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Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer *Trichodesmium*

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[1] Diazotrophic (N_2-fixing) cyanobacteria provide the biological source of new nitrogen for large parts of the ocean. However, little is known about their sensitivity to global change. Here we show that the single most important nitrogen fixer in today's ocean, *Trichodesmium*, is strongly affected by changes in CO_2 concentrations. Cell division rate doubled with rising CO_2 (glacial to projected year 2100 levels) prompting lower carbon, nitrogen and phosphorus cellular contents, and reduced cell dimensions. N_2 fixation rates per unit of phosphorus utilization as well as C:P and N:P ratios more than doubled at high CO_2, with no change in C:N ratios. This could enhance the productivity of N-limited oligotrophic oceans, drive some of these areas into P limitation, and increase biological carbon sequestration in the ocean. The observed CO_2 sensitivity of *Trichodesmium* could thereby provide a strong negative feedback to atmospheric CO_2 increase.

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1. Introduction

[2] Diazotrophic cyanobacteria play an important role in marine ecosystems and biogeochemical cycles [*Mulholland*, 2006]. They provide the biological source of new nitrogen in large parts of the oligotrophic ocean [*Codispoti et al.*, 2001]. *Trichodesmium*, a colony-forming cyanobacterium, fixes nitrogen in an area corresponding to almost half of Earth's surface [*Davis and McGillicuddy*, 2006] and is estimated to account for more than half of the new production in parts of the oligotrophic tropical and subtropical oceans [*Capone et al.*, 2005; *Mahaffey et al.*, 2005]. Future expansion of the oligotrophic subtropical provinces to higher latitudes due to surface ocean warming and increased stratification is expected to change the spatial extent of *Trichodesmium* and hence the magnitude of global N_2 fixation by this organism [*Boyd and Doney*, 2002; *Breitbarth et al.*, 2007].

[3] In addition to sea surface warming, the oceans are experiencing another change of global significance related to CO_2 increase, namely the acidification of seawater due to the massive uptake of fossil fuel CO_2 [*Sabine et al.*, 2004]. The corresponding change in seawater carbonate chemistry results in an increase in hydrogen ion activity and CO_2 concentration, along with a corresponding decrease in carbonate ion concentration and carbonate saturation state. Oceanic CO_2 uptake has already caused a reduction in upper ocean pH by 0.1 units [*Feely et al.*, 2004] and is expected to cause a further decrease by 0.3–0.4 units by the end of this century if CO_2 emissions continue at current trends. While effects of CO_2-related seawater acidification have been demonstrated for a variety of marine microalgae and cyanobacteria [*Giordano et al.*, 2005], mainly focusing on carbon acquisition and concentrating mechanisms [*Burkhardt et al.*, 2001], little is known about it's impact on marine diazotrophs [*Levitan et al.*, 2007]. To examine the influence of CO_2–induced changes in seawater chemistry on *Trichodesmium*, we have grown this species over a range of CO_2 concentrations under controlled laboratory conditions.

2. Material and Methods

2.1. Experimental Setup

[4] Semicontinuous batch cultures of *Trichodesmium* IMS101 were kept in exponential growth at CO_2 partial pressures ranging levels from 140 to 850 μatm. This corresponds to pHt (pH on the total scale) values from 8.5 to 7.8 and, for that reason, a sixfold increase in CO_2, a 1.2-fold increase in bicarbonate (HCO_3^-) and a fourfold decrease in CO_3^{2-} concentrations. Cultures were grown in 0.2 μm sterile filtered YBCII medium [*Chen et al.*, 1996] with 5 μmol kg^{-1} phosphate and no nitrate addition, at 25 °C, a photon flux density of 150 μmol m^{-2} s^{-1} (supplied from cool white fluorescent bulbs, Philips TLD 36W/54) and a 14/10 hour light/dark cycle. CO_2 levels were adjusted by different additions of NaOH to media with equal amount of dissolved inorganic carbon (DIC). Cells were acclimatized to the experimental conditions for approximately two months. In the course of each experiment, cultures were allowed to grow in 1 L polycarbonate bottles for a maximum of three generations, so that DIC would not decrease more than 3%. Cultures were always diluted to the same starting chlorophyll *a* (Chl *a*) concentration (approximately 3 μg/L) in order to

