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Iron Deficiency Increases Growth and Nitrogen-Fixation Rates of Phosphorus-Deficient Marine Cyanobacteria

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ORIGINAL ARTICLE

Iron deficiency increases growth and nitrogen-fixation rates of phosphorus-deficient marine cyanobacteria

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Marine dinitrogen (N_2)-fixing cyanobacteria have large impacts on global biogeochemistry as they fix carbon dioxide (CO_2) and fertilize oligotrophic ocean waters with new nitrogen. Iron (Fe) and phosphorus (P) are the two most important limiting nutrients for marine biological N_2 fixation, and their availabilities vary between major ocean basins and regions. A long-standing question concerns the ability of two globally dominant N_2-fixing cyanobacteria, unicellular *Crocosphaera* and filamentous *Trichodesmium*, to maintain relatively high N_2-fixation rates in these regimes where both Fe and P are typically scarce. We show that under P-deficient conditions, cultures of these two cyanobacteria are able to grow and fix N_2 faster when Fe deficient than when Fe replete. In addition, growth affinities relative to P increase while minimum concentrations of P that support growth decrease at low Fe concentrations. In *Crocosphaera*, this effect is accompanied by a reduction in cell sizes and elemental quotas. Relatively high growth rates of these two biogeochemically critical cyanobacteria in low-P, low-Fe environments such as those that characterize much of the oligotrophic ocean challenge the common assumption that low Fe levels can have only negative effects on marine primary producers. The closely interdependent influence of Fe and P on N_2-fixing cyanobacteria suggests that even subtle shifts in their supply ratio in the past, present and future oceans could have large consequences for global carbon and nitrogen cycles.
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Introduction

The relative degree of iron (Fe) versus phosphorus (P) limitation of marine N_2-fixing cyanobacteria is variable throughout the oceans. Large continental dust inputs from North Africa that deliver Fe to the North Atlantic Ocean are thought to be responsible for the high N_2-fixation rates and low-P concentrations in this region relative to the North Pacific gyre, where the Fe:P concentration ratio is considerably lower (Wu *et al.*, 2000; Falcón *et al.*, 2004; Mahaffey *et al.*, 2005; Mahowald *et al.*, 2009; Karl, 2014). In concordance with this view, there is evidence that P limits N_2-fixation rates by *Trichodesmium* in the Sargasso Sea (Sañudo-Wilhelmy *et al.*, 2001), where this cyanobacterium is abundant (Capone *et al.*, 1997, 2005). In addition, low Fe concentrations in the North Pacific may favor a higher relative dominance of small unicellular N_2 fixers in comparison with *Trichodesmium* (Sohm *et al.*, 2011), because of their lower requirements for Fe to fix N_2 (Berman-Frank *et al.*, 2007).

Some studies, however, do not support the hypothesis that Fe and P are the sole limiting nutrients for N_2 fixation in the North Pacific and North Atlantic, respectively (Mills *et al.*, 2004; Grabowski *et al.*, 2008), suggesting a more complex relationship between these two nutrients. The fact that both Fe and P are at or near limiting concentrations throughout much of the oligotrophic ocean emphasizes the need for an improved understanding of nutrient co-limitation (Saito *et al.*, 2008) of marine N_2 fixation. We examined the consequences of Fe and P co-deficiency for the growth and N_2-fixation rates of *Crocosphaera watsonii* and *Trichodesmium erythraeum*. Together these isolates represent two genera of globally distributed tropical and subtropical marine cyanobacteria that are responsible for a major fraction of total oceanic N_2 fixation (Sohm *et al.*, 2011).

