'] - 0.02 \log[\text{Fe}']^2 \quad (3)$$

The relationship between Fe' and N_2 fixation potential was further constrained by assuming that *Trichodesmium* does not fix N_2 below $\sim 22^\circ\text{C}$ (Carpenter 1983; Capone et al. 1997). Hence, a linear function was applied for -1 to 22°C , with a probability of finding *Trichodesmium* in such waters varying from 0.001 to 0.2. Above 22°C , aeolian iron fluxes dominate the distribution patterns.

The above calculation is clearly sensitive to the assump-

tions made regarding solubility, scavenging rate, and the degree of organic ligand complexation for iron. Given the uncertainty or paucity of measurements for these parameters, we evaluated the success of our model by its ability to recreate realistic surface water $[\text{Fe}]_T$ based on current literature data ($\sim 100 \text{ pM} - 10 \text{ nM}$). Our modeled steady-state Fe concentrations were determined from Fe solubility and scavenging rates. The solubility of Fe in seawater varies from $\sim 10 - 50\%$ (Zhuang et al. 1990; Archer and Johnson 2000) with some reports of minimal solubilities of 0.3% for metal aerosols collected from urban environments (Breslin and Duedall 1987). Zhuang et al. (1990) found that Fe solubility in seawater was limited to $\sim 10 - 17 \text{ nM}$, or $\sim 5 - 8 \text{ nM}$ when the dissolved fraction was defined as Fe that passed through a 0.4- or 0.05- μm filter, respectively. Scavenging rates of Fe are less well constrained. We assumed a first-order, linear scavenging rate with uncomplexed dissolved Fe adopting a constant rate of 0.07 month^{-1} after Archer and Johnson (2000). Our assumption of 1.2% solubility and scavenging rate combined with the aeolian fluxes of Gao et al. (2001) lead to steady-state, total dissolved Fe concentrations that varied between 1 pM in low- and 10 nM in high-deposition areas, similar to the range of reported dissolved Fe in seawater (Johnson et al. 1997) (Fig. 6A,B).

The resulting fields (Fig. 6C,D), generated for winter and

summer reveal large areas of the subtropical Atlantic and Indian ocean that are potentially conducive to N_2 fixation by *Trichodesmium* but show iron limitation in the eastern Pacific. Using an arbitrary cut-off at 80% of the maximum N_2 fixation potential, our results suggest that, given present aeolian iron fluxes and sea surface temperatures, nitrogen fixation is essentially iron-limited in 75% of the world oceans.

Whereas the climatological maps presented in Fig. 6 provide a heuristic representation of nitrogen-fixing potential for *Trichodesmium* in the oceans, these maps do not necessarily correspond to the actual distributions of the organism or of nitrogen fixation. In our simple analysis, we do not consider alternative limiting elements, such as upper ocean phosphate concentrations, that may impose additional constraints, as reported for *Trichodesmium* from the North Pacific subtropical gyre or the central Atlantic (Letelier and Karl 1998; Sanudo-Wilhelmy et al. 2001). Nonetheless, our maps are broadly concordant with global observations of *Trichodesmium* in situ (Carpenter 1983; Capone et al. 1997) and are broadly consistent with estimates of the distribution of nitrogen fixation derived from biogeochemical analyses (Gruber and Sarmiento 1997).

There is a feedback between aeolian iron fluxes and nitrogen fixation that is coupled to the global hydrological cycle (Falkowski et al. 1998). Gao et al. (2001) report that ~75% of the aeolian iron flux is delivered to the upper ocean by wet deposition. Throughout the 20th century, global mean precipitation has increased by an average of 24 mm (i.e., 2.4 mm decade⁻¹; Dai et al. 1997). Such a trend potentially leads to increased iron precipitation closer to the continental sources, thereby restricting the aerial extent of the aeolian fluxes across the ocean basins. This climatological feedback will potentially reduce nitrogen fixation in the Pacific Ocean over the eastern section, where aeolian fluxes are already low. If so, the feedback would lead to a small decrement of nitrogen fixation in coming decades because the hydrological cycle is expected to become even more vigorous (Hennessy et al. 1997).

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