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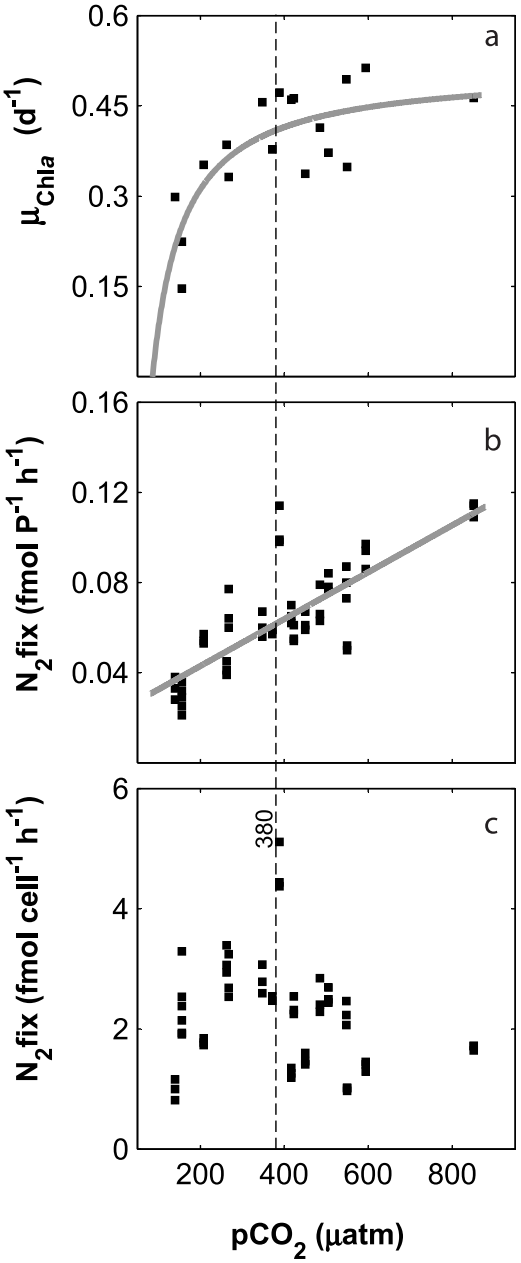

Figure 1. (a) Growth rate based on Chl *a* (μ) of *Trichodesmium* in relation to CO_2 levels (pCO_2). The solid line was obtained by fitting the data to a modified Michaelis-Menten curve which allows for a minimum cellular requirement of CO_2. Rates of nitrogen fixation (N_2fix) (b) per unit of POP and (c) per cell by *Trichodesmium* in relation to CO_2 levels (pCO_2). The solid line in Figure 1b was obtained by linearly fitting the data. In Figures 1b and 1c, triplicate measurements (squares) were made on each sampling day (3 days in total). The vertical dashed line denotes present-day CO_2.

maintain exponential growth. Sampling occurred 4 or 5 days after the previous dilution, when Chl *a* reached approximately 20 μg/L, at a fixed time (10 am) to avoid introducing bias due to diel variability. Cell abundances were determined by cell counts of Lugol preserved samples (2−3% final concentration).

2.2. Carbonate system

[5] CO_2 concentrations were calculated from dissolved inorganic carbon (DIC) and total alkalinity using the temperature- and salinity-dependent dissociation constants given by the *Department of Energy* [1994]. DIC was measured photochemically [*Stoll et al.*, 2001] using an automated segmented-flow analyzer (Quaatro) equipped with an autosampler. Alkalinity was measured according to *Dickson et al.* [2003] in duplicate through potentiometric titration, using a 794 Basic Titrino (Metrohm).

2.3. Nitrogen Fixation

[6] Nitrogen fixation rates were determined by the acetylene reduction assay [*Capone*, 1993], using a gas chromatograph with flame ionization detector (SHIMADZU GC-14B) and calculated according to *Capone* [1993], considering the Bunsen gas solubility coefficient determined for 25°C by *Breitbarth et al.* [2004].