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Materials and methods

To examine interactive effects of Fe and P limitation on growth of the N_2-fixing cyanobacteria *C. watsonii* and *T. erythraeum*, we grew laboratory cultures over a range of P (0.05–4.0 μM) in high-Fe (450 nM) and low-Fe (0.13–0.35 nM) media. Trace metal clean methods were used to grow cultures of *T. erythraeum* (GBRRLI101) and *C. watsonii* (WH0003) across a range of Fe and P concentrations at 28 °C and 125 or 150 μmol quanta $m^{-2} s^{-1}$, respectively. Triplicate cultures were diluted every 3 days to 20×10^3 cells ml^{-1} (*C. watsonii*) or 22×10^3 μm total filament length ml^{-1} (*T. erythraeum*) (counted microscopically) for ~20–50 generations with artificial seawater (Chen *et al.*, 1996) that was microwave sterilized, bubbled with air (24–48 h) and passed through activated Chelex 100 resin (BioRad Laboratories, Hercules, CA, USA) to remove Fe (Price *et al.,* 1989). We added vitamins and trace metals except Fe according to the AQUIL recipe (Sunda *et al.*, 2005), HNa_2PO_4 (0.05–4.0 μM) and $FeCl_3$ (0.45 μM complexed with 5.0 μM ethylenediaminetetraacetic acid, EDTA, to high-Fe cultures (Price *et al.*, 1989). Dissolved Fe was measured in unfiltered seawater (0.13–0.35 nM Fe; Supplementary Table S1) and in stock solutions containing 100 μM and 1.0 mM PO_4^{3-} (0.02–0.12 nM, $n=4$) with a flow injection analysis method (Sedwick *et al.*, 2005). To rule out the possibility of differences between treatments due to potential scavenging of phosphate onto any Fe oxyhydroxide precipitates that may have formed in the medium (Sunda and Huntsman, 1995; Wheat *et al.*, 1996; Liu and Millero, 2002), we measured dissolved phosphate using the MAGIC method (Karl and Tien, 1992) for two P concentrations (100 and 150 nM) at both Fe concentrations used in the medium recipe (Supplementary Table S2). Phosphate concentrations were virtually identical in high- and low-Fe treatments ($P>0.05$), demonstrating that differential P availability was unlikely to have affected our results.

Cultures were acclimated to low-P conditions as described by Garcia *et al.* (2013). Briefly, after establishment of steady-state growth at each P concentration, cultures were then successively transferred to the neighboring lower P treatment until a new steady-state growth rate was achieved before sampling and further transfers. Consequently, cultures, for which we report a growth rate of zero (that is, Fe replete, 0.1 μM P treatments), had initially positive growth rates that diminished with each successive transfer, ultimately resulting in no further accumulation of biomass over the last 3-day dilution period. At this point, cultures were sampled for N_2 fixation and cellular or trichome-specific CNP analysis. Thus, these measurements in the lowest P treatments in experiments with *Trichodesmium* and *Crocosphaera* were considered to represent values where the growth rate approached zero. We calculated growth rates as described previously (Garcia *et al.*, 2013) using volume-specific estimates of cell number or total filament length. Hyperbolic functions of the form $f(x)=ax/(b+x)$ were fit to the data in Figure 1 using an iterative method (Garcia *et al.*, 2013) with Sigma Plot 10 software, where $a=\mu_{max}$, $b=K\mu$ and C_{min} is the minimum concentration of P needed to support growth. Diameters of ~12 cells (*C. watsonii*) were measured microscopically from each treatment (except the 0.6 μM P treatment) with an ocular micrometer. We measured N_2 fixation and particulate C, N and P at the end of a 3-day growth period following the final dilution, as previously described (Garcia *et al.*, 2013). We used growth rates (d^{-1}) and P-quota (fmol per cell) measurements to estimate cell-specific P-uptake rates. To determine statistical significance between treatments, we used a *t*-test or the rank-based, two-tailed, nonparametric Mann–Whitney *U*-test (Zar, 1999).

Results

As expected, growth rates in Fe-deficient cultures were lower than those in Fe-replete cultures under P-replete conditions ($P<0.05$), demonstrating that Fe concentrations in the low-Fe seawater medium limited growth of both *C. watsonii* and *T. erythraeum* (Figure 1). At low-P concentrations,

