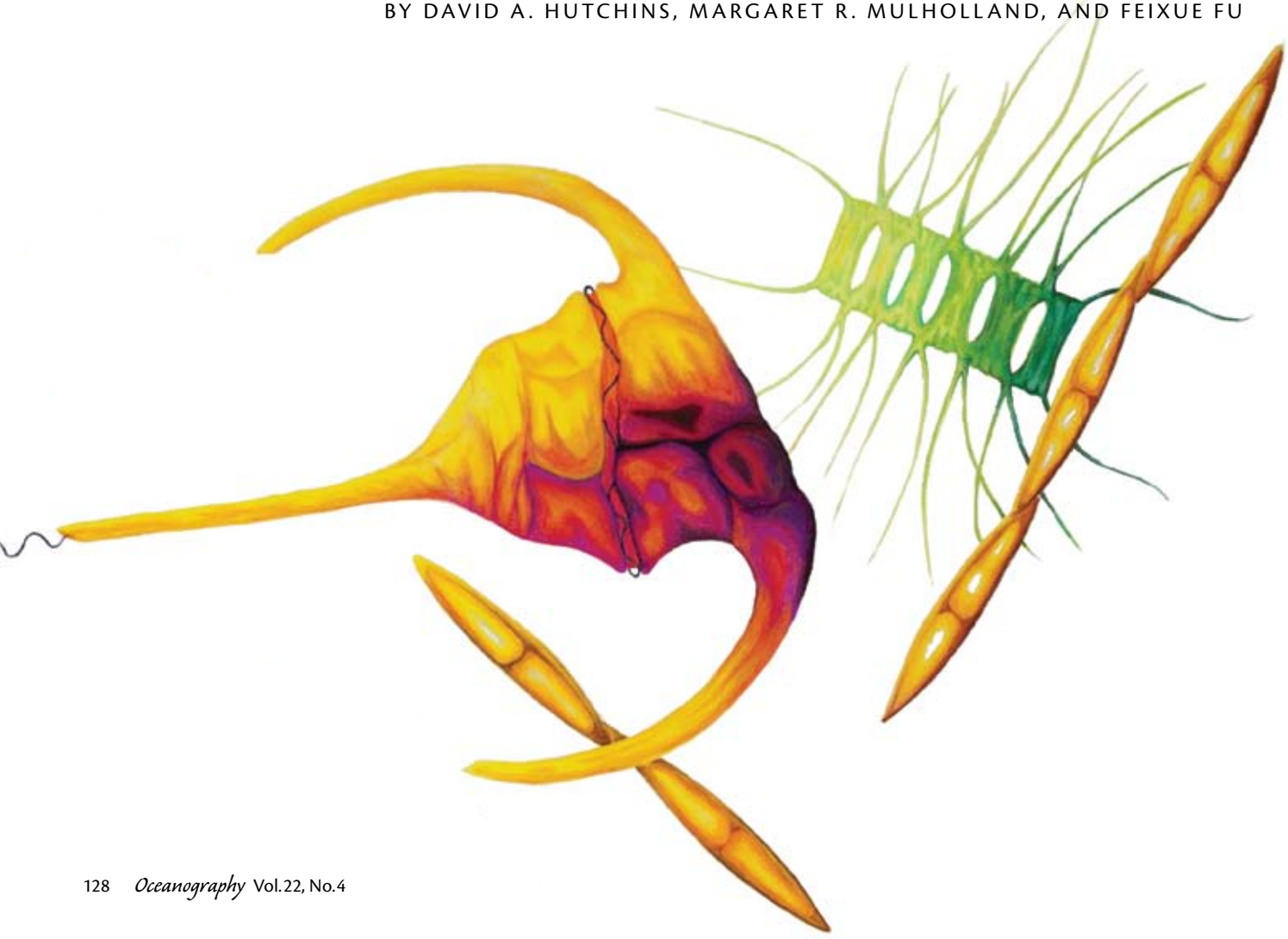
Nutrient Cycles and Marine Microbes in a CO_2-Enriched Ocean

BY DAVID A. HUTCHINS, MARGARET R. MULHOLLAND, AND FEIXUE FU



ABSTRACT

The ocean carbon cycle is tightly linked with the cycles of the major nutrient elements nitrogen, phosphorus, and silicon. It is therefore likely that enrichment of the ocean with anthropogenic CO_2 and attendant acidification will have large consequences for marine nutrient biogeochemistry, and for the microbes that mediate many key nutrient transformations. The best available evidence suggests that the nitrogen cycle may respond strongly to higher CO_2 through increases in global N_2 fixation and possibly denitrification, as well as potential decreases in nitrification. These trends could cause nitrification to become a nitrogen cycle "bottleneck," by increasing the flux of N_2 fixed into ammonium while decreasing the fraction being oxidized to nitrite and nitrate. The consequences could include reduced supplies of oxidized nitrogen substrates to denitrifiers, lower levels of nitrate-supported new primary production, and expansion of the regenerated production system accompanied by shifts in current phytoplankton communities. The phosphorus and silicon cycles seem less likely to be directly affected by enhanced CO_2 conditions, but will undoubtedly respond indirectly to changing carbon and nitrogen biogeochemistry. A review of culture experiments that examined the effects of increased CO_2 on elemental ratios of phytoplankton suggests that for most cyanobacteria and eukaryotes, C:N and N:P ratios will either remain at Redfield values or increase substantially. Natural plankton community CO_2 manipulation experiments show much more mixed outcomes, with both increases and decreases in C:N and N:P ratios reported at future CO_2 levels. We conclude our review with projections of overall trends in the cycles of nitrogen, phosphorus, and silicon over the next century as they respond to the steady accumulation of fossil-fuel-derived CO_2 in a rapidly changing ocean.

INTRODUCTION

The accumulation of fossil-fuel-derived CO_2 in the sea and consequent ocean acidification represents an unprecedented human perturbation of ocean chemistry on a global scale. A fundamental theme of modern oceanography that dates back to the classic work of Redfield (1958) is a recognition of the intimate interweaving of the marine biogeochemical cycle of carbon with the cycles of the major nutrient elements nitrogen, phosphorus, and silicon. Consequently, the ongoing and accelerating enrichment of seawater with anthropogenic CO_2 will likely have far-reaching effects not only on the carbon cycle, but also on the cycles of these biologically required nutrients.

Many of the key pathways and transformations in ocean nutrient cycles are mediated by autotrophs. Photoautotrophs, including cyanobacteria and eukaryotic phytoplankton, fix CO_2 using sunlight as an energy source, while chemoautotrophic bacteria and archaea do the same thing using chemical sources of energy. Together, these two broadly defined functional groups provide the raw materials and energy that fuel all of the ocean's food webs. For autotrophic groups that depend on carbon fixation for a living, rising partial pressure of CO_2 (pCO_2) in the ocean may actually represent an opportunity rather than a misfortune. The term "ocean acidification" carries undeniably negative connotations, and indeed it is hard to envision how most animals and microbial heterotrophs could benefit from lowered ocean pH. However, dissolved inorganic carbon enrichment is an equally accurate description of anthropogenic input of CO_2 to the ocean, and this terminology evokes a much different image of how communities might respond. Much of our view of ocean acidification as an unmitigated stressor for marine life comes from well-justified concern over its negative impacts on corals and other calcifying organisms (Kleypas et al., 1999; Feely et al., 2004; Fabry et al., 2008). It is important to remember, though, that for many of the autotrophs that power the cycles of carbon and nutrients in the ocean and support virtually all marine biological productivity, the term "CO_2 fertilization" may be more appropriate. There are several comprehensive recent reviews on the effects of changing pCO_2

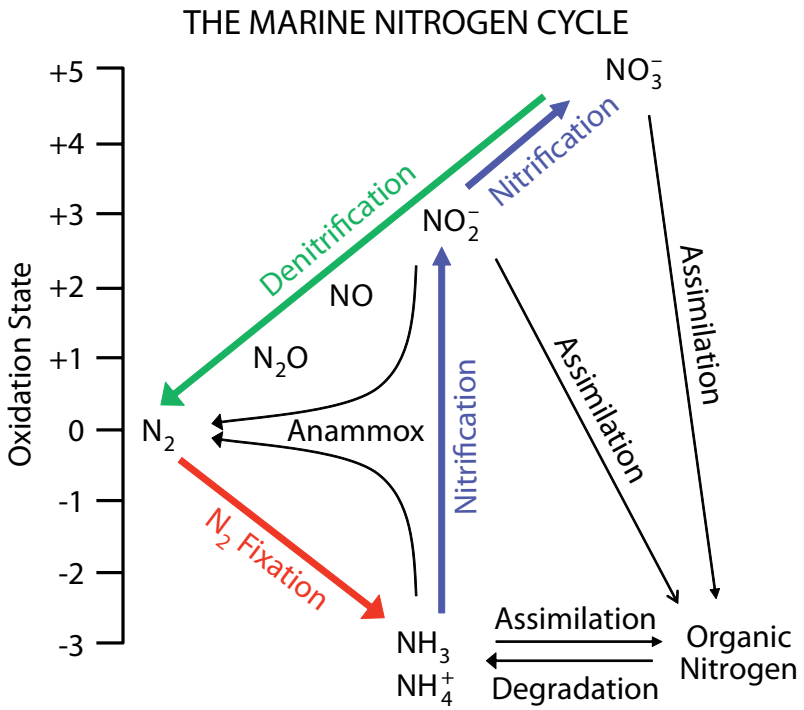


Figure 1. Major chemical forms and transformations of nitrogen in the ocean. The various chemical forms of nitrogen are plotted versus their oxidation states. Here, we consider the potential effects of increased ocean pCO_2 on three of the critical transformations within the N cycle: N_2 fixation (red arrow), nitrification (blue arrows), and denitrification (green arrow).

on phytoplankton physiology, including Riebesell (2004) and Rost et al. (2008).

In this article we explore what is currently known about the implications of ocean acidification and CO_2 fertilization for the biogeochemical cycles of nitrogen, phosphorus, and silicon, and for the keystone marine organisms that mediate them, including photoautotrophs, chemoautotrophs, and heterotrophs. Along with the rapidly mounting concern about ocean acidification, the marine science community has produced a flood of new information from experimental studies in both the laboratory and the field. These novel results are helping to provide the perspective we need to make educated guesses in response to such questions as: How will crucial processes in the marine nitrogen cycle, including nitrogen fixation, nitrification, and denitrification, respond to ocean acidification? Will the cycles of phosphorus and silicon change in response to anthropogenic CO_2 inputs, and if so, how? How will elevated pCO_2 affect the C:N:P stoichiometry of phytoplankton in the ocean? Finally, we conclude this review with a projection of how nutrient biogeochemistry might be altered in the future in response to CO_2 enrichment and concomitant ocean acidification. We acknowledge that our projections are speculative, as we recognize that many large gaps remain in this developing picture, and there is a need for additional work to fill these gaps.

