Aquatic primary production in a high-CO₂ world

Etienne Low-Décarie, Gregor F. Fussmann, and Graham Bell

McGill University, Department of Biology, Stewart Biology Building, 1205 Avenue Docteur-Penfield, Montreal, QC, H3A 1B1, Canada

Here, we provide a review of the direct effect of increasing CO_2 on aquatic primary producers through its function as a source of carbon, focusing our analysis on the interpretation of this increase as an increase in the availability of a resource. This provides an interesting context to evaluate ecological and evolutionary theories relating to nutrient availability and leads us to: the assessment of theories about limitation of productivity and the integration of CO_2 into the co-limitation paradigm; the prediction of community composition and of change in communities from known changes in the environment; and evaluation of the potential for evolutionary adaptation in conditions that increase growth.

The neglected nutrient

Human activity is altering global biogeochemical cycles and might have ushered in a new geological era, the Anthropocene [1]. One of the fastest global changes caused by human activity is the continuing increase in CO_2 levels in the atmosphere: atmospheric CO_2 concentrations have now reached over 400 ppm [2], the highest for millions of years [3,4]. Public interest and research programs have largely been concerned with the incidental effects of this increase in atmospheric CO₂, notably climate change. However, the unique property of CO_2 in the biosphere is its conversion to biomass through photosynthesis. The relatively limited research interest in CO₂ as a key nutrient is related to its high global availability relative to other resources, especially phosphorus. The atmosphere provides an essentially unlimited supply of both nitrogen and carbon (as CO_2) to all living organisms, whereas phosphorus is almost exclusively provided by the slow weathering of mineral reservoirs [5]. The biological availability of nitrogen may be strongly limited by the energetic cost of fixing atmospheric supplies. When the biological fixation rates of both carbon and nitrogen are sufficient, phosphorus is expected to be the main biotic limiting resource globally for aquatic ecosystems over sufficiently large time scales [6].

The limited role assigned to CO_2 in the limitation of productivity is based on the assumption that only a single

0169-5347/\$ - see front matter

CrossMark

nutrient can control productivity at any given time. In addition to varying between systems, the availability of CO_2 in aquatic systems will likely increase with rising atmospheric CO_2 concentrations (Box 1), potentially altering the conditions of life for aquatic primary producers (Box 5). Furthermore, over the past decades, human activity has greatly increased the availability of nitrogen [7], phosphorus [8], and many micronutrients [9] in aquatic ecosystems, thus potentially increasing the role of CO_2 . In this review, we reconsider the paradigm that only a single nutrient limits the productivity of aquatic systems, and challenge the disregard of CO_2 as a potentially important limiting resource. We suggest that CO_2 can function as a rate-limiting resource rather than a yield-limiting resource, and that it can interact with other resources in the co-limitation of productivity. We argue that the ecological responses observed as the result of CO₂ supplementation show that it can act as a limiting resource for phytoplankton. We assess the evidence for changes in phytoplankton community composition resulting from increasing atmospheric CO_2 and suggest that these changes are consistent with predictions based on the principle that the organisms that use a resource least efficiently are those that benefit most from an increase in its supply. Finally, we review the evidence for evolutionary change in response to increasing CO₂ levels, and suggest

Glossary

Carbon concentration mechanism (CCMs): ensemble of physiological systems that contribute to the accumulation of CO₂ around RubisCO. In phytoplankton, this can involve active transport of HCO_3^- , CO₂ (Box 5), and/or H⁺ [103].

Chlorophytes: eukaryotic algae, also known as green algae, that are members of a clade of Chlorophyta [104].

Corresponding author: Bell, G. (graham.bell@mcgill.ca).

^{© 2014} Elsevier Ltd. All rights reserved. http://dx.doi.org/10.1016/j.tree.2014.02.006

Chrysophytes: eukaryotic algae, also known as golden algae, that lack an active CCM and produce siliceous cysts and scales that can be studied in the fossil record.

Diatoms: eukaryotic algae of the class Bacillariophyceae that produce cells enclosed in a silica frustule.

Free air carbon enrichment (FACE) experiments: experiments in which concentrations of CO₂ are increased locally without the use of chambers. CO₂ is pumped directly into the air and local concentrations are controlled through feedback with a sensor. These experiments allow the study of the response of a whole system to CO₂ enrichment under natural conditions, including airflow and mixing.