2.4. Cell Dimensions and Numbers

[7] Fresh samples for cell measurements were collected onto GTBP black filters (0.2 μm) under low vacuum (200 mbar). For each condition, fresh preparations were made with immersion oil (IMMERSION OIL-UVFL, SI, Olympus) and cell dimensions were determined (approximately 100 cells measured) using autofluorescence conditions at 1000x magnification (Zeiss optic microscope with a fluorescence lamp). Cell count samples were preserved with Lugol (2−3% final concentration), filtered on polycarbonate filters (25 mm diameter and 0.2 μm pore size) and photographed systematically (80x magnification) along a transect covering the diameter of the filter with additional supplemental photos (Leica MZ12 Binocular) taken randomly (not in the statistical sense). Individual filaments were enumerated and measured with the computer program Image J. Trichome length was then divided by the cell lengths determined for each condition, providing the corresponding number of cells for each sampling day.

2.5. Cell Contents

[8] Chlorophyll *a* samples were analyzed fluorometrically (10-AU Fluorometer, GAT) according to *Derenbach* [1969] and *Welschmeyer* [1994]. Samples for cellular particulate organic carbon (POC) and nitrogen (PON) were analyzed in a gas chromatograph (EURO EA Elemental Analyser, EUROVECTOR equipped with a thermal conductivity detector and an element analyzer) following *Sharp* [1975]. Particulate organic phosphorus (POP) filters were submitted to alkaline persulphate oxidation (adapted from *Hansen and Koroleff* [1999]) and measured colorimetrically by means of a spectrophotometer (UV-1202, UV-VIS Spectrophotometer, SHIMADZU). Cell morphology was observed by inverted microscopy (Zeiss) at 1000x magnification.

3. Results

[9] Over the experimental CO_2 range (140 to 850 μatm), cell division rate of *Trichodesmium* increased about twofold when based on Chl *a* (Figure 1a), POC, PON, POP and cell numbers (data not shown). Nitrogen fixation rate normal-

