Nutrient Cycles and Marine Microbes in a CO$_2$-Enriched Ocean

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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$_2$
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, chemosynthetic autotrophs, 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.

**THE MARINE 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 oxidation (NH$_3$ 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 2a](image-url)

*Figure 2a. 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 N₂ and carbon fixation responses of *Trichodesmium* represents the largest physiological response yet reported for any marine microbial group (including phytoplankton) to projected pCO₂ changes. If these culture results can be extrapolated to the environment, *Trichodesmium* and perhaps other N₂-fixing cyanobacteria seem poised to be among the biggest "winners" in the future anthropogenically acidified ocean. Such major increases in diazotrophy due to CO₂ 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 CO₂ 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 pCO₂ 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 CO₂ effects on marine N₂ fixers other than *Trichodesmium*. Only one study has examined the response of N₂-fixing unicellular cyanobacteria to elevated CO₂, 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 N₂ fixation rates were enhanced by 40% when cultures were grown at pCO₂ 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 N₂ fixation rates as pCO₂ increased. These results provide a cautionary note, in that we cannot assume that all diazotrophs will respond favorably or uniformly to higher CO₂ levels. These authors suggest that this contrasting CO₂ response by *N. spumigena* could be due to deleterious effects of lower pH on the transport of fixed nitrogen compounds from the site of N₂ 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 N₂ fixers, such as unicellular cyanobacteria, diatom/diazotroph associations, zooplankton/diazotroph symbioses, and N₂-fixing heterotrophic eubacteria or archaea, we are still at the rudimentary stages of understanding and quantifying the contributions of these groups to marine N₂ fixation and assessing where N₂ fixation occurs in the ocean (Carpenter and Capone, 2008). Little or nothing at all is known about how they will react to future pCO₂ changes.

The other limitation to our understanding of overall ocean acidification or CO₂ enrichment effects on N₂ 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 N₂ fixation rates by elevated pCO₂ 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 CO₂/air mixtures. Rate measurements from these incubations using ¹⁵N₂ tracer techniques (Mulholland and Bernhardt, 2005) demonstrated that increased pCO₂ stimulated N₂ 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
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.

**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 CO₂ limitation in many prokaryotic and eukaryotic phytoplankton at present-day pCO₂ is the low affinity of the Rubisco enzyme for CO₂ (Rost et al., 2008). Consequently, nitrifying proteobacteria should be equally vulnerable to CO₂ limitation, because both the NH₃-oxidizing genus *Nitrosomonas* and the NO₂-oxidizing genus *Nitrobacter* also fix carbon using the Calvin cycle (Ward, 2008). Thus, there is the possibility that the CO₂ 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 CO₂ by bubbling seawater with CO₂-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 pCO₂ 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 CO₂ concentrations of 1000 ppm. Their model output suggested that the ratio of nitrate:nitrite + 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 pCO₂ increases was presented by Beman et al. (2008) at the American Society of Limnology and Oceanography (ASLO) meeting in Nice, France. They found that pCO₂ 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 pCO₂. More studies are needed before we will be able to make reliable quantitative predictions of changes in nitrification rates in a high CO₂ 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 pCO₂ 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 pCO₂ 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 CO₂-fixing, hydrogenotrophic denitrifying bacteria in sewage sludge are inhibited when elevated pCO₂ 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 CO₂-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 CO₂ (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-CO₂ 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 pCO₂ 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 pCO₂ 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-CO₂ ocean. Because there are quite a number of contrasting and sometimes conflicting reports on how phytoplankton elemental stoichiometry may change in response to higher pCO₂ (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). CO₂-driven enhancement of ocean N₂ 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 P AND Si CYCLES