Redfield ratio: the average atomic ratio of carbon, nitrogen, and phosphorus (106:16:1) found in phytoplankton. This ratio can be considered as the optimal ratio for phytoplankton growth and deviations from it suggest nutrient limitation.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO): a key enzyme for the fixation of atmospheric CO₂ during photosynthesis and for the oxygenation of the resulting compounds during photorespiration.

Box 1. Increasing atmospheric CO_2 and carbon availability in aquatic ecosystems

Several biotic and abiotic factors regulate inorganic carbon concentration in aquatic ecosystems. Thorough reviews of carbon chemistry in aquatic systems and its relation to acidity have been provided elsewhere (for freshwater, see [87,88]; and for saltwater, see [89,90]). In many aquatic systems, dissolved inorganic carbon (DIC) is mostly present as bicarbonate, with <1% of DIC found as free CO₂. However, most phytoplankton can actively take up bicarbonate through their CCM and can facilitate the conversion of bicarbonate to free CO₂ within or outside the cell with the enzyme carbonic anhydrase (CA) [91].

Aquatic ecosystems can be either CO_2 sources (supersaturated and releasing CO_2 to the atmosphere) or sinks (taking up CO_2 from the atmosphere). Marine systems were historically near equilibrium with the atmosphere, but are now net sinks of CO_2 as they reequilibrate with the increasing atmospheric concentrations [92]. The presence of large amounts of organic matter from terrestrial systems causes freshwater systems to be dominated by heterotrophic processes, and these systems act as net sources of CO_2 to the atmosphere ([93–95], but see [96,97]). Outgassing of CO_2 from freshwater systems is comparable to both total global net productivity and the total emissions from burning of fossil fuel [98].

The rate of gas exchange between water and air is directly proportional to the concentration gradient of the gas across the phase boundary [99]. Thus, assuming the ratio of photosynthetic and respiration rates remains constant under elevated CO₂₀ increasing atmospheric CO₂ will lead to increases in CO₂ and DIC concentrations and decrease pH in aquatic systems, including supersaturated freshwater systems. However, increasing CO₂ can affect rates of photosynthesis and respiration, potentially leading to complex feedbacks between DIC and its production and consumption (e.g., the priming effect [58]). In addition, other global changes may alter CO₂ concentrations in aquatic systems. Rising temperatures can increase the mineralization of dissolved organic matter [100], which could increase the amount of dissolved CO_2 and thermal stratification, which could alter the range and distribution of CO₂ concentrations within the water column [101]. The complex interplay of processes in the water and the atmosphere suggests that experiments that emulate the free air carbon enrichment (FACE) experiments performed in terrestrial systems [102] would be required to determine the exact changes in water chemistry that will be caused by increasing atmospheric CO₂ concentrations. Such experiments may reveal unforeseen tipping points in the response of aquatic ecosystems to increasing atmospheric CO₂.

that specific adaptation to elevated CO_2 and other environmental changes that increase growth is unlikely to evolve.

CO₂ in limiting nutrient paradigms

Liebig's law and CO₂ as a limiting resource

Phosphorus is the main limiting resource for phytoplankton growth over geological time, because both nitrogen and carbon, which can limit growth on shorter time scales in some systems [10], are provided in abundance by the atmosphere and phosphorus is only made naturally available from the weathering of rock.

In freshwater, chlorophyll concentration is tightly correlated with phosphorus concentration both in space, across lakes, and in time, within lakes across seasons [6]. Whole-lake experiments first showed that supplementation with phosphorus and nitrogen increased algal yield [11] and induced short-term carbon limitation [12]; subsequently, it was shown that phosphorus addition was the crucial factor [13]. The identification of phosphorus as the main limiting resource in freshwater systems is widely credited with the successful limitation of the spread of eutrophication in many lakes and river estuaries [14].

In the oceans, the Redfield ratio (see Glossary) characterizes a fixed stoichiometric ratio of carbon and nitrogen relative to phosphorus [15]. Despite the variation in these stoichiometric ratios that has now been documented [16,17], the Redfield ratio remains a central principle for understanding biogeochemical cycles and nutrient limitation (although alternatives are being proposed; e.g., [18]). The prevailing paradigm for saltwater is nitrogen limitation due to lower rates of nitrogen uptake and assimilation than in freshwater systems [19]. Phosphorus might limit the net primary production of oceans on geological timescales [20,21], but few experiments have shown that phosphorus supplementation increases yield, whereas several experiments have shown that the addition of silicon, nitrogen, or iron causes increases in productivity [22]. This highlights the importance of time scale when trying to identify experimentally the processes that determine nutrient limitation in oceans [23].