David A. Hutchins *(dahutch@usc.edu) is Professor of Marine Environmental Biology, Department of Biological Sciences, University of Southern California, Los Angeles, CA, USA.* **Margaret R. Mulholland** *is Associate Professor, Department of Ocean, Earth, and Atmospheric Sciences, Old Dominion University, Norfolk, VA, USA.* **Feixue Fu** *is Research Assistant Professor, Department of Biological Sciences, University of Southern California, Los Angeles, CA, USA.*

THE NITROGEN CYCLE

The potential for ocean acidification to affect multiple processes within the marine nitrogen cycle is obvious, because this complex cycle involves compounds that are stable across eight oxidation states (Figure 1). Microbes are the primary mediators of the cycling of nitrogen between compounds, and through their metabolisms the nitrogen and carbon cycles are inextricably linked. Consequently, CO_2 enrichment or acidification-related shifts in both cell physiology and biological community structure could have major impacts on ocean biogeochemical cycles.

New nitrogen enters the ocean through the fixation of atmospheric dinitrogen (N_2) by marine N_2 fixers, also known as diazotrophs (Figure 1, red arrow). Nitrogen losses from the ocean are through organisms that reduce oxidized N (primarily NO_3^-) to N_2 through classical denitrification, an anaerobic process (Figure 1, green arrow). Further losses of fixed N from the ocean occur through anaerobic ammonium (NH_4^+) oxidation via so-called "anammox." Once input into the ocean, nitrogen is cycled through microbial and food web interactions. Nitrogen taken up into particulate material and microbial biomass is released and regenerated

as ammonium and dissolved organic compounds. In aerobic waters, nitrifying bacteria and archaea can then oxidize ammonium, producing first nitrite (NO_2^-) and then nitrate (NO_3^-) (Figure 1, blue arrows). Here, we evaluate the state of our current knowledge about how ocean uptake of anthropogenic CO_2 and attendant acidification could affect three of the fundamental nitrogen cycle processes shown in Figure 1: N_2 fixation (red arrow), nitrification (blue arrows), and denitrification (green arrow).

N_2 Fixation

The effects of rising CO_2 on N_2 fixation by marine cyanobacteria have probably been better documented than any other part of the marine nitrogen cycle, although our knowledge is still limited to only a few taxa. Most studies to date have focused on the ubiquitous subtropical and tropical diazotroph *Trichodesmium*, which is amenable to culture and believed to be responsible for up to 50% of total marine N_2 fixation (Mahaffey et al., 2005). Four recently published studies have presented similar results showing that increasing pCO_2 stimulates N_2 fixation by *Trichodesmium* cultures (Hutchins et al., 2007; Levitan et al., 2007; Barcelos e Ramos et al., 2007; Kranz et al., 2009). The reported increases in rates of N_2 fixation from present-day pCO_2 (375–380 ppm) to projected year 2100 pCO_2 (~ 750 ppm) range from 35% to 65%, although Levitan et al. (2007) reported an increase in N_2 fixation of 121% between 400 and 900 ppm (Figure 2a). Several of these culture studies also observed similar degrees of stimulation of *Trichodesmium* carbon fixation and/or growth rates by increased CO_2.

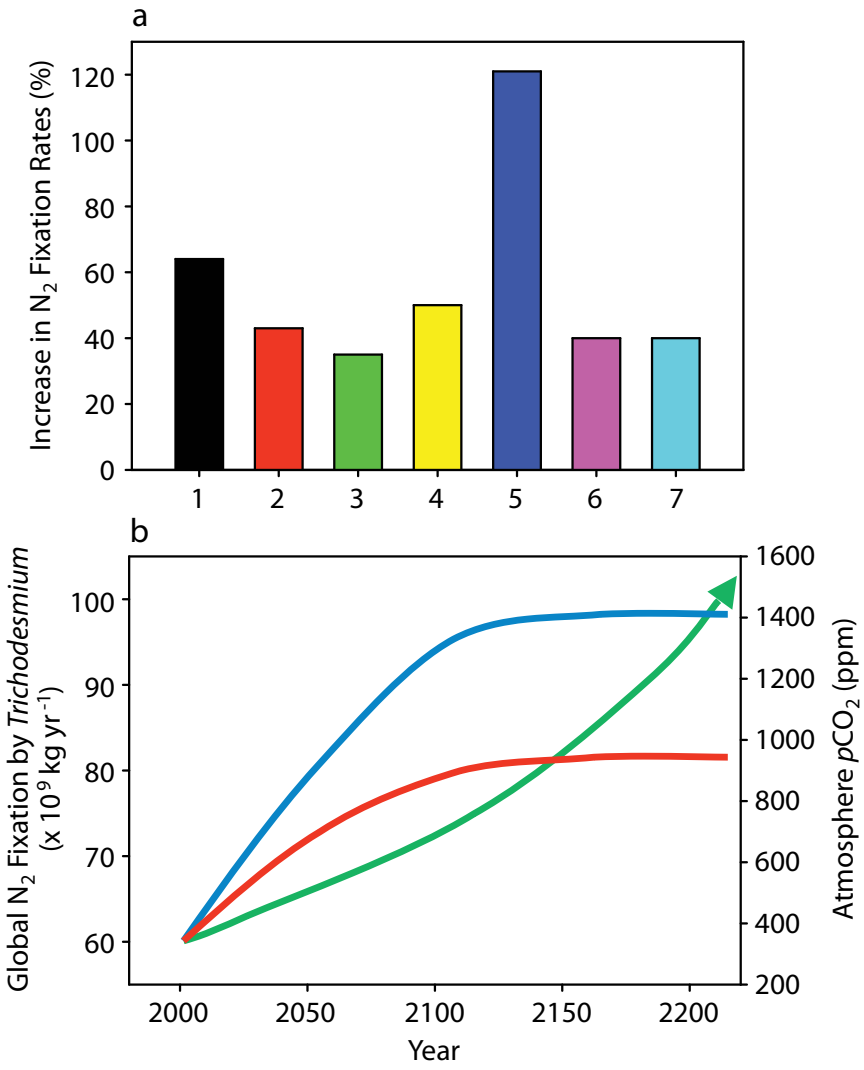


Figure 2. (a) Enhanced N_2 fixation rates of cultured marine cyanobacteria at projected future pCO_2 (750–1000 ppm), expressed as a percent (%) increase over N_2 fixation rates at current pCO_2 (370–400 ppm). Data are taken from experiments using: (1) and (2) *Trichodesmium erythraeum* strain GBR (Great Barrier Reef) grown at 29°C or 25°C across a range of 380–750 ppm CO_2 (Hutchins et al., 2007); (3) *T. erythraeum* strain IMS 101 grown at both 25°C and 29°C across a range of 380–750 ppm CO_2 (Hutchins et al., 2007); (4) *T. erythraeum* strain IMS 101 grown at 25°C across a range of 380–750 ppm CO_2 (Barcelos e Ramos et al., 2007); (5) *T. erythraeum* strain IMS 101 grown at 25°C across a range of 400–900 ppm CO_2 (Levitan et al., 2007); (6) *T. erythraeum* strain IMS 101 grown at 25°C across a range of 370–1000 ppm CO_2, (Kranz et al., 2009); and (7) *Crocosphaera watsonii* strain WH8501 grown at 28°C across a range of 380–750 ppm CO_2 (Fu et al., 2008). (b) Potential future increases in annual global N_2 fixation by *Trichodesmium* spp. (left axis), based on maximum (blue) and minimum (red) levels of CO_2 stimulation observed in laboratory cultures by Hutchins et al. (2007). Estimated atmospheric pCO_2 (right axis) is plotted in green. *CO_2 data from Royal Society (2005)*

The magnitude of these N_2 and carbon fixation responses of *Trichodesmium* represents the largest physiological response yet reported for any marine microbial group (including phytoplankton) to projected pCO_2 changes. If these culture results can be extrapolated to the environment, *Trichodesmium* and perhaps other N_2-fixing cyanobacteria seem poised to be among the biggest "winners" in the future anthropogenically acidified ocean. Such major increases in diazotrophy due to CO_2 fertilization of the ocean could have immense implications for the marine nitrogen cycle over the next century.

However, a closer examination of the data presented in Figure 2a reveals that our picture of CO_2 impacts on marine diazotrophy is still far from complete. To date, all of the published *Trichodesmium* culture studies have used only one species, *T. erythraeum,* and we know that physiological responses sometimes vary greatly between even closely related species. Consequently, the responses of other common species within this genus to high pCO_2 remain completely unknown. In fact, with the exception of one study that compared *T. erythraeum* isolates from the Atlantic and Pacific (Hutchins et al., 2007), all of the currently published data come from a single *T. erythraeum* culture strain, *Trichodesmium* IMS 101 (Figure 2a). This commonly cultivated "lab weed" was isolated about 20 years ago from North Carolina coastal waters (Prufert-Bebout et al., 1993). Consequently, it is unclear how representative these studies are, given the broad diversity of uncultivated *Trichodesmium* species and ecotypes growing in the tropical and subtropical seas.

In addition, there is still very little information available regarding CO_2 effects on marine N_2 fixers other than *Trichodesmium*. Only one study has examined the response of N_2-fixing unicellular cyanobacteria to elevated CO_2, despite the fact that this group is now thought to fix at least as much nitrogen as *Trichodesmium* on a global basis (Zehr et al., 2001; Montoya et al., 2004). This investigation, using cultures of the unicellular diazotroph *Crocosphaera watsonii*, demonstrated that N_2 fixation rates were enhanced by 40% when cultures were grown at pCO_2 levels of 750 ppm relative to those grown at 380 ppm (Figure 2a; Fu et al., 2008a). Thus, the general trends observed were very similar to those reported for *Trichodesmium*.

In contrast to the results of these studies, a recent culture study by Czerny et al. (2009) of the bloom-forming heterocystous cyanobacterium *Nodularia spumigena* from the brackish Baltic Sea found large decreases in cell division rates and slight decreases in N_2 fixation rates as pCO_2 increased. These results provide a cautionary note, in that we cannot assume that all diazotrophs will respond favorably or uniformly to higher CO_2 levels. These authors suggest that this contrasting CO_2 response by *N. spumigena* could be due to deleterious effects of lower pH on the transport of fixed nitrogen compounds from the site of N_2 fixation in the heterocysts to neighboring vegetative cells.

Estuaries and brackish waters may experience more dramatic pH changes than the open ocean, due to their lower buffering capacities (Najjar et al., in press). Heterocystous cyanobacteria generally do not occur in the marine environment and are more common in estuaries and coastal systems. Therefore, it is not clear that the Czerny et al. (2009) results have large ecosystem implications, except in specific environments such as the Baltic Sea where harmful blooms of these organisms can occur at fairly low salinities. As for the other important groups of marine N_2 fixers, such as unicellular cyanobacteria, diatom/diazotroph associations, zooplankton/diazotroph symbioses, and N_2-fixing heterotrophic eubacteria or archaea, we are still at the rudimentary stages of understanding and quantifying the contributions of these groups to marine N_2 fixation and assessing where N_2 fixation occurs in the ocean (Carpenter and Capone, 2008). Little or nothing at all is known about how they will react to future pCO_2 changes.