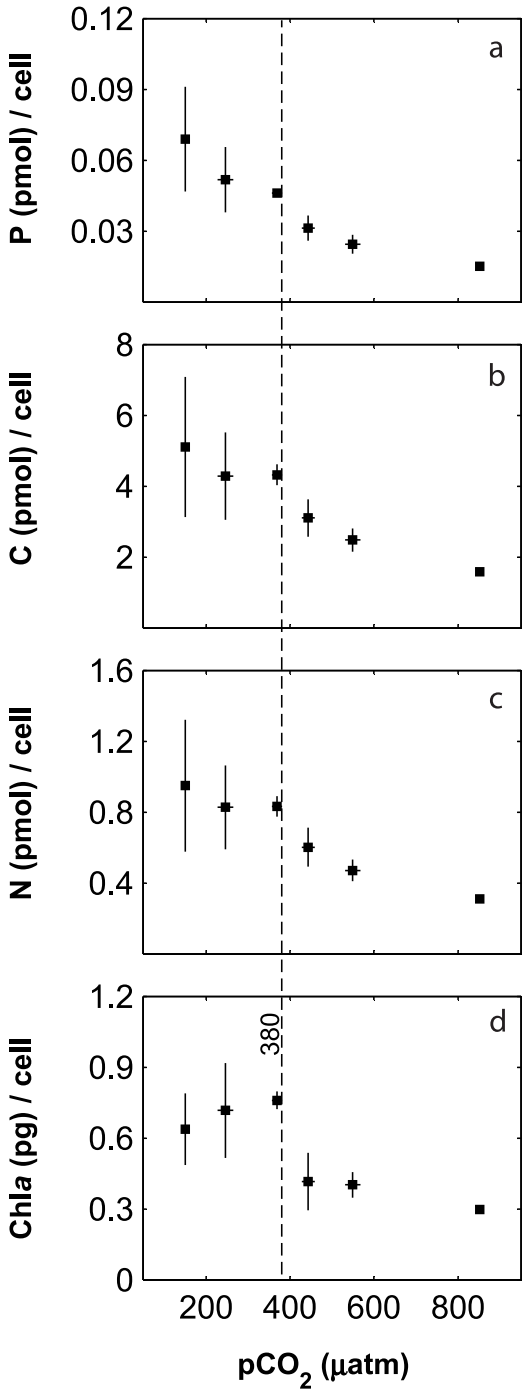

Figure 2. Cellular elemental contents of *Trichodesmium* in relation to CO_2 levels (pCO_2). Cellular contents presented are (a) phosphorus, (b) carbon, (c) nitrogen, and (d) chlorophyll *a*. The squares are averages of defined pCO_2 groups (100 μatm) with vertical and horizontal errors bars representing standard errors for each group. Dashed vertical line denotes present-day CO_2.

ized to cellular phosphorus quota (Figure 1b) and Chl *a* content (data not shown) increased threefold over the experimental CO_2 range. This corresponds to a 50% increase in P-normalized N_2 fixation for atmospheric CO_2 increasing from its present value (380 μatm) to that projected for 2100 (750 μatm) assuming a business as usual CO_2 emission scenario. Owing to the strong reduction in cell content with increasing CO_2 (see below), C and N_2 fixation rate demonstrate no trend with CO_2 when normalized on a per cell basis (Figure 1c).

[10] Increased cell division rate at elevated CO_2 was associated with lower cellular content of C, N, P, and Chl *a* (Figure 2). On average, cell length in high CO_2 grown cultures was ca. 20% lower than under low CO_2 conditions, with no significant difference in cell width. This can be

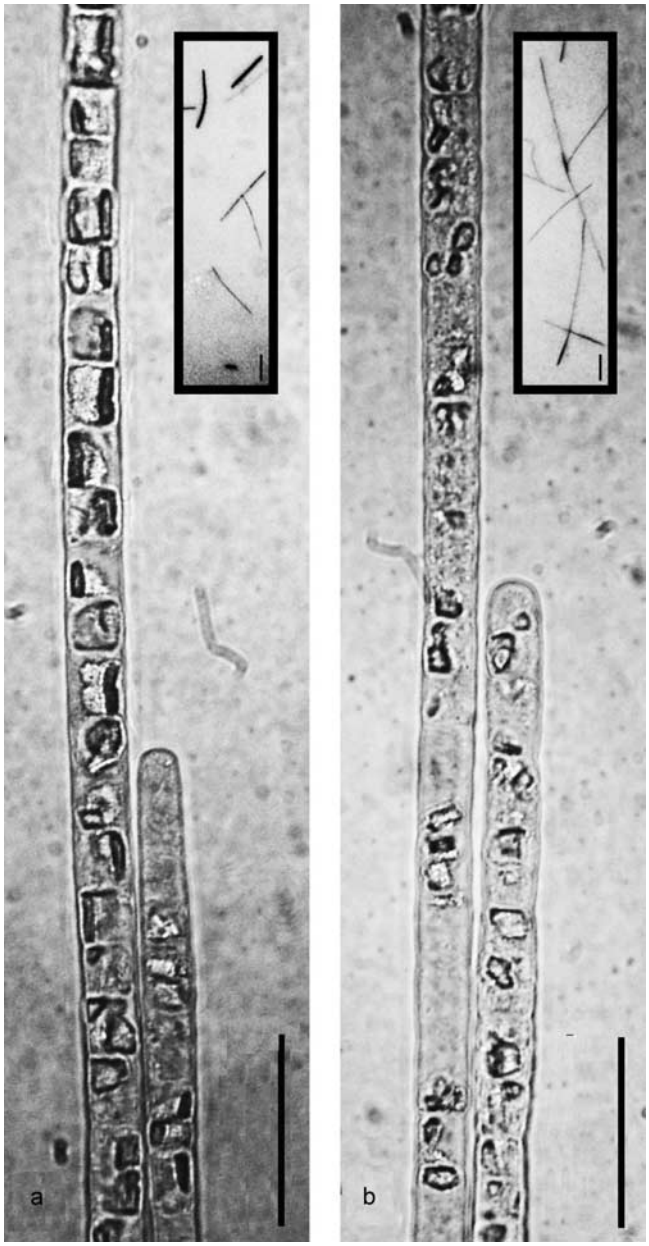

Figure 3. Cell morphology of *Trichodesmium* IMS101 visualized at 1000x magnification acclimatized to (a) 167 μatm and (b) 700 μatm CO_2 levels. Insert: corresponding trichome view at 80x magnification. Scale bars: 25 μm (Figures 3a and 3b), 200 μm (bottom right of inserts).