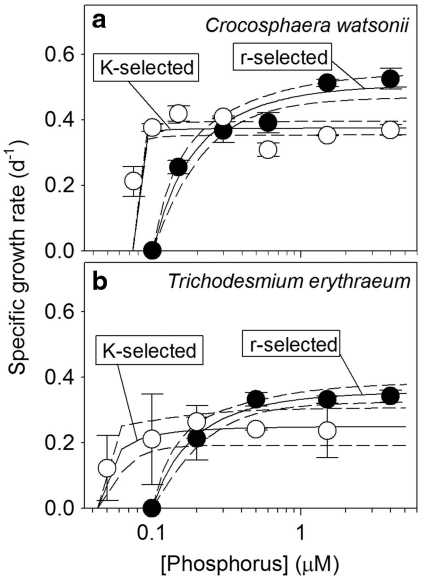

Figure 1 Growth of two dinitrogen (N_2)-fixing cyanobacteria relative to variations in iron (Fe) and phosphorus (P) concentrations. Mean cell-specific growth rates (with s.d.) in cultures of *Crocosphaera watsonii* (WH0003) (**a**) and *Trichodesmium erythraeum* (GBRRLI101) (**b**) grown over a range of P concentrations (0.05–4.0 μM) under high (450 nM; closed symbols) and low (0.12–0.35 nM; open symbols) Fe concentrations. K-selection yields faster growth in low-P water, whereas r-selection yields higher maximum growth rates. Monod kinetic constants and parameters of the hyperbolic functions (solid lines) were best fit to the data with 95% confidence intervals on hyperbolas (dashed lines).

however, this effect was reversed, with Fe-deficient cultures of both *C. watsonii* (Figure 1a) and *T. erythraeum* (Figure 1b) maintaining significantly higher cell-specific growth rates than Fe-replete cultures ($P<0.05$). Thus, cultures that were co-deficient in both Fe and P grew faster than those deficient in P alone, revealing an unexpected interactive relationship between Fe and P availability and cell-specific growth rates.

Fe deficiency allowed both species to maintain positive growth rates at and below the P concentrations at which Fe-replete growth rates fell to zero (0.1 μM, Figure 1, Table 1). Half-saturation constants for growth with respect to P were reduced in Fe-deficient cultures relative to Fe-replete cultures of both *C. watsonii* and *T. erythraeum* (Table 1). Although maximum growth rates of *C. watsonii* were higher than those of *T. erythraeum*, the minimum concentration of P that was required to sustain growth was lowest in Fe-deficient cultures of *T. erythraeum* (Table 1).

Similar to growth rates, the effects of Fe availability on N_2-fixation rates were also reversed in low-P seawater in comparison with high P treatments (Figure 2). In low-P treatments, mean C-specific (Figures 2a and c) and N-specific (Figures 2b and d) N_2-fixation rates by both species were higher in Fe-deficient cultures in comparison with Fe-replete cultures ($P<0.05$). In addition, Fe-deficient cultures of both species were able to fix N_2 (Figure 2) and maintain cell biomass in the form of particulate organic carbon standing stocks (Figure 3) at low P concentrations where Fe-replete cultures were unable to survive (standing stocks of particulate organic carbon at the end of the dilution period integrate differences in growth rates and cellular C quotas between treatments).

This reversal of the expected effects of Fe availability on growth and N_2 fixation at low P concentrations was associated with significant reductions in cell size and elemental quotas under Fe/P co-deficiency in *Crocosphaera*. At low P concentrations (0.1–0.3 μM), mean cell volume in Fe-deficient cultures of *C. watsonii* was 38–61% lower (Figure 4a) and mean weight of combined C, N and P (pg per cell) was 29–57% lower (Figure 4b), relative to Fe-replete cultures ($P<0.05$). The Fe-deficient culture grown at the lowest P level (0.075 μM P) was an exception to this general trend (Figure 4a), likely due to severely reduced growth rates associated with extreme P starvation. Fe-replete cultures, however, were unable to grow at all at 0.075 μM P (Figure 1a). We could not

Table 1 Monod kinetic parameters calculated from hyperbolic functions fitted to data in Figure 1

	K_μ (μM P)[a]	μ_{max} (d^{-1})[b]	C_{min} (μM P)[c]	r^2
C. watsonii				
Fe deficient	0.075	0.37	0.074	0.66
Fe replete	0.16	0.51	0.10	0.94
T. erythraeum				
Fe deficient	0.050	0.25	0.04	0.30
Fe replete	0.16	0.35	0.10	0.95

[a]The half-saturation constant for growth with respect to phosphorus (P).
[b]The maximum growth rate with respect to P.
[c]The minimum concentration of P needed to support growth.