The other limitation to our understanding of overall ocean acidification or CO_2 enrichment effects on N_2 fixation stems from the fact that all of the published work so far has been from laboratory culture studies. Field evidence is still lacking for stimulation of N_2 fixation rates by elevated pCO_2 in *Trichodesmium* or other diazotrophs. We recently carried out a set of three preliminary incubation experiments using natural *Trichodesmium* colonies collected from a bloom along the west coast of Florida. The colonies were collected with net tows, then incubated on deck for four to six daylight hours in filtered seawater bubbled with 380 ppm or 750 ppm CO_2/air mixtures. Rate measurements from these incubations using $^{15}N_2$ tracer techniques (Mulholland and Bernhardt, 2005) demonstrated that increased pCO_2 stimulated N_2 fixation rates by 6%, 21%, and 41% above

rates measured in colonies maintained at ambient pCO_2 (Figure 3). The very rapid (hours) responses of these natural *Trichodesmium* samples suggest that CO_2 was a proximate limitation on N_2 fixation rates, in a manner very analogous to a limiting nutrient. The short time frame of their responses further suggests that it was unlikely that the colonies needed to induce new cellular biochemical systems to take advantage of the CO_2 enrichment. To our knowledge, these are the first field data supporting the results from the laboratory studies referenced above, but much more evidence from natural diazotroph populations will be needed before we can robustly assess the potential importance of CO_2 stimulation of marine N_2 fixation for global biogeochemical cycles.

If we assume that the results of previous laboratory culture experiments can legitimately be scaled up to the whole ocean, we can calculate the potential increase in future new N inputs by *Trichodesmium* due to CO_2 fertilization. Current annual global N_2 fixation by *Trichodesmium* spp. has been estimated to be about 60 x 10^9 kg N yr^{-1} (Mahaffey et al., 2005). Calculations using estimates of predicted atmospheric pCO_2 increases over the next two centuries (Royal Society, 2005), along with the N_2 fixation rate relationships determined across a range of pCO_2 for the Pacific *Trichodesmium* isolate GBR (for Great Barrier Reef) by Hutchins et al. (2007), yield the potential trends illustrated in Figure 2b. They suggest that the minimum levels of CO_2 stimulation of N_2 fixation we observed in cultures would result in a global increase of about 20 x 10^9 kg N yr^{-1}, or a total of 80 x 10^9 kg N yr^{-1} fixed by *Trichodesmium* alone by the end of this century. Using the maximum values obtained from our culture experiments in this calculation boosts our estimate by another 20 x 10^9 kg N yr^{-1} to nearly 100 x 10^9 kg N yr^{-1} by the year 2100 (Figure 2b). Because current estimates of total global N_2 fixation by all oceanic diazotrophs combined are about 100–200 x 10^9 kg N yr^{-1} (Galloway et al., 2004), this suggests the possibility that by 2100, CO_2 fertilization could increase new N inputs by *Trichodesmium* alone to levels approaching those estimated for the entire marine diazotrophic community today. Active growth and N_2 fixation by *Trichodesmium* are restricted to waters > 20°C (Carpenter and Capone, 2008), so expansion of their subtropical habitat into higher latitudes due to sea surface warming will tend to further increase this estimate as well. Adding in the contributions of unicellular cyanobacteria and other groups of N_2 fixers might greatly augment this estimate, if CO_2 increases also stimulate N_2 fixation by these less-understood marine diazotrophs.

Although Hutchins et al. (2007) found that N_2 fixation rates of *Trichodesmium* increased with pCO_2 up to about 750 ppm, N_2 fixation rates did not continue to rise as pCO_2 was further elevated to 1250 and 1500 ppm. For this reason, we believe that the increase in the total amount of potential *Trichodesmium* N_2 fixation could level off by about the year 2100 (Figure 2b). This saturation response also emphasizes the similarity of *Trichodesmium* pCO_2 responses to classical phytoplankton nutrient limitation kinetics; examination of the Hutchins et al. (2007) data set suggests an N_2 fixation half-saturation constant of about 330 ppm CO_2. The CO_2 concentration of the atmosphere passed this level during the 1970s (Royal Society, 2005).

Of course, the major assumption behind these calculations of future trends is that other limiting factors will not constrain *Trichodesmium* N_2 fixation before the CO_2 response saturates. There has been a lively debate in the literature about the relative importance of iron and phosphorus as limiting nutrients for

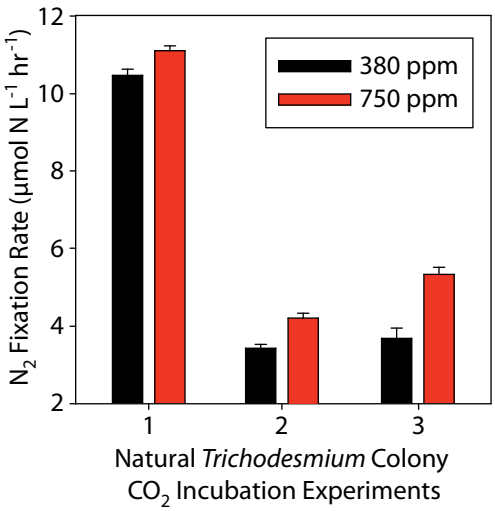


Figure 3. N_2 fixation rates of collected natural *Trichodesmium* spp. colonies from the Gulf of Mexico in three separate experiments, incubated for four to six hours at current pCO_2 (380 ppm, black) and projected year 2100 pCO_2 (750 ppm, red). Error bars are the standard deviations of triplicate bottles in each treatment. N_2 fixation rates at elevated pCO_2 in these three experiments were increased by 6%, 21%, and 41% above rates at ambient pCO_2.

diazotrophs in the present-day ocean (Hutchins and Fu, 2008), and adding pCO_2 into the mix as another potentially limiting factor is likely to muddy these waters even further. It is therefore crucial that we understand how the availability of these two other potentially limiting nutrients may interact with observed CO_2 effects on N_2 fixers.

The interaction of CO_2 with P has been tested using a factorial experimental approach in which both P-limited and P-replete *Trichodesmium* cultures were grown at two pCO_2 levels (380 and 750 ppm; Hutchins et al., 2007). These results were intriguing, in that higher pCO_2 still stimulated N_2 fixation rates even in severely P-limited cultures. Because either phosphate or CO_2 additions resulted in higher N_2 and CO_2 fixation and growth rates, this suggests a form of independent co-limitation between the two (Saito et al., 2008). *Trichodesmium* populations that are apparently P-limited at present-day pCO_2 (Wu et al., 2000; Sanudo-Wilhelmy et al., 2001) may in fact be co-limited by CO_2 as well, and thus future CO_2-mediated increases in N_2 fixation could happen regardless of limiting concentrations of P in the central gyre ecosystems.

In contrast, Fu et al. (2008a) found a very different relationship between pCO_2 and iron availability for *Crocosphaera*. N_2 fixation rates scaled with pCO_2 in iron-replete cultures (Figure 4a), but in iron-limited cultures, increasing pCO_2 had no effect on rates of N_2 fixation (Figure 4b). This result suggests a more traditional Liebig-type single limiting nutrient relationship, in which CO_2 can stimulate N_2 fixation only if iron limitation is first relieved. Thus, it is possible that iron limitation may constrain the projected future increases in global N_2 fixation suggested in Figure 2b. Clearly, more experiments are needed to examine responses of a wider variety of marine diazotrophs to the interactive effects of changing pCO_2 and other potentially limiting factors, such as iron, phosphorus, light, and temperature, before we can make confident predictions about the future of N_2 fixation in a high-CO_2 ocean.

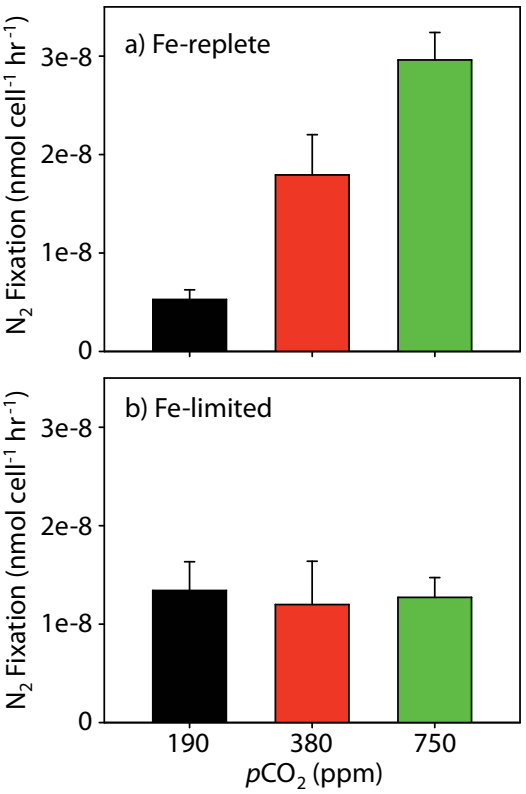


Figure 4. N_2 fixation rates of cultures of the unicellular cyanobacterium *Crocosphaera* grown at 190 ppm CO_2 (black), 380 ppm CO_2 (red), and 750 ppm CO_2 (green), under both (a) Fe-replete and (b) Fe-limited growth conditions. N_2 fixation rates are linearly correlated with pCO_2 in Fe-replete cultures, but are unaffected by pCO_2 changes in Fe-limited cultures. *From Fu et al. (2008a)*

Nitrification

The physiology of nitrifying proteobacteria and archaea could be directly influenced by ocean acidification in at least two ways. There is evidence suggesting that the actual enzymatic substrate during the first step of nitrification by *Nitrosomonas* may be NH_3, rather than NH_4^+ (Ward, 2008). Because the ammonia/ammonium (NH_3/NH_4^+) buffer system in seawater has a pK_a of about 9.19 (Millero, 2007), the projected reduction in seawater pH over the next century will reduce the fraction of NH_3 by nearly 50%, from ~ 6% to ~ 3% (Bange, 2008). This suggests a possible negative impact of higher acidity on marine nitrification rates, due to reductions in available substrate concentrations.

On the other hand, because nitrifiers are autotrophs, like many phytoplankton they could also benefit from the increased availability of CO_2 in seawater. Almost nothing is presently known about the physiology of inorganic-carbon-concentrating mechanisms in

marine nitrifying chemoautotrophs or the potential for carbon limitation of these organisms. The ultimate cause of CO_2 limitation in many prokaryotic and eukaryotic phytoplankton at present-day pCO_2 is the low affinity of the Rubisco enzyme for CO_2 (Rost et al., 2008). Consequently, nitrifying proteobacteria should be equally vulnerable to CO_2 limitation, because both the NH_3-oxidizing genus *Nitrosomonas* and the NO_2^--oxidizing genus *Nitrobacter* also fix carbon using the Calvin cycle (Ward, 2008). Thus, there is the possibility that the CO_2 fertilization effect could positively affect the carbon fixation rates of marine nitrifiers, as it does for some photoautotrophs.