The disregard for the potential role of resources other than phosphorus, including CO2, is based on Liebig's law of the minimum [24], which states that only one resource can limit productivity at any one time. Liebig's law of the minimum remains the main principle of models investigating growth and competition for resources (e.g., [25,26], but see 'Co-limitation by several resources') and is an active topic of investigation in terrestrial systems [27,28]. The increasing availability of phosphorus [8] and nitrogen [29] in many ecosystems might reduce the limitation of productivity by these resources, thereby making it more likely that CO₂ limits productivity. Furthermore, Liebig's law of the minimum was intended to guide the provision of nutrients to agricultural monocultures, and such a simple theory might be misleading when it is applied to diverse phytoplankton communities [30].

There is ample evidence that CO_2 can be a limiting resource, at least locally or on short temporal scales. CO_2 can limit productivity of eutrophic lakes in instances where photosynthetic demand exceeds diffusive influx from the atmosphere [12]. CO_2 supersaturation in lakes increases primary productivity up to tenfold when compared with the same system in equilibrium with the atmosphere, even in lakes where phosphorus limitation is expected [31]. In oceans, the experimental results are less clear, but CO₂ concentrations below current levels do reduce productivity [32] and higher CO_2 concentration, manipulated using acidification, increased productivity even at nutrient-poor sites [33]. Increased CO_2 greatly increases the productivity of benthic algae in marine systems [34]. Furthermore, most marine and freshwater phytoplankton have inducible carbon concentration mechanisms (CCMs) that serve to increase the concentration of CO_2 at the site of photosynthesis [35]. Although the effectiveness of CCMs varies across taxonomic groups (Figure 1), the evolution of such a mechanism across groups suggests a selective advantage to having a CCM. CO_2 availability can be limiting even when carbon is plentifully available in other forms, as is the case in aquatic systems with high pH.



Figure 1. Physiological differences in the capacity of phytoplankton to uptake and use CO_2 . Major taxonomic groups of phytoplankton differ in their CO_2 physiology, suggesting that predictable changes in community composition should arise as a result of increasing CO_2 concentration. Error bars represent one standard deviation of the mean after averaging value by species. Based on a plot by [50], but using data directly collected from the literature (see references provided in the supplemental material online).

Rate-limiting and yield-limiting resources. The concept of a single limiting resource can be reconciled with the ability of CO_2 to limit productivity. The solution is to integrate the concept of time scale [36] by distinguishing between ratelimiting resources and yield-limiting resources (Figure 2, [37,38] and implications for nutrient limitation measurements [39]). Rate-limiting resources are defined as resources whose concentration affects the speed of biomass accretion [growth rate (r)], whereas yield-limiting resources are resources whose total amount limits the potential maximum of biomass present in a system [carrying capacity (K)]. Rate-limiting resources have a low uptake rate compared with the uptake rate that would maximize growth. This can be caused by physiological limitations, such as the low affinity of transport systems, or by a low rate of supply to the local environment from a reservoir, such as the atmosphere in the case of CO₂. Resources directly involved in the uptake and storage of energy, such as CO_2 (Box 5), are likely to be rate-limiting resources. Yield-limiting resources become limiting when they are depleted from the environment because the provision of these resources from reservoirs is negligible on ecological time scales. Phosphorus is a vield-limiting resource. Dynamic systems with short boombust cycles, such as diel or seasonal cycles, might seldom have the time to reach the potential maximum biomass allowed by the availability of the yield-limiting resource. In these systems, realized yield might be controlled by the rate-limiting resource. Early models of phosphorus and CO₂ limitation of growth, which qualitatively match results from whole-lake experiments, describe CO_2 as a rate-limiting resource that can be important on short time scales, whereas phosphorus limits maximum yield and is important on larger time scales [40,41].