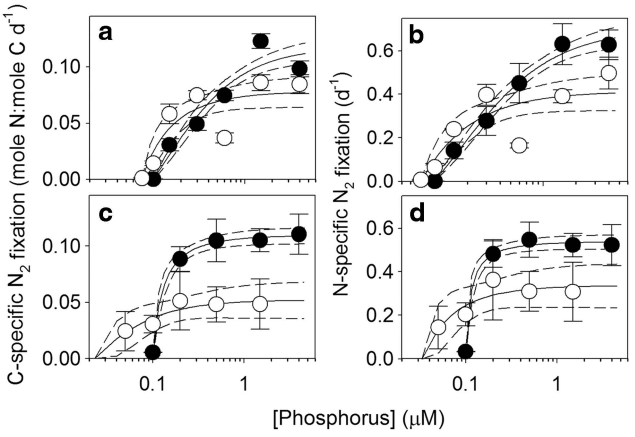

Figure 2 Dinitrogen (N_2)-fixation rates of two N_2-fixing cyanobacteria relative to variations in iron (Fe) and phosphorus (P) concentrations. Mean carbon (C)-specific and N-specific N_2-fixation rates (with s.d.) of *Crocosphaera watsonii* (WH0003) (**a**, **b**) and *Trichodesmium erythraeum* (GBRRLI101) (**c**, **d**) grown over a range of P concentrations (0.05–4.0 μM) under high (450 nM; closed symbols) and low (0.12–0.35 nM; open symbols) Fe concentrations. Monod kinetic constants and parameters of the hyperbolic functions (solid lines) were best fit to the data with 95% confidence intervals on hyperbolas (dashed lines).

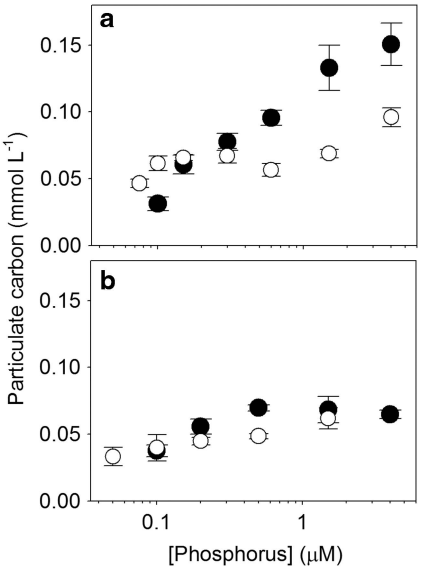

Figure 3 Particulate organic carbon standing stocks in cultures at the time when N_2-fixation rates shown in Figure 2 were estimated. Particulate organic carbon concentrations in cultures of *Crocosphaera watsonii* (WH0003) (**a**) and *Trichodesmium erythraeum* (GBRRLI101) (**b**) grown over a range of added P concentrations (0.05–4.0 μM P) under high (450 nM; closed symbols) and low (0.12–0.35 nM; open symbols) Fe concentrations. Means are plotted with s.d.

accurately estimate *Trichodesmium* cell volume due to its filament-forming habit, but mean weight of combined C, N and P per unit of filament length (pg μm^{-1}) was also significantly lower in Fe-deficient cultures in comparison with Fe-replete cultures ($P<0.05$), with the largest differences (18–59%) in low-P treatments (0.1–0.2 μM P; Figure 4c). In general, the difference in cell volume and cell-specific or filament length-specific masses between Fe-deficient and Fe-replete cultures was largest under very low-P conditions.

Cellular P quotas in Fe-deficient *C. watsonii* were in general slightly higher at high P concentrations and slightly lower at low P concentrations, relative to Fe-replete cultures (Supplementary Figure S1A). Thus, cellular P quotas and growth rates had opposite trends relative to Fe availability. Consequently, P-uptake rates calculated using these two values were not significantly different between Fe-replete and Fe-deficient cultures ($P<0.05$; except when growth rates fell to zero, Supplementary Figure S1B). Along with dissolved P measurements (Supplementary Figure S1A), the similar P-uptake rates further support the idea that phosphate availabilities did not differ substantially between Fe treatments.

To evaluate effects of Fe availability on P-deficient growth, we compared growth affinities with respect to P (μ_{max} /K_μ) between Fe-replete and Fe-deficient cultures (Figure 5). Growth affinities with respect to P were higher for *Crocosphaera* than for *Trichodesmium* in Fe-replete cultures ($P<0.05$), but increased greatly for both species (by 57% for *Crocosphaera* and 129% for *Trichodesmium*; $P<0.05$) to nearly identical elevated values at low Fe concentrations (Figure 5). Thus, Fe limitation provided a demonstrable advantage during P limitation by increasing the efficiency at which both cyanobacteria use P to support their growth.