In fact, the limited experimental evidence currently available suggests that lower pH probably has a net inhibitory effect on nitrification. Huesemann et al. (2002) measured nitrification rates in two seawater samples in which they simulated the effects of deliberate ocean disposal of waste CO_2 by bubbling seawater with CO_2-amended air. Although they examined a pH range over 0.5 unit increments that extended much lower than any foreseeable levels of "passive" anthropogenic acidification, they found that ammonium oxidation rates showed an almost linear decrease from pH 8 down to pH 6.5, at which point nitrification was nearly 100% inhibited. Although the pH increments used by Huesemann et al. (2002) were coarse, because the relationship was linear between pH 8 and 6.5, it may be justifiable to use it to extrapolate to the smaller pH changes expected over the next century from acidification (roughly -0.3 to -0.5 units or so).

Using this linear relationship to calculate changes in nitrification at projected year 2100 pCO_2 levels, we estimate a possible reduction in the global median ammonium-specific oxidation rate in the surface ocean of 0.195 d^{-1} (from a data compilation by Yool et al., 2007) to about 0.171 d^{-1} by the end of this century. Using this same pH/nitrification relationship (Huesemann et al., 2002), a modeling study by Blackford and Gilbert (2007) projected a 20% reduction in North Sea nitrification rates at atmospheric CO_2 concentrations of 1000 ppm. Their model output suggested that the ratio of nitrate:nitrate + ammonium in the region would decrease by 5–10% as a consequence. Blackford and Gilbert (2007) discussed the potential ecosystem implications of this change, including shifts in phytoplankton community structure and reductions in the fluxes of oxidized nitrogen species that support denitrification.

New preliminary work measuring nitrification rates in both open ocean and coastal marine environments as a function of realistically anticipated pCO_2 increases was presented by Beman et al. (2008) at the American Society of Limnology and Oceanography (ASLO) meeting in Nice, France. They found that pCO_2 increases from 390 to 750 ppm had generally negative effects on nitrification rates, and that the abundance of both proteobacterial and archaeal nitrifiers was lower in samples incubated at the higher pCO_2. More studies are needed before we will be able to make reliable quantitative predictions of changes in nitrification rates in a high CO_2 ocean, but such ongoing work promises to help shed new light on the future responses of this crucial component of the marine nitrogen cycle.

Denitrification

At present, there seems to be little reason to expect ocean acidification to have direct consequences for the physiology of most marine denitrifying eubacteria and archaea. Denitrification and anammox occur in anaerobic environments, and these areas in the ocean typically already have very elevated pCO_2 and low pH values. These conditions, coupled with the slow ventilation rates of most marine sediments and water column suboxic zones, suggest that near-term effects of increasing atmospheric pCO_2 on their biology and chemistry may be limited. However, the microbes capable of various modes of denitrification are extremely phylogenetically diverse (Devol, 2008), and these assorted groups could respond in different ways to ocean acidification. For instance, nitrate reduction rates by some CO_2-fixing, hydrogenotrophic denitrifying bacteria in sewage sludge are inhibited when elevated pCO_2 causes pH values to drop below 6 (Ghafari et al., 2009). However, to our knowledge there are no studies demonstrating effects of realistically expected future ocean pH levels on denitrification rates of ecologically important marine prokaryotes.

Indirect effects of ocean acidification on marine denitrification seem much more probable, such as those that may be driven by changes in the fluxes of organic carbon that support their heterotrophic metabolisms, and/or the supply of oxidized nitrogen substrates for respiration. A recent modeling study by Oschlies et al. (2008) examined the possible effects of CO_2-driven changes in the C:N stoichiometry of phytoplankton on dissolved oxygen levels in the tropical ocean. Based on the results

of a mesocosm experiment that demonstrated higher C:N export ratios at high CO_2 (Riebesell et al., 2007, see below), they predict a global expansion of suboxic water volume by up to 50% over the next 100 years as this "excess" organic carbon is remineralized in subsurface waters. If this prediction proves correct, and the areal extent of denitrifying zones greatly expands, loss of fixed nitrogen from the ocean due to denitrification could increase dramatically over just decades in a high-CO_2 ocean. Evidence has been found in the sedimentary nitrogen isotope record for large increases in suboxic zone denitrification during glacial/interglacial transitions (Altabet et al., 2002). Because deglaciations are a time when atmospheric pCO_2 increases rapidly, this evidence seems consistent with the scenario proposed by Oschlies et al. (2008). However, most paleoceanographic studies attribute the expansion of suboxic zones during deglaciation to factors such as enhanced export due to changing ocean circulation patterns (Robinson et al., 2007), rather than to a direct effect of rising pCO_2 on carbon export.

The Oschlies et al. (2008) modeling results were very sensitive to the assumed changes in particle flux C:N stoichiometry, and these authors actually predicted a contrary trend of increased dissolved oxygen levels in the tropical thermocline if carbon export remained at Redfield ratios in the high-CO_2 ocean. Because there are quite a number of contrasting and sometimes conflicting reports on how phytoplankton elemental stoichiometry may change in response to higher pCO_2 (see below), we suggest that the jury is still out on this hypothesis about future acidification-driven higher C:N ratios resulting in significantly increased global denitrification.

Basic changes in other critical processes both within and external to the ocean N cycle could have big implications for trends in ocean denitrification over the next century as well. Several investigators have predicted large increases in ocean suboxia as a result of future sea surface warming and intensified stratification (Sarmiento et al., 1998; Matear and Hirst, 2003; Gnanadesikan et al., 2007). CO_2-driven enhancement of ocean N_2 fixation, as discussed above, would greatly increase the ultimate source of fixed nitrogen to denitrifiers. In contrast, the potential decreases in nitrification rates at lower pH previously mentioned would tend to decrease the availability and supply of oxidized nitrogen compounds to serve as substrates for denitrification. Although it seems that denitrification will almost certainly respond strongly to an acidifying ocean, these large uncertainties suggest that more information will be needed to reliably determine the magnitude and even the likely direction of many of these responses.

> "...THE ONGOING AND ACCELERATING ENRICHMENT OF SEAWATER WITH ANTHROPOGENIC CO_2 WILL LIKELY HAVE FAR-REACHING EFFECTS NOT ONLY ON THE CARBON CYCLE, BUT ALSO ON THE CYCLES OF... BIOLOGICALLY REQUIRED NUTRIENTS [N, P, Si]."

THE P AND Si CYCLES

Compared to nitrogen, relatively little research effort has been directed toward understanding the effects of high CO_2 on the marine phosphorus cycle. The dominant form of inorganic phosphorus in seawater at pH 8 is HPO_4^{2-} (~ 87%), but the fraction of $H_2PO_4^-$ will increase marginally with future acidification. The implications of this chemical speciation shift for phosphorus bioavailability are uncertain (Hutchins and Fu, 2008). The only study we are aware of that examined rates of phosphate uptake (using the isotopic tracer ^{33}P) and potential dissolved organic phosphorus utilization (by assaying alkaline phosphatase activity) as a function of pCO_2 is that of Tanaka et al. (2008). They measured these P cycling parameters in a Norwegian fjord mesocosm experiment at 350, 700, and 1050 ppm CO_2, and although a few minor trends emerged, they found no statistically significant effects of pCO_2 on P biogeochemistry over the course of a 24-day incubation period.

Gervais and Riebesell (2001) grew the common bloom-forming diatom

Skeletonema costatum in P-replete and P-limited cultures and found no differences in C:P ratios at pH values of 8.0 and 8.6, although they did not examine lower pH values relevant to anthropogenic ocean acidification. Burkhardt et al. (1999) also found no major impacts or consistent influence of low pH on the C:P ratios of six diatoms and a dinoflagellate. Fu et al. (2008b) showed that cellular P quotas in the harmful bloom flagellate *Heterosigma akashiwo* were unchanged by increasing pCO_2 from 375 to 750 ppm, while those of the dinoflagellate *Prorocentrum minimum* were slightly lower at the higher pCO_2. Likewise, Hutchins et al. (2007) and Fu et al. (2007) found that a similar pCO_2 increase had no effect on the P cell quotas of the cyanobacteria *Trichodesmium erythraeum*, *Synechococcus*, and *Prochlorococcus*. In contrast, Czerny et al. (2009) documented a slight increasing trend in cellular P quotas with higher pCO_2 for the estuarine cyanobacterium *Nodularia spumigena*. At this point, the available evidence suggests negligible or minor effects of projected future changes in pCO_2 on most phytoplankton phosphorus requirements.

The story for silicon is similar to that of phosphorus. The dominant form of dissolved silicon in the ocean is silicic acid (H_4SiO_4), which, like phosphorus, comprises a buffer system in seawater. The relatively basic pK_a values for the dissociation of H_4SiO_4 ($pK_{a1} = 9.84$, $pK_{a2} = 13.2$) mean that it is nearly all present in the fully protonated form at pH 8. For this reason, further decreases in seawater pH are unlikely to substantially shift the chemical speciation of dissolved silicon.

Only a handful of studies have examined acidification effects on diatom silicification, and these few suggest that direct pCO_2 impacts are generally small. Milligan et al. (2004) saw no difference between the Si quotas of the diatom *Thalassiosira weissflogii* when cultures were grown at 370 or 750 ppm CO_2, although the quota increased somewhat at very low (100 ppm) pCO_2. Similarly, our own unpublished results using a variety of pennate and centric diatoms isolated from a range of marine environments suggest that large changes in diatom silicification are unlikely to occur between present-day and projected year 2100 pCO_2 levels. The biggest effect of pCO_2 that Milligan et al. (2004) observed was not on live diatom cells but on dissolution rates of empty silica frustules; dissolution rates were much higher at 750 ppm CO_2 than at 370 ppm. They were uncertain about the reasons for these low-pH-enhanced silica dissolution rates, but their results do suggest the possibility that increasing pCO_2 could enhance Si remineralization rates from sinking particles, and thus potentially cause shoaling of silicate vertical profiles and increased Si availability in surface waters.

Although the evidence for direct effects is sparse, changing pCO_2 could certainly affect silicon biogeochemistry indirectly through shifts in algal community structure. A natural community pCO_2 manipulation experiment in the equatorial Pacific showed lower dissolved Si:N consumption ratios at 150 ppm CO_2 compared to 750 ppm (Tortell et al., 2002). These low ratios were attributed to a community shift away from diatoms and toward *Phaeocystis* spp. at the lower pCO_2. CO_2-induced dominance changes within the diatom community have been reported in the Ross Sea, with large, heavily silicified centric diatoms replacing smaller, lightly silicified pennates at high pCO_2 (Tortell et al., 2008; Feng et al., in press). In another natural community experiment during the North Atlantic spring bloom, Feng et al. (2009) combined two pCO_2 treatments (375 and 750 ppm) with two temperature treatments (ambient and ambient + 4°C) and saw lower particulate Si:N ratios due to reduced diatom abundance at the higher temperature, but no effect on silicification due to pCO_2. Similarly, Bellerby et al. (2008) found that silicate drawdown was virtually identical in 350-, 700-, and 1050-ppm CO_2 treatments in the same Norwegian mesocosm experiment in which Tanaka et al. (2008) examined phosphorus utilization (see above).