Compared to nitrogen, relatively little research effort has been directed toward understanding the effects of high CO₂ on the marine phosphorus cycle. The dominant form of inorganic phosphorus in seawater at pH 8 is HPO₄²⁻ (~ 87%), but the fraction of H₂PO₄⁻ 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 ³²P) and potential dissolved organic phosphorus utilization (by assaying alkaline phosphatase activity) as a function of pCO₂ 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 CO₂, and although a few minor trends emerged, they found no statistically significant effects of pCO₂ 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\textsubscript{2} from 375 to 750 ppm, while those of the dinoflagellate Prorocentrum minimum were slightly lower at the higher pCO\textsubscript{2}. Likewise, Hutchins et al. (2007) and Fu et al. (2007) found that a similar pCO\textsubscript{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\textsubscript{2} for the estuarine cyanobacterium Nodularia spumigena. At this point, the available evidence suggests negligible or minor effects of projected future changes in pCO\textsubscript{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\textsubscript{4}SiO\textsubscript{4}), which, like phosphorus, comprises a buffer system in seawater. The relatively basic pK\textsubscript{a} values for the dissociation of H\textsubscript{4}SiO\textsubscript{4} (pK\textsubscript{a1} = 9.84, pK\textsubscript{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\textsubscript{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\textsubscript{2}, although the quota increased somewhat at very low (100 ppm) pCO\textsubscript{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\textsubscript{2} levels. The biggest effect of pCO\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2} could certainly affect silicon biogeochemistry indirectly through shifts in algal community structure. A natural community pCO\textsubscript{2} manipulation experiment in the equatorial Pacific showed lower dissolved Si:N consumption ratios at 150 ppm CO\textsubscript{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\textsubscript{2}. CO\textsubscript{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\textsubscript{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\textsubscript{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 silification due to pCO\textsubscript{2}. Similarly, Bellerby et al. (2008) found that silicate drawdown was virtually identical in 350-, 700-, and 1050-ppm CO\textsubscript{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\textsubscript{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 $p$CO$_2$ 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 $p$CO$_2$ 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 $p$CO$_2$ 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, $p$CO$_2$ 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 pCO2 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 pCO2; 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) CO2-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 CO2 (Fu et al., 2007)
- 3 and 4, Fe-replete and Fe-limited Crocosphaera grown at 380 and 750 ppm CO2 (Fu et al., 2008a)
- 5 and 6, P-replete and P-limited Trichodesmium grown at 380 and 750 ppm CO2
  (Hutchins et al., 2007)
- 7, Trichodesmium grown at 400 and 900 ppm CO2 (Levitan et al., 2007)
- 8, Trichodesmium grown at 380 and 750 ppm CO2 (Barcelos e Ramos et al., 2007)
- 9, Trichodesmium grown at 370 and 1000 ppm CO2 (Kranz et al., 2009)

(b) CO2-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 CO2 and Skeletonema grown at 400 and 720 ppm CO2 (Burkhart et al., 1999)
- 12 and 13, the Antarctic diatom Chaetoceros and the Antarctic colonial prymnesiophyte Phaeocystis grown at 430 and 820 ppm CO2 (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 pCO2 (Feng et al., 2008)
- 16, the coccolithophorid Emiliania huxleyi grown at 490 and 750 ppm pCO2 (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 CO2 (Leonardos and Geider, 2005)
- 19 and 20, the toxic raphidophyte Heterosigma and the dinoflagellate Prorocentrum grown at 375 and 750 ppm pCO2 (Fu et al., 2008b)
- 21 and 22, P-replete and P-limited cultures of the toxic dinoflagellate Karlodinium grown at 430 and 745 ppm CO2 (recent work of author Fu and colleagues)

(c) CO2-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 CO2 (Feng et al., 2009)
- 24, a shipboard continuous culture incubation in the Ross Sea, Antarctica, at 380 and 750 ppm CO2 (Feng et al., in press)
- 25, a shipboard semicontinuous culture incubation in the equatorial Pacific at 150 and 750 ppm CO2 (Tortell et al., 2002)
- 26 and 27, mesocosm batch culture incubations in a Norwegian fjord at 350 and 700 ppm CO2 and 410 and 710 ppm pCO2 (Riebesell et al., 2007; Engel et al., 2005)
- 28, a mesocosm batch incubation in Korean coastal waters at 400 and 750 ppm CO2 (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 pCO2 (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 pCO2 (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 pCO2. 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 pCO2; 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 pCO2 in Prochlorococcus. The C:N ratios of Fe-replete cultures of the unicellular diazotroph Crocosphaera increased with higher pCO2 (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 pCO2 (Figure 5a).

Eukaryotic Phytoplankton Cultures

Figure 5b shows a compendium of results from laboratory CO2 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 pCO2 levels, but there were several exceptions. Burkhart 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 pCO₂. N:P ratios in *E. huxleyi* did not respond to changing pCO₂ 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 pCO₂ 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 pCO₂; 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 pCO₂ 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 pCO₂, 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 pCO₂ 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 pCO₂ 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 pCO₂ 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 pCO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 N₂ fixation rates over the next century. This large influx of new nitrogen will be due to the CO₂ 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 pCO₂, although we suggest that the evidence for the latter is still not conclusive (see below). Interestingly, the predicted century-time-scale increases in N₂ 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
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 CO₂-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 pico-cyanobacteria 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.

Interactions of Other Global Change Factors With Ocean Acidification

Another large uncertainty is the interaction of rising pCO₂ 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 CO₂ 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 N₂ 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 pCO₂ 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 CO₂ fertilization in a rapidly changing ocean.

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