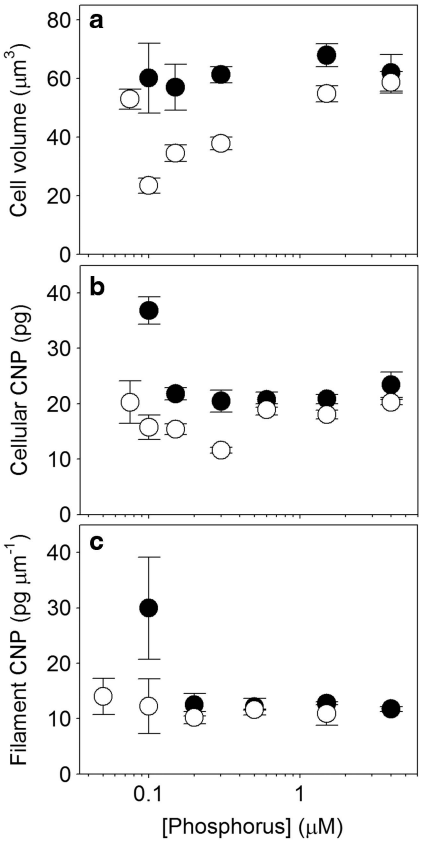

Figure 4 Cell size and major elemental mass of two dinitrogen (N_2)-fixing cyanobacteria relative to variations in both iron (Fe) and phosphorus (P) concentrations. Cell volume of *Crocosphaera watsonii* (WH0003) (**a**) and total summed mass of cellular carbon (C), N and P (pg per cell) of *C. watsonii* (**b**) and summed mass of C, N and P per unit of filament length (pg μm^{-1} filament length) of *Trichodesmium erythraeum* (GBRRLI101) (**c**) grown over a range of added P concentrations (0.05–4.0 μM P) under high (450 nM; closed symbols) and low (0.12–0.35 nM; open symbols) Fe concentrations. s.d. are plotted on treatment means.

Discussion

Our surprising finding is that two widely distributed and ecologically important oceanic N_2-fixing cyanobacteria are able to fix N_2 and grow faster when co-deficient in both Fe and P, than when deficient in P alone. For *Crocosphaera*, one possible mechanism for this unexpected response is a drastic reduction in cell size and cellular elemental quotas in Fe/P co-deficient environments. Both of these species elicited nearly identical responses to changes in relative Fe and P co-deficiency, suggesting that the concentration ratio of Fe:P may be more important in determining oceanic N_2-fixation rates than the concentration of either nutrient alone.

In general, Fe limitation is known to reduce cell size, and this effect has recently been documented for a different strain of *Crocosphaera* (Jacq *et al.*, 2014). Cell size of the *C. watsonii* isolate that we examined (WH0003) declines with decreasing light as well, which is also associated with lower half-saturation constants for growth with respect to P and lower minimum concentrations of P required to

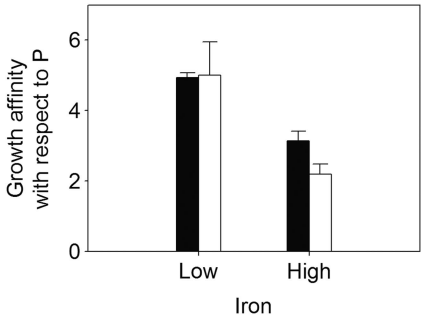

Figure 5 Growth affinities with respect to phosphorus (P) of two dinitrogen-fixing cyanobacteria as a function of iron concentration. Growth affinities (μ_{max}/K_μ) were calculated from Monod hyperbolic parameters in Table 1 for *Crocosphaera watsonii* (WH0003; filled bars) and *Trichodesmium erythraeum* (GBRRLI101; open bars) grown at high (450 nM) and low (0.12–0.35 nM) iron concentrations. Error bars represent propagation of the standard error on μ_{max} and K_μ with respect to P.

maintain growth and N_2 fixation (Garcia *et al.*, 2013). In our experiment, neither P nor Fe had an independent effect on cell size of WH0003, but the combined effect of P and Fe together drastically reduced the cell volume of *Crocosphaera*. The exact mechanisms that restrict flexibility of cell size in Fe-replete cells experiencing P deficiency and P-replete cells experiencing Fe deficiency is not known, but may involve changes in several intracellular pools of C, N, P and Fe.