PHYTOPLANKTON ELEMENTAL STOICHIOMETRY

The elemental ratios of phytoplankton represent the pivotal intersection of the cycles of carbon, nitrogen, and phosphorus in the ocean. Because of the central role that algal C:N:P ratios play in our understanding of global ocean biogeochemistry (Redfield, 1958), the effects of changing pCO_2 on phytoplankton stoichiometry have been examined in many experiments dating back at least a decade, to some of the earliest publications in the relatively new field of ocean acidification studies (Burkhardt et al., 1999). Despite this fairly large body of results, there is still no consensus on whether phytoplankton elemental ratios are likely to be altered in a systematic, predictable manner in a future acidified ocean.

We gathered a collection of the

available experimental data on how pCO_2 changes the elemental ratios of both cultured phytoplankton and mixed natural phytoplankton communities (Figure 5). These data include published studies, as well as some of our unpublished data. We attempted as much as possible to constrain our summary to studies that examined C:N and/or N:P ratio changes from present day to roughly expected year 2100 pCO_2 levels (between ~ 380 and ~ 750 ppm), although a few of these studies used other concentrations ranging from 150 to 2000 ppm. The unialgal culture data offer a glimpse of how the stoichiometry of individual phytoplankton species may respond to changing pCO_2 under carefully controlled laboratory conditions (Figure 5a,b). The field data are from experiments carried out using entire natural plankton assemblages, and so these results integrate the net responses and species composition shifts of many algal species as well as any other organisms (zooplankton, bacteria) present in the incubations (Figure 5c). The details of each experiment differ in important ways, including different marine regimes, algal taxa, pCO_2 levels, and incubation methodology, as well as other co-variables examined, such as light and nutrients. Therefore, the absolute magnitude of C:N and N:P ratio changes are not directly comparable between experiments. Nevertheless, this summary offers some interesting insights into what the major directions and trends in phytoplankton elemental ratios could potentially be as the ocean continues to take up anthropogenic CO_2 over the next century, as well as some cautionary notes about interpretation of experimental results.

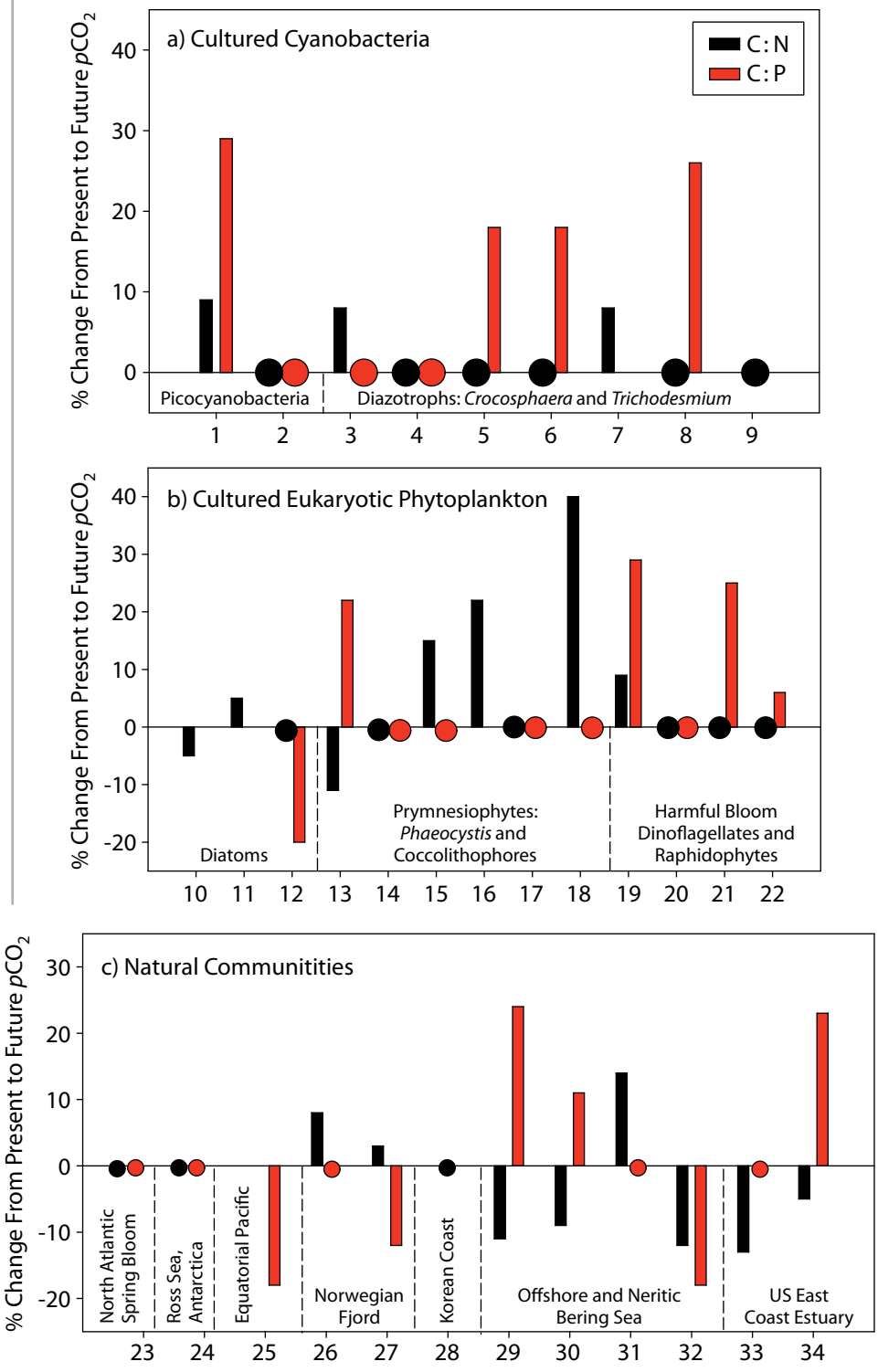


Figure 5. A compilation of data from the literature as well as some of the authors' own unpublished experimental results on how increasing pCO_2 from past or present-day levels (150–420 ppm) to projected future levels (750–2000 ppm) affects the C:N ratios (black) and N:P ratios (red) of important phytoplankton groups in cultures and in mixed natural communities. The trends in these elemental ratios are presented as the percent (%) change from present-day to future pCO_2; positive bars indicate increasing ratios, negative bars indicate declining ratios, and a circle symbol on the 0% line indicates no change in C:N (black) or N:P (red). Note that not every study measured both parameters.

(a) CO_2-mediated changes in C:N and N:P ratios of cultured marine cyanobacteria, including:
- 1 and 2, *Synechococcus* and *Prochlorococcus* grown at 380 and 750 ppm CO_2 (Fu et al., 2007)
- 3 and 4, Fe-replete and Fe-limited *Crocosphaera* grown at 380 and 750 ppm CO_2 (Fu et al., 2008a)
- 5 and 6, P-replete and P-limited *Trichodesmium* grown at 380 and 750 ppm CO_2 (Hutchins et al., 2007)
- 7, Trich*odesmium* grown at 400 and 900 ppm CO_2 (Levitan et al., 2007)
- 8, *Trichodesmium* grown at 380 and 750 ppm CO_2 (Barcelos e Ramos et al., 2007)
- 9, *Trichodesmium* grown at 370 and 1000 ppm CO_2 (Kranz et al., 2009)

(b) CO_2-mediated changes in C:N and N:P ratios of cultured marine eukaryotic phytoplankton, including:
- 10 and 11, the diatoms *Asterionella* grown at 430 and 820 ppm CO_2 and *Skeletonema* grown at 400 and 720 pCO_2 (Burkhardt et al., 1999)
- 12 and 13, the Antarctic diatom *Chaetoceros* and the Antarctic colonial prymnesiophyte *Phaeocystis* grown at 430 and 820 ppm CO_2 (recent work of author Fu and colleagues)
- 14 and 15, the coccolithophorid *Emiliania huxleyi* grown under low and high light at 375 and 750 ppm pCO_2 (Feng et al., 2008)
- 16, the coccolithophorid *Emiliania huxleyi* grown at 490 and 750 ppm pCO_2 (Iglesias-Rodriguez et al., 2008)
- 17 and 18, a noncalcifying strain of the coccolithophorid *Emiliania huxleyi* grown under low and high light at 360 and 2000 ppm CO_2 (Leonardos and Geider, 2005)
- 19 and 20, the toxic raphidophyte *Heterosigma* and the dinoflagellate *Prorocentrum* grown at 375 and 750 ppm pCO_2 (Fu et al., 2008b)
- 21 and 22, P-replete and P-limited cultures of the toxic dinoflagellate *Karlodinium* grown at 430 and 745 ppm CO_2 (recent work of author Fu and colleagues)

(c) CO_2-mediated changes in C:N and N:P ratios of incubated natural phytoplankton communities, including:
- 23, a shipboard continuous culture incubation during the North Atlantic spring bloom at 390 and 690 ppm CO_2 (Feng et al., 2009)
- 24, a shipboard continuous culture incubation in the Ross Sea, Antarctica, at 380 and 750 ppm CO_2 (Feng et al., in press)
- 25, a shipboard semicontinuous culture incubation in the equatorial Pacific at 150 and 750 ppm CO_2 (Tortell et al., 2002)
- 26 and 27, mesocosm batch culture incubations in a Norwegian fjord at 350 and 700 ppm CO_2 and 410 and 710 ppm pCO_2 (Riebesell et al., 2007; Engel et al., 2005)
- 28, a mesocosm batch incubation in Korean coastal waters at 400 and 750 ppm CO_2 (Kim et al., 2006)
- 29 and 30, shipboard continuous culture incubations in Bering Sea shelf waters at 10°C and 15°C, and 31 and 32, in Bering Sea offshore waters at 10°C and 15°C, all incubated at 370 and 750 ppm pCO_2 (unpublished data from the study presented in Hare et al., 2007)
- 33 and 34, two semicontinuous incubations of a US East Coast estuarine phytoplankton community at 380 and 750 ppm pCO_2 (unpublished data of author Fu and colleagues)

Cyanobacteria Cultures

Figure 5a presents the collected data from laboratory cultures of marine cyanobacteria on stoichiometry changes in response to higher pCO_2. A trend that is immediately apparent is that in all of these studies, cyanobacterial C:N and N:P ratios either increased or remained the same with increased pCO_2; there are no reports of decreases in either of these ratios. Fu et al. (2007) found that both C:N and N:P ratios increased (by 9% and 29%) in *Synechococcus*, but neither ratio responded to changing pCO_2 in *Prochlorococcus*. The C:N ratios of Fe-replete cultures of the unicellular diazotroph *Crocosphaera* increased with higher pCO_2 (by 8%) but N:P ratios did not, while both ratios were unchanged in Fe-limited treatments (Fu et al., 2008a). Among the laboratory studies using *Trichodesmium*, Hutchins et al. (2007) reported proportionately identical large N:P ratio increases in both P-replete and P-limited cultures (18%), similar to the 26% increase seen by Barcelos e Ramos et al. (2007). Neither Levitan et al. (2007) nor Kranz et al. (2009) reported N:P ratios, and among the four studies, only Levitan et al. (2007) found increases in C:N ratios with higher pCO_2 (Figure 5a).