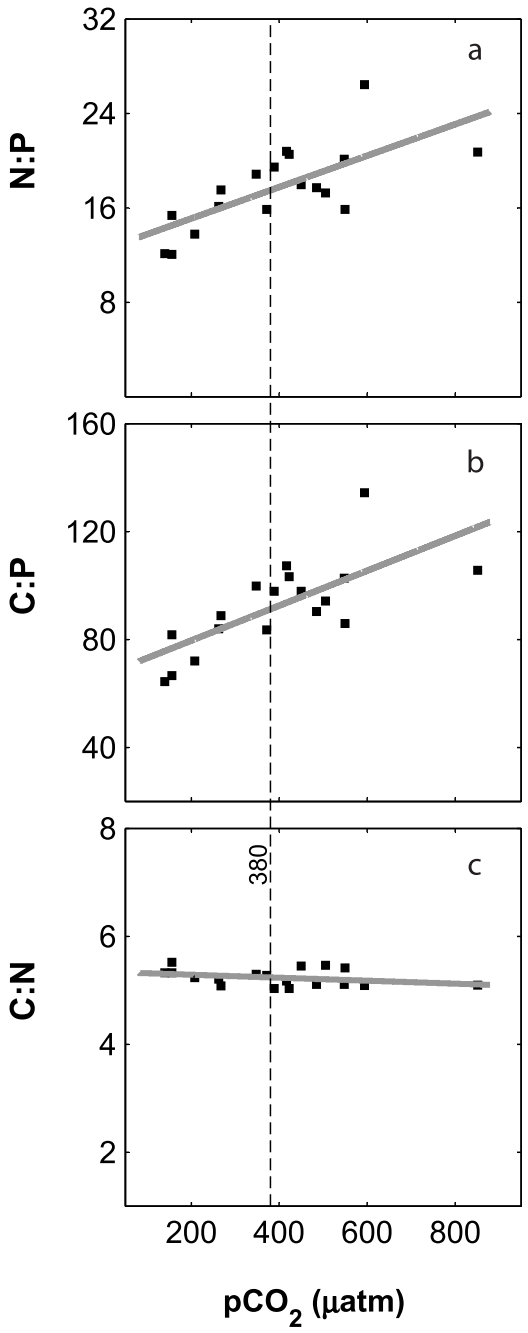

Figure 4. Particulate organic matter ratios of *Trichodesmium* in relation to CO_2 levels (pCO_2). (a) Nitrogen to phosphorus ratio. (b) Carbon to phosphorus ratio. (c) Carbon to nitrogen ratio. The solid lines were obtained by a linear fit through the respective data. The vertical line denotes present-day CO_2.

explained by the larger fraction of newly divided cells in the faster growing high CO_2 cultures. There was also a concomitant 7−35% increase in trichome length (Figure 3 inserts) caused by a higher number of cells per trichome. Consistent with the reduction of cell quotas, cells grown at higher CO_2 appeared less granulated (Figure 3).

[11] CO_2-stimulated C and N_2 fixation in relation to P assimilation caused a distinct shift in cellular stoichiometry. Over the experimental range, N:P and C:P ratios increased from approximately 13:1 and 69:1, respectively, at the low CO_2 to 24:1 and 124:1, respectively, at high CO_2 (Figures 4a and 4b). For the projected increase in atmospheric CO_2 over the course of the 21st century (380 to 750 μatm), this corresponds to an approximately 40−50% increase in N:P and C:P ratios, i.e., consistent with the independently determined change in P normalized N_2 fixation rates. In contrast, the C:N ratio remained unaffected at about 5.2 over the entire CO_2 range (Figure 4c).