Two widely recognized advantages of small cells in low nutrient environments are a high cell surface area:volume quotient and a thin diffusion boundary layer, both of which facilitate cross-membrane transport of required elements such as Fe, P, N and C (Sunda and Hardison, 2010). Cell size reductions can relieve uptake-rate limitation and diffusion limitation imposed by any of these required elements, allowing cells to obtain resources more efficiently to support cell growth. For example, P-uptake rates increased as a function of decreasing cell size of *Crocosphaera* WH0003 (Garcia *et al.*, 2013).

Another less commonly acknowledged advantage is that the material and energetic investment for reproduction is also considerably reduced for smaller cells. Because elemental quotas are lower in small cells, the total mass of C, N, P and Fe that must be accumulated before cell division can occur is significantly reduced. This may be an additional mechanism that allows miniaturized Fe-deficient *Crocosphaera* cells to maintain faster growth rates in low-P environments relative to larger, Fe-replete cells. To determine if this reduction in cell volume and elemental quotas could account for the observed changes in growth rates, we compared the relative magnitude of changes in these parameters between Fe-deficient and Fe-replete *Crocosphaera* cells in cultures growing at steady state with 0.15 μM P. Cellular P and total major elemental mass (C + N + P) were both reduced by 29%, and cell volume by 39% (Figure 4) in Fe-deficient cultures relative to Fe-replete cultures. In comparison, Fe-deficient cultures grew 64% faster than Fe-replete ones (Figure 1). Thus, reductions in elemental quotas could potentially account for a large fraction of the higher growth rates in miniaturized *Crocosphaera*, with other recognized mechanisms like faster nutrient uptake rates likely accounting for the rest.

The minimum diameter achieved by *Crocosphaera* cells that were co-deficient in Fe and P in our experiment approached the optimal cell diameter/growth ratio for a range of phytoplankton species documented by Bec *et al.* (2008) and Marañón *et al.* (2013), as originally predicted by Raven (1994). Since cell size is positively correlated with sinking rates (Boyd and Newton, 1999), such shifts in cell size towards optimal size/growth ratios may affect organic carbon drawdown into the deep ocean (Finkel *et al.*, 2007). Thus, cell size plasticity may be important for modeling responses and feedbacks to global change (Morán *et al.*, 2010).

Although there was also a significant difference in CNP mass between Fe-replete and Fe-deficient filaments of *Trichodesmium*, this was caused by increases in Fe-replete, P-limited elemental mass, and not by decreases in CNP mass in Fe-deficient cultures. Thus, our data do not support a strong reduction in CNP mass of *Trichodesmium* cells in cultures grown in low-P, low-Fe seawater, as they do for *Crocosphaera*. Although the mechanism(s) behind the *Trichodesmium* response are unknown, they may be related to other morphological changes such as longer filaments in Fe-deficient cultures in comparison with Fe-replete cultures (data not shown), or to as yet undetermined physiological responses of cellular nutrient acquisition and utilization pathways.

In consideration of the ecological and evolutionary implications of our findings, we examined differences in growth kinetics in terms of classic biological growth modes. Fe-replete cultures had high maximum growth rates (μ_{max}) and high half-saturation constants for growth with respect to P (K_μ), typical of r-selected species, and Fe-deficient cultures had low μ_{max} and K_μ values with respect to P, characteristics of K-selected growth (Figure 1, Table 1). Variable r- and K- growth strategies have been documented among strains and species of marine N_2-fixing cyanobacteria relative to other nutrient resources such as CO_2 (Hutchins *et al.*, 2013), but an intraspecific ability to switch strategies relative to limiting nutrients has not been described previously within single microbial isolates. Rather, this environmental response is assumed in paradigms that describe evolution of species, where flexible growth strategies within strains likely precede selection for more permanent species-specific changes in cell size and growth rate relationships (Litchman *et al.*, 2007).

Clearly, Fe-deficient cells of both species use limiting concentrations of P to support their growth more efficiently than do Fe-replete cells. In our experiments, high concentrations of Fe effectively raised the minimum concentration of P that was needed to support positive N_2-fixation rates, growth and standing stocks of particulate organic carbon (Table 1; Figure 5). Thus, the effect of Fe availability on growth affinities with respect to P for photosynthetic N_2 fixers may have broad implications for linking the C, N, P and Fe biogeochemical cycles. Although a dustier climate has been hypothesized to yield high N_2 fixation and primary production rates over geological time scales (Falkowski, 1997; Michaels *et al.*, 2001), our data suggest that increasing Fe input to regions where P is chronically low could actually have a negative effect on N_2 fixation. Our results imply that N_2-fixation rates, primary production and carbon export may all be sensitively attuned to small changes in Fe:P input ratios to the sunlit layers of the oceans.