Eukaryotic Phytoplankton Cultures

Figure 5b shows a compendium of results from laboratory CO_2 experiments using eukaryotic phytoplankton. As with the cyanobacteria, most studies using eukaryotic photoautotrophs found either increases or no change in C:N and N:P ratios at higher pCO_2 levels, but there were several exceptions. Burkhardt et al. (1999) saw that the C:N ratios of one diatom increased by 5%, while that of

another decreased by 5%; several other species examined had similar variable outcomes (data not shown), and this study did not report N:P ratios. Our unpublished results using Antarctic isolates found no change in C:N ratios in the chain-forming centric diatom *Chaetoceros,* but a large decrease in N:P ratios (-20%). This same investigation also documented decreases in C:N ratios (-11%) coupled with increases in N:P ratios (22%) in the ecologically dominant colonial prymnesiophyte *Phaeocystis antarctica* (Figure 5b). Three studies using the cosmopolitan coccolithophorid *Emiliania huxleyi* all showed increases in C:N ratios, except that both Leonardos and Geider (2005) and Feng et al. (2008) found that C:N ratios in light-limited cultures were unaffected by pCO_2. N:P ratios in *E. huxleyi* did not respond to changing pCO_2 in any of these studies. Experiments using harmful bloom flagellates found increases in both C:N (9%) and N:P (29%) in the toxic estuarine raphidophyte *Heterosigma akashiwo,* but no change in either ratio in the co-occurring dinoflagellate, *Prorocentrum minimum* (Fu et al., 2008b). The toxic dinoflagellate *Karlodinium veneficum* exhibited increased N:P ratios at higher pCO_2 in both P-replete and P-limited cultures, but unaltered C:N ratios in both treatments (Figure 5b; recent work of author Fu and colleagues).

Natural Assemblage Experiments

The collection of natural assemblage experimental results suggests a much more mixed picture in terms of the direction of changes in C:N and N:P with pCO_2; both increases and decreases in these ratios have been commonly reported (Figure 5c). Feng et al. (2009, in press) found no changes in either C:N or N:P ratios as a function of pCO_2 in two shipboard continuous culture incubations carried out using a coccolithophore/diatom community during the North Atlantic spring bloom, and a diatom/*Phaeocystis* assemblage in the Ross Sea, Antarctica (Figure 5c). Tortell et al. (2002) used differences in nutrient drawdown to calculate much lower N:P utilization ratios (-18%) at high pCO_2, coinciding with a shift from diatoms to *Phaeocystis* in an equatorial Pacific assemblage. A mesocosm experiment using a diatom-dominated assemblage from a Norwegian fjord showed an 8% increase in C:N ratios but no change in N:P ratios; these results were used to suggest large potential shifts in future global C:N export stoichiometry (Riebesell et al., 2007) and the consequent increases in ocean suboxia discussed in the denitrification section above (Oschlies et al., 2008). A previous experiment using the same mesocosm facility, but with an assemblage dominated instead by coccolithophores (Engel et al., 2005), showed a small increase in C:N (3%) but a large decrease in N:P (-12%). Another mesocosm experiment in Korean coastal waters found no response of C:N ratios to increasing pCO_2 but did not report N:P ratios (Kim et al., 2006). Two continuous culture incubations with communities collected from the continental shelf and offshore waters in the Bering Sea (Hare et al., 2007), and carried out at both ambient (10°C) and elevated (15°C) temperatures, found large decreases in C:N ratios at higher pCO_2 levels in three out of four cases (C:N increased in the offshore, ambient temperature experiment). In the same study, N:P ratios increased at both temperatures in the neritic experiment, while they were unchanged at 10°C and decreased at 15°C (-18%) in the offshore incubation (Figure 5c). Finally, our unpublished results from two separate semi-continuous experiments carried out in estuarine waters of the Delaware Inland Bays along the US East Coast showed that higher pCO_2 depressed community C:N ratios in both cases (-5% and -13%), while N:P ratios were either steady or increased (22%).

The results of these collected investigations suggest several trends. With a few exceptions, both the C:N and N:P ratios of unialgal cultures of cyanobacteria and eukaryotic algae usually increased, or did not change, when pCO_2 was elevated from present-day to projected future levels. In contrast, incubations of natural populations showed a much greater degree of variability in both the direction and magnitude of elemental stoichiometry changes. These differences could be due in part to differences in incubation methods, which have included small volume shipboard continuous cultures similar in principle to laboratory chemostats (Hare et al., 2007; Feng et al., 2009, in press), semi-continuous incubations in which part of the seawater is removed periodically and replaced with fresh filtered water (Tortell et al., 2002; recent work of author Fu and colleagues), and very large volume mesocosms in which plankton are grown in "batch" mode until nutrients are exhausted (Engel et al., 2005; Kim et al., 2006; Riebesell et al., 2007). It is also highly likely that the major differences in plankton community composition in the various regimes where experiments have been conducted, ranging from the equator to

the polar seas and from estuaries to the remote open ocean, are also responsible for the variable results from these natural assemblage incubations. Large variations between experiments in biological factors like zooplankton grazing, algal interspecific competition, and bacterial metabolism are also almost certainly the case. Overall, though, it is clear that extrapolation of the results from any particular natural assemblage experiment to other regimes, or to the global ocean in general, needs to be undertaken with extreme caution and with these major qualifiers in mind.

CONCLUSIONS

Marine scientists agree that the ocean has mitigated some of the climatic effects of anthropogenic CO_2 emissions by absorbing a large fraction of the fossil-fuel-derived carbon emitted to the atmosphere. We now also recognize that ocean ecosystems are paying a steep price for this service through the resulting acidification of seawater. Like any major ecological shift, though, our global perturbation of ocean carbonate chemistry is not all negative. It will favor some organisms while selecting against others. In nature, environmental changes that spell disaster for one species always represent an opportunity for another. Ocean food webs will persist by shifting to accommodate the changed conditions, although there is certainly no guarantee that the human species will be happy with the results.

Considering the comprehensive chemical and biological changes now underway as anthropogenic CO_2 continues to accumulate in the ocean, it would be surprising indeed if linked marine biogeochemical cycles were not also perturbed. It is universally recognized that the cycle of carbon in the ocean is inextricably tied to the cycles of the major nutrient elements. While the details and even the directions of particular biogeochemical responses are still being debated, some plausible outlines of future marine nutrient cycles are beginning to emerge.

The Future Nitrogen Cycle

The available evidence suggests that the ocean nitrogen cycle may be altered more than any other nutrient biogeochemical cycle in response to ocean acidification and CO_2 fertilization. The numerous biologically mediated redox transformations within this cycle seem especially likely to be sensitive to changes in the acid/base balance of seawater. Many key organisms such as phytoplankton and nitrifiers are autotrophs, and so may be directly influenced by increasing CO_2 availability. Other critical microbial components of the nitrogen cycle such as denitrifiers and anammox organisms are heterotrophic, and so could be secondarily affected by changes in both the quantity and quality of organic carbon reaching them.

Based on the best experimental evidence that we have available at this time, we project that there may be large increases in global N_2 fixation rates over the next century. This large influx of new nitrogen will be due to the CO_2 fertilization effect, and to diazotrophic range expansion in a warmer ocean. At the same time, global denitrification could also increase substantially, following increases in ocean suboxia due to changes in ocean physics and surface ocean productivity. Oxygen depletion and denitrification could possibly be greatly enhanced through non-Redfieldian "carbon overconsumption" by phytoplankton growing at high pCO_2, although we suggest that the evidence for the latter is still not conclusive (see below). Interestingly, the predicted century-time-scale increases in N_2 fixation (35–65%; Figure 2) are of about the same magnitude as the 50% increase in denitrification predicted by the Oschlies et al. (2008) model. Although both of these estimates certainly have large uncertainties, if both input and output fluxes accelerate roughly equally, there could be a faster throughput but a relatively unchanged total fixed nitrogen inventory in the ocean.

One factor that may prevent such a

> "LIKE ANY MAJOR ECOLOGICAL SHIFT...OUR GLOBAL PERTURBATION OF OCEAN CARBONATE CHEMISTRY IS NOT ALL NEGATIVE. IT WILL FAVOR SOME ORGANISMS WHILE SELECTING AGAINST OTHERS."

balanced but faster spinning nitrogen cycle scenario is the apparent negative effect of lower pH on nitrification. Although the deep ocean will not experience significant anthropogenic acidification for some time (Royal Society, 2005), recent estimates place a large fraction of global nitrification within near-surface waters just below the euphotic zone (Yool et al., 2007). These shallow nitrifying organisms will almost certainly encounter strong acidification impacts within this century. Thus, inhibition by rising acidity could turn nitrification into a nitrogen cycle bottleneck, constricting the flow of fixed N inputs from nitrogen fixation to N losses through denitrification and annamox. Decreases in nitrification rates at low pH could negate the trends discussed above that would have otherwise tended to increase denitrification, because the supplies of nitrate that are required by denitrifiers as a respiratory electron acceptor would be substantially reduced.

The potentially increased fluxes of ammonium coming from CO_2-fertilized nitrogen fixers will have only two ways to go; if nitrification is progressively inhibited by acidification, more ammonium will likely be diverted into the algal assimilatory pathway (Figure 1). Annamox could also possibly help to consume some of this excess ammonium supply, but because anaerobic ammonium oxidation also requires supplies of oxidized nitrite, it is also vulnerable to acidification-driven decreases in nitrification (Figure 1). This scenario would ultimately reduce surface ocean nitrate concentrations and nitrate-supported new primary production, while increasing the relative importance of the ammonium-fueled regenerated production system. Such an outcome would undoubtedly have large consequences for phytoplankton community structure, as many species have preferences for specific forms of nitrogen (Mulholland and Lomas 2008). One prediction would be increased global biomass of regenerated production specialists like picocyanobacteria and nanoflagellates, and reduced importance of new production specialists like diatoms. These ammonium-driven increases in the relative size of the microbial food web at the expense of nitrification and new production may well negatively impact higher-trophic-level production and fisheries harvests in the future acidified ocean.

> "CONSIDERING THE COMPREHENSIVE CHEMICAL AND BIOLOGICAL CHANGES NOW UNDERWAY AS ANTHROPOGENIC CO_2 CONTINUES TO ACCUMULATE IN THE OCEAN, IT WOULD BE SURPRISING INDEED IF LINKED MARINE BIOGEOCHEMICAL CYCLES WERE NOT ALSO PERTURBED."

Interactions of Other Global Change Factors With Ocean Acidification

Another large uncertainty is the interaction of rising pCO_2 with other global change processes such as global warming, stratification, changes in ocean circulation and ice cover, and shifts in light and nutrient availability (Boyd et al., in press). For example, the interactions between CO_2 fertilization of nitrogen fixation and growth limitation by phosphorus and/or iron are still very much unresolved. The availability of P is likely to be reduced with intensified stratification of the future surface ocean. The same is true of iron in upwelling zones where phytoplankton receive most of their inputs from below, but future trends in iron supplies from continental aerosols in the central gyres where most N_2 fixation occurs are more uncertain. Some modeling studies predict changes in precipitation patterns over the continents that will reduce iron inputs to the ocean from dust (Mahowald and Luo, 2003), while other investigators predict potentially increased aeolian iron supply to the ocean from accelerating agricultural desertification (Takeda and Tsuda, 2005) or industrial pollution (Sedwick et al., 2007). Although we have not extensively considered iron or other micronutrients in this review, higher pCO_2 could alter ocean iron cycles through direct changes in phytoplankton iron requirements (Figure 3; Fu et al., 2008a), and through pH-driven changes in the chemical

speciation of organically complexed iron (Millero et al., 2009). There is a clear need for more holistic experimental and modeling studies that attempt to incorporate feedbacks between rising ocean pCO_2 and acidity, and all of these other concurrent and often linked environmental change variables.