4. Discussion

[12] CO_2 concentration has been previously reported to affect photosynthetic carbon fixation of marine phytoplankton [*Hein and Sand-Jensen*, 1997; *Leonardos and Geider*, 2005; *Raven*, 2003; *Riebesell et al.*, 1993], but its significance in modifying oceanic primary production remains uncertain [*Riebesell*, 2004]. More importantly, the magnitude of the observed CO_2 effect on *Trichodesmium* by far exceeds those previously seen in other photoautotrophs. A twofold to threefold increase in N_2 fixation rate per unit of phosphorus utilization and a doubling in growth rate as CO_2 increases from glacial (180 μatm) to year 2100 CO_2 levels (750 μatm) makes *Trichodesmium* one of the most CO_2 sensitive primary producer tested to date [*Giordano et al.*, 2005].

[13] An unusually strong response also occurred with respect to changes in the cellular elemental composition, with C:P and N:P ratios increasing approximately twofold over the experimental CO_2 range (Figure 4). Unlike previous studies [*Burkhardt et al.*, 1999; *Gervais and Riebesell*, 2001], our results for *Trichodesmium* do not indicate a CO_2 saturation level in the range tested, above which elemental ratios remain constant. Moreover, contrary to other phytoplankton groups, which generally increase cellular carbon quotas with increasing CO_2 availability [*Riebesell*, 2004; *Rost et al.*, 2002], C quota (as well as cellular N, P, and Chl *a* contents) of *Trichodesmium* decreases with increasing CO_2. This difference is due to the strong CO_2 effect on cell division rate in *Trichodesmium*, which again is generally not found in other phytoplankton groups to the same extent [*Rost et al.*, 2002].

[14] The nature of the observed CO_2 effect on carbon and nitrogen fixation in *Trichodesmium* is presently unknown. Both photosynthetic carbon fixation and diazotrophic nitrogen fixation are energy demanding processes, which compete for energy and reducing power with a variety of other cellular processes, such as protein synthesis [*Geider and MacIntyre*, 2001] and carbon acquisition [*Kaplan and Reinhold*, 1999]. Owing to the relatively low affinity of their main carboxylating enzyme Rubisco (ribulose bisphosphate carboxylase oxygenase) [*Tortell*, 2000], cyanobacteria have to invest significant amounts of energy to concentrate CO_2 at the site of carboxylation. This so-called CO_2 concentrating mechanism (CCM) includes the cost of active HCO_3^- and CO_2 transport into the cell and to the site of carboxylation, and the synthesis of the proteins involved

in CCM activity (e.g., transporters and carbonic anhydrase). Cyanobacteria are known to down-regulate their CCM in response to increasing CO_2 availability [*Giordano et al.*, 2005], thereby allowing allocation of energy to other cellular processes.

[15] *Trichodesmium* has a low-affinity Na^+/HCO_3^- symport [*Badger et al.*, 2006], which is likely to be dependent on an energy demanding Na^+/H^+ antiport system [*Badger et al.*, 2006; *Giordano et al.*, 2005]. Moreover, this species also has a low-affinity CO_2 uptake complex dependent on reductive power present in the thylakoid membrane [*Badger et al.*, 2006]. Two processes possibly affected by ambient CO_2 concentration are diffusive uptake of CO_2 by the cell and CO_2 leakage out of the cell. Elevated CO_2 concentrations could reduce the diffusive loss of CO_2 from the cell and/or increase the proportion of diffusive CO_2 uptake into the cell, resulting in a down-regulation of CCM activity. As a consequence, competing processes such as C and N_2 fixation may benefit by receiving additional energy and reductive power. In fact, the surprisingly high CO_2 sensitivities of C and N_2 fixation imply major changes in energy allocation in response to changing CO_2 availability. A possible explanation may be found in the low CO_2 affinity of cyanobacterial Rubisco. As one of the oldest life forms on planet Earth [*Falkowski et al.*, 2004] cyanobacteria rely on a Rubisco with lower affinity to CO_2 in relation to O_2 when compared to more recently evolved phytoplanktonic groups [*Tortell*, 2000]. To compensate for this, cyanobacteria need to invest considerable amount of energy in concentrating CO_2 at the site of carboxylation. Thus the energetic gain at elevated CO_2 may be higher in cyanobacteria compared to other phytoplanktonic groups with Rubiscos characterized by higher CO_2 affinities. Clearly, further studies are needed to examine CCM regulation of this ecologically and biogeochemically important diazotroph.