In addition to cell physiology and ocean biogeochemistry, the linkage between Fe and P availability could also affect marine ecology. Small cells are

more vulnerable to grazing (Sunda and Hardison 2010), but faster growth rates could assist in compensating for such increased grazing mortality. Conversely, in high-Fe, low-P environments, larger cells might offset grazing mortality, bolstering survivability despite slower growth rates. In response to long-term exposure to specific Fe:P conditions, simultaneous bottom-up and top-down selection of N_2-fixing ecotypes and species could result from phenotypic tradeoffs between growth and cell size. Our experimental results indicate that Fe and P control the expression of size phenotypes in *Crocosphaera* and r- and K-selected growth in both species, demonstrating a possible means by which divergent N_2-fixing strains and species might evolve in contrasting biogeochemical environments (Finkel *et al.*, 2007). The general high abundance of larger N_2-fixing phototrophic taxa in high-Fe waters of the North Atlantic relative to low-Fe waters of the North Pacific Ocean (Wu *et al.*, 2000; Sohm *et al.*, 2011) seems to support selection of N_2-fixing cyanobacteria cell size based on Fe input. Our results suggest that Fe:P ratios may be more important than the absolute concentration of either nutrient in selecting for strain and species dominance in various ocean basins and regions, and could thereby control bulk N_2-fixation rates and affect plankton community structure.

Several field studies indicate dynamic relationships between Fe and P in controlling N_2 fixation. In the eastern tropical North Atlantic Ocean, experimental Fe and P additions to natural plankton communities suggest Fe and P co-limitation of N_2 fixation (Mills *et al.*, 2004). Other studies, however, indicate that N_2-fixation rates are relatively high in the western North Atlantic in comparison with the eastern portion of this basin, despite decreasing Fe inputs with increasing distance from North Africa (Capone *et al.*, 2005; Mather *et al.*, 2008; Mahowald *et al.*, 2009; Moore *et al.*, 2009). A close balance between Fe and P availability in controlling N_2-fixation rates may also be implicit in studies from the North Pacific Subtropical Gyre, where Fe and P additions to natural phytoplankton communities yielded variable responses between study sites (Grabowski *et al.*, 2008). In these types of short-term field experiments, it may be important to distinguish between nutrient co-deficiency and co-limitation, as short and long-term responses to nutrient supplies may be very different. Responses such as the N_2-fixation rate and cell size changes we observed in our steady-state cultures may not be manifested in short shipboard incubation experiments, as they likely depend on the acclimated phenotype and long-term plasticity of cells.

Overall, our results suggest that these N_2-fixing cyanobacteria share a common strategy that allows them to maintain relatively high growth rates in Fe- and P-co-deficient environments, conditions that characterize vast areas of the oligotrophic regions where these species grow. Varying ratios of Fe and P may also create a range of ecological niches for at least the unicellular N_2-fixing cyanobacteria, through tradeoffs between cell size and growth rates. Fe deficiency appears to be advantageous during P limitation because it affords *Crocosphaera* a viable strategy to help maintain higher cell-specific growth rates–cellular miniaturization–that is not available to Fe-replete cells. For *Trichodesmium*, the cell size response appears instead to consist of increases in filament mass under Fe-replete, P-limited conditions. Various major cellular elemental pools could be involved in controlling cell size plasticity and Fe and P-use efficiencies for growth, including those associated with the nitrogenase complex, photosynthetic electron transport (Raven 1988), polyphosphates (Rao *et al.,* 2009), Fe storage compounds such as ferritin (Keren *et al.*, 2004) or other proteins and nucleic acids (Raven *et al.,* 2013).

Future work should examine the physiological, biochemical and genetic mechanisms involved in cell morphological responses to Fe concentrations, as biogeochemical models may need to understand these mechanisms in order to better parameterize nutrient co-limitation and co-deficiency and their effects on the biological pump (Moore *et al.,* 2013). Regardless of the mechanism, our results suggest that if Fe:P supply ratios change as the future surface ocean becomes warmer and more stratified with lower P fluxes from below (Sarmiento *et al.*, 2004), we may expect corresponding changes in cell size of N_2-fixing cyanobacteria, new N inputs, standing stocks of organic carbon and overall biological community structure.

Conflict of Interest

The authors declare no conflict of interest.

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