Future Phosphorus and Silicon Cycles

At present, the P and Si cycles seem less likely to be directly affected by rising ocean pCO_2, but they undoubtedly will react indirectly to the expected changes in the C and N cycles. Phytoplankton community composition changes may be especially important in determining the way the silicon cycle will respond in the future ocean. The apparent lack of a strong influence of pCO_2 on most algal cellular P requirements should allow oceanographers to use P as a relatively conservative denominator to which changing C and N quotas can be compared; fortuitously, we already routinely use P in this way as a normalizer in the Redfield ratio.

Future Algal Elemental Ratios and Nutrient Stoichiometry

Far-reaching impacts on future nutrient cycles and biological productivity are to be expected if algal elemental ratios are systematically influenced by pCO_2, as some studies suggest. Our survey of laboratory culture data suggests that the C:N and N:P ratios of most cyanobacteria and many eukaryotic phytoplankton will either remain near Redfield values, or increase as pCO_2 rises during the coming century. These responses appear to be species-specific, making community and ecosystem predictions problematic. Higher global average algal N:P ratios would tend to deplete the ocean inventory of fixed N relative to P, thus favoring increased nitrogen fixation above and beyond the direct effects of CO_2 fertilization. Higher algal C:N ratios would provide negative feedback on rising atmospheric pCO_2 (Riebesell et al., 2007), but could also potentially lead to worldwide expansion of oxygen-depleted zones (Oschlies et al., 2008).

Not surprisingly, the collected results of experiments that manipulated pCO_2 in complex natural plankton assemblages are not quite as clear-cut as those from monospecific culture studies. In fact, C:N ratios increased with higher pCO_2 in only three of the natural community data sets we examined; in five cases they declined, and in three experiments they were unchanged. Trends in N:P ratios were similarly mixed. We need to be very cautious about making broad global generalizations about stoichiometry changes from any particular experiment, and much more information will be needed to confidently extrapolate incubation results to century-scale, ocean-wide trends.

Evolution, Adaptation, and Changing Biology in the Future Ocean

Another thing that cannot be reliably predicted from the short-term experiments discussed here is how populations of biogeochemically critical marine microbes may adapt and evolve in response to higher pCO_2. Most previous laboratory CO_2 experiments have been careful to fully acclimate the cultures to the experimental conditions over multiple generations, but still have not been carried out long enough for true natural selection and evolution to occur. The even shorter time frame of most natural community experiments means they probably are not able to fully acclimate to elevated pCO_2, and so are best interpreted in terms of the proximate responses of present-day communities to pCO_2 changes, in a manner analogous to a diagnostic iron or nutrient addition experiment.

Classic natural selection is driven by environmental changes that reduce an organism's chances of survival or reproductive fitness; thus, marine microbes that are negatively impacted by higher pCO_2 (such as the nitrifiers and calcifiers) may indeed evolve more rapidly in an acidifying ocean. It is not clear, though, how natural selection in a changing environment may affect those organisms that are instead favored by higher pCO_2 (such as *Trichodesmium*). In fact, the few studies that have looked at the long-term responses (thousands of generations) of phytoplankton to high pCO_2 have found no evidence for the evolution of new traits (Collins and Bell, 2006). However, through random mutational degradation, some cell lines in these studies did lose existing traits that operate only at low pCO_2, such as high-affinity dissolved inorganic carbon acquisition systems. This observation raises the possibility that some marine microbes may adapt to a high pCO_2 ocean in ways that will make them less competitive if human remediation efforts are able to eventually lower atmospheric pCO_2 once again. More of these types of experiments examining the long-term responses of key marine functional groups are needed before we will be able to fully understand how nutrient biogeochemistry and biology will respond

to the combined impacts of acidification and CO_2 fertilization in a rapidly changing ocean.

ACKNOWLEDGEMENTS

We thank P.W. Bernhardt, N. Garcia, M. Warner, and C. Heil for their help with our field research, and D. Bronk and an anonymous reviewer for their helpful comments. Grant support was provided by NSF OCE 0722337 and 0825319 and EPA ECOHAB R83-3221 to D.A. Hutchins, NSF OCE 0722395 to M.R. Mulholland, and NSF OCE 0850730 to F.-X. Fu. Special thanks to C. Baranyk for contributing her original artwork.

REFERENCES

Altabet, M.A., M.J. Higginson, and D.W. Murray. 2002. The effect of millennial-scale changes in Arabian Sea denitrification on atmospheric CO_2. *Nature* 415:159–162.

Bange, H.W. 2008. Gaseous nitrogen compounds (NO, N_2O, N_2, NH_3) in the ocean. Pp. 51–94 in *Nitrogen in the Marine Environment*, 2nd ed. D.G. Capone, D.A. Bronk, M.R. Mulholland, and E.J. Carpenter, eds, Elsevier Press, Amsterdam.

Barcelos e Ramos, J.B.E., H. Biswas, K.G. Schulz, J. LaRoche, and U. Riebesell. 2007. Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer *Trichodesmium*. *Global Biogeochemical Cycles* 21, GB2028, doi:10.1029/2006GB002898.

Bellerby, R.G.J., K.G. Schulz, U. Riebesell, C. Neill, G. Nondal, E. Heegaard, T. Johannessen, and K.R. Brown. 2008. Marine ecosystem community carbon and nutrient uptake stoichiometry under varying ocean acidification during the PeECE III experiment. *Biogeosciences* 5:1,517–1,527.

Beman, J.M., C.E. Chow, B.N. Popp, J.A. Fuhrman, Y. Feng, and D.A. Hutchins. 2008. Alteration of oceanic nitrification under elevated carbon dioxide concentrations. Paper presented at ASLO meeting, Nice, France, January 25–30, 2008. Abstract available online at: http://www.sgmeet.com/aslo/nice2009/viewabstract2.asp?AbstractID=5886 (accessed November 17, 2009).

Blackford, J.C., and F.J. Gilbert. 2007. pH variability and CO_2 induced acidification in the North Sea. *Journal of Marine Systems* 64:229–241.

Boyd, P.W., R. Strzepek, R., F.-X. Fu, and D.A. Hutchins. In press. Environmental control of open ocean phytoplankton groups: Now and in the future. *Limnology and Oceanography*.

Burkhardt, S., I. Zondervan, and U. Riebesell. 1999. Effect of CO_2 concentration on C:N:P ratio in marine phytoplankton: A species comparison. *Limnology and Oceanography* 44(3):683–690.

Carpenter, E.J., and D.G. Capone. 2008. Nitrogen fixation. Pp. 141–198 in *Nitrogen in the Marine Environment*, 2nd ed. D.G. Capone, D.A. Bronk, M.R. Mulholland, and E.J. Carpenter, eds, Elsevier Press, Amsterdam.

Collins, S., and G. Bell. 2006. Evolution of natural algal populations at elevated CO_2. *Ecological Letters* 9:129–135.

Czerny, J., J. Barcelos e Ramos, and U. Riebesell. 2009. Influence of elevated CO_2 concentrations on cell division and nitrogen fixation rates in the bloom-forming cyanobacterium *Nodularia spumigena*. *Biogeosciences* 6:1,865–1,875.

Devol, A.H. 2008. Denitrification including anammox. Pp. 263–302 in *Nitrogen in the Marine Environment*, 2nd ed. D.G. Capone, D.A. Bronk, M.R. Mulholland, and E.J. Carpenter, eds, Elsevier Press, Amsterdam.

Engel, A., I. Zondervan, K. Aerts, L. Beaufort, A. Benthien, L. Chou, B. Delille, J.-P. Gattuso, J. Harlay, C. Heemann, and others. 2005. Testing the direct effect of CO_2 concentration on a bloom of the coccolithophorid *Emiliania huxleyi* in mesocosm experiments. *Limnology and Oceanography* 50(2):493–507.

Fabry, V.J., C. Langdon, W.M. Balch, A.G. Dickson, R.A. Feely, B. Hales, D.A. Hutchins, J.A. Kleypas, and C.L. Sabine. 2008. Ocean acidification's effects on marine ecosystems and biogeochemistry. *Eos, Transactions, American Geophysical Union* 89:143–144.

Feely, R.A., C.L. Sabine, K. Lee, W. Berelson, J. Kleypas, V.J. Fabry, and F.J. Millero. 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science* 305:362–366.

Feng, Y., W.E. Warner, Y. Zhang, J. Sun, F.-X. Fu, and D.A. Hutchins. 2008. Interactive effects of increased pCO_2, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae). *European Journal of Phycology* 43:87–98, doi:10.1080/09670260701664674.

Feng, Y., C.E. Hare, K. Leblanc, J. Rose, Y. Zhang, G.R. DiTullio, P.A. Lee, S.W. Wilhelm, J.M. Rowe, J. Sun, and others. 2009. The effects of increased pCO_2 and temperature on the North Atlantic Spring Bloom. I. The phytoplankton community and biogeochemical response. *Marine Ecology Progress Series* 388:13–25.

Feng, Y., C.E. Hare, J.M. Rose, S.M. Handy, G.R. DiTullio, P.A. Lee, W.O. Smith Jr., J. Peloquin, S. Tozzi, J. Sun, and others. In Press. Interactive effects of iron, irradiance, and CO_2 on Ross Sea phytoplankton. *Deep-Sea Research Part I.*

Fu, F-X., M.R. Mulholland, N.S. Garcia, A. Beck, P.W. Bernhardt, M.E. Warner, S.A. Sañudo-Wilhelmy, and D.A. Hutchins. 2008a. Interactions between changing pCO_2, N_2 fixation, and Fe limitation in the marine unicellular cyanobacterium *Crocosphaera*. *Limnology and Oceanography* 53:2,472–2,484.

Fu, F.-X., M.E. Warner, Y. Zhang, Y. Feng, and D.A. Hutchins. 2007. Effects of increased temperature and CO_2 on photosynthesis, growth and elemental ratios of marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria). *Journal of Phycology* 43:485–496.

Fu, F.-X., Y. Zhang, M.E. Warner, Y. Feng, and D.A. Hutchins. 2008b. A comparison of future increased CO_2 and temperature effects on sympatric *Heterosigma akashiwo* and *Prorocentrum minimum*. *Harmful Algae* 7, doi:10.1016/j.hal.2007.05.006.

Galloway, J.N., F.J. Dentener, D.G. Capone, E.W. Boyer, R.W. Howarth, S.P. Seitzinger, G.P. Asner, C.C. Cleveland, P.A. Green, E.A. Holland, and others. 2004. Nitrogen cycles: Past, present, and future. *Biogeochemistry* 70:153–226.