Co-limitation by several resources. Although different resources can limit growth rate and yield, a single resource can be the main limiting resource at any given time. However, there is growing evidence that neither growth rate nor maximum yield is always limited by a single resource [42]. A review of lake experiments indicated that the simultaneous addition of both nitrogen and phosphorus had a larger effect than the addition of either resource separately [43,44]. Synergistic responses to phosphorus and nitrogen addition have since been shown to be widespread in all major biomes [42,45]. The theory of a single limiting resource (Liebig's law of the minimum) can still explain cases of synergistic responses, provided that one of the two added nutrients has an effect when added



Figure 2. Conceptual separation of rate-limiting and yield-limiting resources. The unbroken line represents phytoplankton biomass increase under both rate and yield limitation. The dashed line indicates biomass increase under pure yield limitation due to addition of the rate-limiting resource, which increases growth rate initially without affecting carrying capacity. The dash-and-dot line represents pure rate limitation due to addition of the yield-limiting resource, which affects carrying capacity without affecting growth rate. The dotted line represents release from both yield and rate limitation due to addition of both types of resource, which increases both growth rate and carrying capacity. Interpretation of the effect of each resource is dependent on the time of observation after resource addition. CO₂ might not have an apparent effect on biomass when biomass levels are already at the maximum yield afforded by phosphorus.



Figure 3. Types of co-limitation. Darkness of the tiles indicates productivity (rate or yield) standardized by maximum potential yield. Axes are relative amounts of each resource. (A) Serial co-limitation is consistent with Liebig's law of the minimum. The addition of the primary resource (black arrow) is required for the addition of the secondary resource (gray arrow) to have an effect. (B) Simultaneous co-limitation: at the time of nutrient addition, each resource might be in equilibrium with demand or below uptake thresholds, the addition of ne resource will not increase productivity (separate black and gray arrows) but the addition of both resources will (overlapping black and gray arrows). In the data simulation, productivity is determined by the same function as for Liebig's law of the minimum: the resource with the lowest availability limits productivity. (C) Aiding and substitutable resources with independent co-limitation: each resource can take the place of the other or can increase the rate of uptake of the other resource. Adding either resource independently increases productivity (separate black and gray arrows). Adding both resources would increase productivity further. (D,E) If individual species are each limited by a single resource, the addition of either resource increases the growth of one of the two species. (F) Community independent co-limitation: the community formed by species 1 and 2 can respond to the addition of both resources, and the addition of both resources might increase capacities), productivity can be determined by the sum (additive response to nutrient addition) or product (used here, synergistic responses to nutrient addition) of the limitation on productivity of each resource. If two essential nutrients are both below the uptake threshold of the organisms, there is 'simultaneous co-limitation' and the addition of either resource will have no effect, but the combined addition of both resources will increase productivity, although the addition of both resources will increase

individually (Figure 3). This has been termed 'serial colimitation' (for a visual explanation of serial co-limitation, see [46]) because only a single nutrient is limiting at any given time. As traditional drivers of eutrophication become more abundant, the potential for serial co-limitation involving CO_2 grows. However, other types of synergistic response to nutrient addition represent true deviations from Liebig's law of the minimum (Figure 3 and Box 2)

Although co-limitation involving nitrogen and phosphorus appears to be widespread, the prevalence of colimitation involving nutrient pairs that include CO_2 is unknown (Box 5). Few studies that have investigated the response of phytoplankton to increasing CO_2 have integrated the availability of another resource as a treatment. Most of these (eight out of nine experiments) showed responses that are in line with a synergistic response to CO_2 and the addition of the other nutrient; three experiments showed a pronounced increase with the addition of both resources compared with the addition of the secondary resource alone (nitrogen, phosphorus, and vitamin B_{12} ; Figure 4). Independent co-limitation has been found for CO_2 and phosphorus in a laboratory study of a phytoplankton species at low levels of both resources [47,48].

Species-specific limitation

One of the mechanisms for community-level co-limitation is provided by differences in the nutrient requirements of species within the community [49]. Major taxonomic groups of phytoplankton differ in both their ribulose-1,5bisphosphate carboxylase/oxygenase (RubisCO) specificity factor (the preference of the RubisCO enzyme for CO₂ rather than O₂ as substrate, which controls the ratio of carboxylation and oxygenation activity of the enzyme) and in the ability of their CCM to increase internal carbon concentrations ([50]; Figure 1). Cyanobacteria are extremely efficient at raising their internal CO₂ concentration