[16] If representative of the natural environment, the observed CO_2 sensitivity of *Trichodesmium* would have broad implications in a changing ocean. The increase of N:P and C:P in *Trichodesmium*-dominated oceanic regimes may affect the nutritional value of primary produced organic matter as well as the stoichiometry of particulate matter sinking to depth. On the ecosystem level this could impact the efficiency of bacterial degradation and zooplankton reproduction, with possible cascading effects on the pelagic food web [*Sterner and Elser*, 2002]. From a biogeochemical point of view, changing C:P and C:N ratios may affect the remineralization depth, the pool of bioavailable nitrogen and consequently carbon sequestration via the biological carbon pump. Extrapolation of this finding to the natural environment should be done with caution, however, since other factors (e.g., iron and phosphorus availability and temperature) may influence *Trichodesmium*'s response to rising CO_2.

[17] Global *Trichodesmium* N_2 fixation, is estimated from direct measurements at 60 to 85 Tg N a^{-1} [*Galloway et al.*, 2004; *LaRoche and Breitbarth*, 2005; *Mahaffey et al.*, 2005], accounting for half of the new production in parts of the tropical and subtropical oceans [*Capone et al.*, 2005]. On the basis of our results, this could rise to 90 to 128 Tg N a^{-1} by 2100 in a business-as-usual CO_2 emission scenario (IS92a) [*Intergovernmental Panel on Climate Change*, 2001]. Moreover, recent estimates of *Trichodesmium* subsurface abundances raise the basin-scale average nitrogen fixation rate by a factor of 2.7 to 5.0 [*Davis and McGillicuddy*, 2006].

[18] In view of recent studies, suggesting that global N_2 fixation by unicellular cyanobacteria may be as high or higher than that of *Trichodesmium* [*Montoya et al.*, 2004; *Zehr et al.*, 2001], it appears important to examine the extent to which the observed CO_2 sensitivity of *Trichodesmium* N_2 fixation also applies to other diazotrophic cyanobacteria.

[19] A CO_2-induced increase in N_2 fixation would increase bioavailable nitrogen in N-limited oligotrophic oceans, either by release of ammonia and dissolved organic nitrogen [*Mulholland et al.*, 2004] or by cell death (programmed cell death, grazing and/or viral attack), possibly fueling productivity of other phytoplanktonic groups. This may enhance phosphate utilization, driving some of these areas to P limitation under future high CO_2 conditions. However, given that other environmental changes are expected with rising CO_2 such as temperature and atmospheric dust deposition, the relative importance of the CO_2 effect on nitrogen fixation needs to be further assessed in the context of the future ocean. The expected rise in global sea surface temperature, leading to enhanced stratification, decreased mixed layer depth and decreased nutrient availability, has been suggested to result in an increase in nitrogen fixation [*Boyd and Doney*, 2002]. Because the increase in temperature may also result in a reduction of the area characterized by optimum nitrogen fixation and growth [*Breitbarth et al.*, 2007], the overall effect of ocean warming on diazotrophs is still uncertain.

[20] We here show that not only *Trichodesmium* responds to rising CO_2, but as one of the oldest life forms on planet Earth it is more sensitive then other groups previously considered (e. g., coccolithophores and diatoms). If the observed effect on *Trichodesmium* is a general phenomenon in diazotrophic cyanobacteria our results would predict an increase in global ocean N_2 fixation at CO_2 levels expected for the future ocean. This in turn, would increase the nitrogen inventory, resulting in increase future primary productivity and oceanic carbon sequestration.

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