Gervais, F., and U. Riebesell. 2001. Effect of phosphorus limitation on elemental composition and stable carbon isotope fractionation in a marine diatom growing under different CO_2 concentrations. *Limnology and Oceanography* 46(3):497–504.

Gnanadesikan, A., J.L. Russell, and F. Zang. 2007. How does the ocean ventilation change under global warming? *Ocean Science* 3:43–53.

Ghafari, S., M. Hasan, and M.K. Aroua. 2009. Effect of carbon dioxide and bicarbonate as inorganic carbon sources on growth and adaptation of autohydrogenotrophic denitrifying bacteria. *Journal of Hazardous Materials* 162:1,507–1,513.

Hare, C.E., K. Leblanc, G.R. DiTullio, R.M. Kudela, Y. Zhang, P.A. Lee, S. Riseman, P.D. Tortell, and D.A. Hutchins. 2007. Consequences of increased temperature and CO_2 for algal community structure and biogeochemistry in the Bering Sea. *Marine Ecology Progress Series* 352:9–16.

Huesemann, M.H., A.D. Skillman, and E.A. Crecelius. 2002. The inhibition of marine nitrification by ocean disposal of carbon dioxide. *Marine Pollution Bulletin* 44:142–148.

Hutchins, D.A., and F.-X. Fu. 2008. Linking the oceanic biogeochemistry of iron and phosphorus with the marine nitrogen cycle. Pp. 1,627–1,653 in *Nitrogen in the Marine Environment*, 2nd ed. D.G. Capone, D.A. Bronk, M.R. Mulholland, and E.J. Carpenter, eds, Elsevier Press, Amsterdam.

Hutchins, D.A., F.-X. Fu, Y. Zhang, M.E. Warner, Y. Feng, K. Portune, P.W. Bernhardt, and M.R. Mulholland. 2007. CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth rates, and elemental ratios: Implications for past, present, and future ocean biogeochemistry. *Limnology and Oceanography* 52:1,293–1,304.

Iglesias-Rodriguez, M.D., P.R. Halloran, R.E.M. Rickaby, I.R. Hall, E. Colmenero-Hidalgo, J.R. Gittins, D.R.H. Green, T. Tyrrell, S.J. Gibbs, P. von Dassow, and others. 2008. Phytoplankton calcification in a high-CO_2 world. *Science* 320(5874):336–340, doi:10.1126/science.1154122.

Kleypas, J.A., R.W. Buddemeier, D. Archer, J.-P. Gattuso, C. Langdon, and B.N. Opdyke. 1999. Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science* 284:118–120.

Kim, J.-M., K. Lee, K. Shin, J.-H. Kang, H.-W. Lee, M. Kim, P.-G. Jang, and M.-C. Jang. 2006. The effect of seawater CO_2 concentration on growth of a natural phytoplankton assemblage in a controlled mesocosm experiment. *Limnology and Oceanography* 51(4):1,629–1,636.

Kranz, S.A., D. Sültemeyer, K.-U. Richter, and B. Rost. 2009. Carbon acquisition by *Trichodesmium*: The effect of pCO_2 and diurnal changes. 2009. *Limnology and Oceanography* 54(2):548–559.

Leonardos, N., and R.J. Geider. 2005. Elevated atmospheric carbon dioxide increases organic carbon fixation by *Emiliania huxleyi* (Haptophyta) under nutrient-limited high-light conditions. *Journal of Phycology* 41:1,196–1,203.

Levitan, O., G. Rosenberg, I. Setlik, E. Setlikova, J. Grigel, J. Klepetar, O. Prasil, and I. Berman-Frank. 2007. Elevated CO_2 enhances nitrogen fixation and growth in the marine cyanobacterium *Trichodesmium*. *Global Change Biology* 13:531–538.

Mahaffey, C., A.F. Michaels, and D.G. Capone. 2005. The conundrum of marine N_2 fixation. *American Journal of Science* 305:546–595.

Mahowald, N.M., and C. Luo. 2003. A less dusty future? *Geophysical Research Letters* 30(17), 1903, doi:10.1029/2003GL017880.

Matear, R.J., and R.C. Hirst. 2003. Long-term changes in dissolved oxygen concentrations in the ocean caused by protracted global warming. *Global Biogeochemical Cycles* 17(4):1,125, doi:10.1029/2002GB001997.

Millero, F.J. 2007. The marine inorganic carbon cycle. *Chemical Reviews* 107:308–341.

Millero, F.J., R. Woosley, B. DiTrolio, and J. Waters. 2009. Effect of ocean acidification on the speciation of metals in seawater. *Oceanography* 22(4):72–85.

Milligan, A.J., D.E. Varela, M.A. Brzezinski, and F.M.M. Morel. 2004. Dynamics of silicon metabolism and silicon isotopic discrimination in a marine diatom as a function of pCO_2. *Limnology and Oceanography* 49(2):322–329.

Montoya, J.P., C.M. Holl, J.P. Zehr, A. Hansen, T.A. Villareal, and D.G. Capone. 2004. High rates of N_2 fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean. *Nature* 430:1,027–1,032.

Mulholland, M.R., and M.W. Lomas. 2008. Nitrogen uptake and assimilation. Pp. 303–385 in *Nitrogen in the Marine Environment*, 2nd ed. D.G. Capone, D.A. Bronk, M.R. Mulholland, and E.J. Carpenter, eds, Elsevier Press, Amsterdam.

Mulholland, M.R., and P.W. Bernhardt. 2005. The effect of growth rate, phosphorus concentration, and temperature on N_2 fixation, carbon fixation, and nitrogen release in continuous cultures of *Trichodesmium* IMS101. *Limnology and Oceanography* 50:839–849.

Najjar, R.G., C.R. Pyke, M.B. Adams, D. Breitburg, M. Kemp, C. Hershner, R. Howarth, M. Mulholland, M. Paolisso, D. Secor, K. Sellner, D. Wardrop, and R. Wood. In press. Potential climate-change impacts on the Chesapeake Bay. *Estuarine, Coastal, and Shelf Science*.

Oschlies, A., K.G. Schulz, U. Riebesell, and A. Schmittner. 2008. Simulated 21st century's increase in oceanic suboxia by CO_2-enhanced biotic carbon export. *Global Biogeochemical Cycles* 22, GB4008, doi:10.1029/2007GB003147.

Prufert-Bebout, L., H.W. Paerl, and C. Lassen. 1993. Growth, nitrogen fixation, and spectral attenuation in cultivated *Trichodesmium* species. *Applied and Environmental Microbiology* 59:1,367–1,375.

Redfield, A.C. 1958. The biological control of chemical factors in the environment. *American Scientist* 46(3):205–221.

Riebesell, U. 2004. Effects of CO_2 enrichment on marine phytoplankton. *Journal of Oceanography* 60(4):719–729.

Riebesell, U., K.G. Schulz, R.G.J. Bellerby, M. Botros, P. Fritsche, M. Meyerhofer, C. Neill, G. Nondal, A. Oschlies, J. Wohlers, and E. Zollner. 2007. Enhanced biological carbon consumption in a high CO_2 ocean. *Nature* 450:545–548, doi:10.1038/nature06267.

Robinson, R.S., A. Mix, and P. Martinez. 2007. Southern Ocean control on the extent of denitrification in the southeast Pacific over the last 70 ky. *Quaternary Science Review* 26:2,001–2,212.

Rost, B., I. Zondervan, and D. Wolf-Gladrow. 2008. Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: Current knowledge, contradictions and research directions. *Marine Ecology Progress Series* 373:227–237.

The Royal Society. 2005. Ocean acidification due to increasing atmospheric carbon dioxide. Policy document 12/05 ISBN 0 85403 617 2. Abstract available online at: http://www.royalsoc.ac.uk/Report_WF.aspx?pageid=9633&terms=Ocean+acidification+due+to+increasing+atmospheric+carbon+dioxide (accessed November 17, 2009).

Saito, M.A., T.J. Goepfert, and J.T. Ritt. 2008. Some thoughts on the concept of colimitation: Three definitions and the importance of bioavailability. *Limnology and Oceanography* 53:276–290.

Sañudo-Wilhelmy, S.A., A.B. Kustka, C.J. Gobler, D.A. Hutchins, M. Yang, K. Lwiza, J. Burns, D.G. Capone, J.A. Raven, and E.J. Carpenter. 2001. Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central North Atlantic Ocean. *Nature* 411:66–69.

Sarmiento, J.L., T.M.C. Hughes, R.J. Stouffer, and S. Manabe. 1998. Simulated response of the ocean carbon cycle to anthropogenic climate warming. *Nature* 393:245–249, doi:10.1038/30455.

Sedwick, P.N., E.R. Sholkovitz, and T.M. Church. 2007. Impact of anthropogenic combustion emissions on the fractional solubility of aerosol iron: Evidence from the Sargasso Sea. *Geochemistry Geophysics Geosystems* 8, Q10Q06, doi:10.1029/2007GC001586.

Takeda, S., and A. Tsuda. 2005. An in situ iron-enrichment in the western subarctic Pacific (SEEDS): Introduction and summary. *Progress in Oceanography* 64:95–109.

Tanaka, T., T.F. Thingstad, T. Løvdal, H.-P. Grossart, A. Larsen, M. Allgaier, M. Meyerhofer, K.L. Schulz, J. Wohlers, E. Zollner, and U. Riebesell. 2008. Availability of phosphate for phytoplankton and bacteria and of glucose for bacteria at different pCO_2 levels in a mesocosm study. *Biogeosciences* 5:669–678.

Tortell, P.D., G.R. DiTullio, D.M. Sigman, and F.M.M. Morel. 2002. CO_2 effects on taxonomic composition and nutrient utilization in an Equatorial Pacific phytoplankton assemblage. *Marine Ecology Progress Series* 236:37–43.

Tortell, P.D., C.D. Payne, Y. Li, S. Trimborn, B. Rost, W.O. Smith, C. Riesselman, R.B. Dunbar, P. Sedwick, and G.R. DiTullio. 2008. CO_2 sensitivity of Southern Ocean phytoplankton. *Geophysical Research Letters* 35, L04605, doi:10.1029/2007GL032583.

Ward, B.B. 2008. Nitrification in marine systems. Pp. 199–262 in *Nitrogen in the Marine Environment*, 2nd ed. D.G. Capone, D.A. Bronk, M.R. Mulholland, and E.J. Carpenter, eds, Elsevier Press, Amsterdam.

Wu, J., W. Sunda, E.A. Boyle, and D.M. Karl. 2000. Phosphate depletion in the western North Atlantic Ocean. *Science* 289:759–762.

Yool, A., A.P. Martin, C. Fernandez, and D.R. Clark. 2007. The significance of nitrification for oceanic new production. *Nature* 447:1,000–1,002, doi:10.1038/nature05885.

Zehr, J.P., J.B. Waterbury, P.J. Turner, J.P. Montoya, E. Omoregie, G.F. Steward, A. Hansen, and D.M. Karl. 2001. Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean. *Nature* 412:635–638.