Box 2. Co-limitation: cases of true departure from Liebig's law

Three types of true deviation from Liebig's law of the minimum are widespread across all biomes for nitrogen and phosphorus [42] (Figure 3, main text): (i) low nutrient levels with several nutrients whose concentration is lower than the minimum at which they can be taken up by the cell (Figure 3B, main text). In dense algal blooms, both phosphorus and CO₂ can be drawn down to levels below the uptake capacity of aquatic primary producers; (ii) substitutable or aiding nutrients (reviewed in [105,106]; Figure 3C, main text). Substitutable resources are nutrients that can have the same biological role. For example, cadmium or cobalt can act as substitutes for zinc [107,108]. Aiding resources are those that enable the uptake or use of another resource, as has been found for copper and its function in the uptake of iron [109]. It can be postulated that CO₂ is the ultimate aiding resource and is co-limiting with all nutrients that enter the cell through active transport. Active transport is dependent on the energy produced through photosynthesis, which itself is directly dependent on CO₂ concentration and thus the uptake of resources requiring active transport might be dependent on CO₂ availability. Conversely, zinc, and its substitutes cobalt and cadmium, can act as aiding nutrients in the uptake of carbon as zinc is required for the functioning of carbonic anhydrase (CA) that is necessary to make use of the high availability of bicarbonate [110]; and (iii) different resources limiting growth of some species or genotypes belonging to the community (Figure 3F, main text, [49]). The productivity of each species conforms to Liebig's law, but the productivity of the community does not. Although CO₂ concentrations might not limit overall community productivity, they might limit the productivity of certain types of organism (major taxa, species, or genotypes) within the community. This mechanism implies differences between types within a community and will lead to changes in community composition with increasing CO₂. This mechanism also highlights the inadequacy of applying Liebig's law to diverse communities.

relative to their environment, reaching concentration differences of around 1000-fold. Given their efficient CCM, some cyanobacteria can reach their maximal growth rate at current ambient CO_2 concentrations [51,52]. By contrast, chlorophytes have inefficient mechanisms for both carbon concentration and utilization. If we grant that phytoplankton communities were near ecological equilibrium at historical concentrations of CO₂, chlorophytes would be expected to benefit the most from an increase in CO₂ concentration because they have the largest growth response and, consequently, will tend to increase in frequency in the community (Box 3). Other traits might influence carbon uptake and use, including: cell size, which influences the surface:volume ratio and, hence, the rate of diffusion into the cell; motility and buoyancy, which both influence the size of the CO₂-poor boundary layer around the cell [53]; and evolutionary constraints in the physiological requirements for carbon relative to other resources, which can be imposed by several factors, including cell size and even codon usage in the genome [54].

The effect of CO₂ on community dynamics

Differences between species or groups of species in their carbon economy are expected to lead to changes in community composition as atmospheric CO_2 concentration increases. The general principle that governs changes in community composition is simple: the group of phytoplankton that is currently most limited in its growth by CO_2 availability would stand the most to gain from an increase in CO_2 concentration and, thus, would be expected to



Figure 4. Potential co-limitation of CO_2 and other resources. Relative response to the addition of elevated CO_2 , the addition of a second resource, and the addition of both CO_2 and the secondary resource (combination). Most experiments (eight out of nine) showed a synergistic response to CO_2 and the addition of the other nutrient. Data compiled from the literature (see references provided in the supplemental material online).

increase in frequency in the community as CO_2 concentrations increase (Box 3). However, the response of the composition of phytoplankton communities to increasing CO_2 concentration is still uncertain and might differ between marine and freshwater systems.

Experiments in the sea have largely been designed to estimate parameters important in modeling global nutrient cycles (compiled and reviewed by [55]). In one mesocosm experiment, the frequency of fucoxanthin-containing phytoplankton (including diatoms and prymnesiophytes) was found to decrease with increasing CO_2 [56]. By contrast, two similar mesocosm experiments of the Pelagic Ecosystem CO_2 Enrichment (PeECE) program failed to show any detectable effect on community composition [57,58]. In the third experiment of the PeECE series, the response of the community to elevated CO_2 was not constant through time: once other nutrients in the mesocosms had been depleted, there was a linear increase in the frequency of chlorophytes with increasing CO₂ concentration [59,60]. The expansion of chlorophytes came at the expense of coccolithophores. These shifts in community composition following an artificially induced algal bloom are consistent with predictions from the physiology of major taxonomic groups (Figure 1). Cell size can also affect the response to increasing CO_2 [61]. Elevated CO_2 increases the relative abundance of larger species within diatom communities [32] and decreases the relative abundance of picophytoplankton [62].

In freshwater systems, community composition appears to be more closely linked with CO_2 availability. Phytoplankton blooms can reduce CO_2 concentrations in freshwater far below atmospheric concentrations and this might be one of the mechanisms that maintain a high frequency

Box 3. Predictability of community response to change in resource availability

Resource ratio-competition theory states that the type of organism (functional group, species, or genotype) in a system that has the lowest requirement for the resource provided at the lowest rate in that system will outcompete other types and that coexistence of types is possible when a different resource limits the growth of each type [111,112]. Testing of the resource-ratio theory, despite its widespread use, has been dominated by simplified aquatic laboratory systems [113-115]. For predicting patterns of biodiversity, the ratio of available resources and the ratio of demand for these resources is not sufficient and information on total availability of resources and total demand is required [116]. Furthermore, resource-ratio theory has been strongly influenced by Liebig's law of the minimum, with only a single nutrient limiting the growth of each type at one time and, at this level, might encounter the same problems as for the prediction of total yield from single resource limitation.

Although resource-ratio competition theory might not be sufficient to predict species composition from nutrient ratios in natural systems, its principles provide strong predictions of changes in the relative frequency of major taxonomic groups (functionally classified by their nutrient requirements) within communities, the classical example being plant succession on new soil or fertilized soil [117]. If there are differences between major taxonomic groups in their demand for specific nutrients, such as is the case for phytoplankton groups and CO₂, then changes in the availability of these resources should lead to predictable changes in the frequency of these major taxa. The types that utilize the resource least efficiently are those that are most likely to benefit from an increase in the supply of the latter and would be expected to increase in frequency in the community.

For plant succession following increasing nitrogen availability, this prediction is contentious and has often failed [118–120]. Increasing atmospheric CO_2 might present a special case for which the response of community composition, both for plants and phytoplankton, can be adequately predicted.

of cyanobacteria during blooms of freshwater phytoplankton [63]. As in marine systems, the effect of CO_2 concentration on community composition in freshwater systems can be attributed in part to a pH effect [64]. However, studies using laboratory cultures buffered against pH changes showed changes in community composition that were consistent with predictions from the physiology of major taxonomic groups: chlorophytes usually increased under elevated CO_2 at the expense of cyanobacteria [65]. Change in phytoplankton community composition in freshwater systems might even be used to infer changes in CO_2 concentration. The abundance of species of diatoms appears to be strongly and differentially determined by CO_2 concentrations even when differences in pH are taken into account, to the extent that diatom community composition is a reliable predictor of geographic variations in CO₂ concentration across lakes [66]. Throughout the paleolimnological record, chrysophytes, which do not have CCMs, were most abundant during the middle Eocene, a period of elevated atmospheric CO₂ concentrations, and have increased in abundance over the recent past with increasing CO₂ [67].

In both marine and freshwater environments, the effect of increasing CO_2 on community composition can also act indirectly through its effect on pH [68]. Coccolithophores, which are calcifying phytoplankton, are poor at concentrating and utilizing CO_2 and, thus, would be expected to Few experiments have explored the response of organisms to environmental changes that increase growth. The only long-term experiment involving the addition of nutrients to plant communities and allowing for the potential of evolutionary change (and arguably the longest running ecology and evolution experiment) is the Rothamsted Park Grass Experiment (PGE) [121]. In these experiments, historical phosphorus concentration did not affect plant growth response to phosphorus and magnesium in the test environment [122], whereas adaptation to calcium levels might have been detected [122]. The reciprocal transplant experiments designed to detect adaption to nutrient levels using material from the PGE lasted only a single generation and, thus, could not exclude maternal effects or carry-over from the source environment. In studies of the effect of CO2 on plants, 15 years of Free-Air-CO2enrichment (FACE) experiments did not elicit evolutionary change in any observed plant species [102,123]. Thus, adaptation to elevated nutrient levels might be unlikely in general. A significant proportion of ongoing global change increases the growth rate of certain organisms and a better understanding of the evolutionary consequences of such changes is essential for making adequate predictions of the response of ecosystems to a continuously changing environment.

benefit from increased CO_2 availability (Figure 1). However, the decrease in pH resulting from an increase in CO_2 causes a decrease in the ability of coccolithophores to calcify and an associated decrease in their growth rate.

Hence, it might be feasible to predict how the composition of phytoplankton communities will respond to increasing atmospheric CO_2 (Box 5). These predictions can rest on the simple principle that the types of phytoplankton least efficient at taking up and utilizing CO_2 , whether it be large phytoplankton or chlorophytes, will increase in frequency with higher CO_2 concentrations, provided that these types are not disproportionately harmed by a decrease in pH.

Evolution and adaptation to elevated CO₂

The possibility of changes in the frequency of species within a community in response to increasing CO_2 leads naturally to the possibility of changes in the frequency of genotypes within a species and, hence, to the possibility of evolutionary change in response to increasing CO_2 . However, most examples of evolutionary change have concerned adaptation to stressful environments where growth is reduced (e.g., [69]). Few studies have investigated the direct effect of elevated CO_2 under constant pH in aquatic systems (for a review of the evolutionary response of phytoplankton to ocean acidification, see [70]) or the evolutionary consequence of any other environmental change that results in increased growth rate (Boxes 4 and 5). Three evolutionary scenarios can be postulated to arise as a direct response to elevated CO_2 .

The first scenario is that organisms might adapt specifically to elevated CO_2 . Such adaptation could arise from the sorting of existing variation within a species or through the spread of novel mutations. The fact that there can be as much variation in response to CO_2 enrichment between ecotypes of the same species as there is between major taxonomic groups, indicates that variation on which selection can act to elicit adaptation is already present in some cases [71].

Box 5. Outstanding questions

- How readily will an increase in atmospheric CO₂ concentration translate into a biologically significant increase in dissolved CO₂ concentration in aquatic systems?
- Does Liebig's law hold when dealing with CO₂?
- Is the distinction between rate-limiting and yield-limiting resources ecologically important?
- Can CO₂ co-limit productivity?
- Predictions of change in the composition of phytoplankton communities in response to increasing CO₂ can be predicted from physiology. Can such predictions be made for other environmental changes?
- Are environmental changes that cause an increase in growth as likely to lead to evolutionary changes as deleterious environmental changes?

The second scenario is that organisms might accumulate mutations that are not detrimental under elevated CO₂ but would be costly under current ambient conditions (so-called 'conditionally deleterious mutations'). The potential for such mutations is illustrated by the numerous mutagenesis experiments that led to the creation of genotypes that are capable of growth under elevated CO_2 but which have depressed fitness under ambient CO_2 conditions (e.g., [72,73]). Alternatively, the physiological mechanisms underlying the direct response to elevated CO_2 might necessarily reduce growth at current ambient CO₂ (functional interference or 'antagonistic pleiotropy'). Both mutational degradation and functional interference lead to a negative indirect response to elevated CO₂ when evolved lines are exposed to current ambient concentrations.

The third scenario is that there might be no specific evolutionary change, direct or indirect. In addition to their ecological importance in aquatic ecosystems, microalgae including phytoplankton are good model organisms to study potential adaptation to elevated nutrients because large populations can be grown for hundreds of generations in small volumes within brief periods of time. After culture at ambient and elevated CO₂ concentrations for 1000 generations, no specific adaptation was detected in selection lines of Chlamydomonas reinhardtii. However, some selection lines grew poorly in ambient CO_2 , suggesting the accumulation of conditionally deleterious mutations in the CCM [74,75]. Similar patterns were found in microalgae naturally exposed to elevated CO₂ concentrations [76]. Efforts to extend these results to other species and taxonomic groups failed to elicit any specific evolutionary response to elevated CO_2 , either direct or indirect, even after over 750 generations of exposure [77]. Finally, even when the consequences of evolution were tested in the more ecologically relevant context of a community, long-term selection under elevated CO₂ and warming failed to cause detectable evolutionary changes [78].

The experiments that were conducted to detect evolutionary response to elevated CO_2 were performed in such a way as to maximize the probability that a response would occur. For example, long-term selection of a single species in isolation is expected to be more likely to cause adaptation to elevated CO_2 than is selection in the wild in complex communities (reviewed in [79] and shown specifically for the evolution of phytoplankton to elevated CO_2 [80]). Hence, the failure to observe specific adaptation in the laboratory suggests that it is unlikely to evolve in the field, at least in the short term of a few thousands of generations.

Specific adaptation to elevated CO_2 might also be obstructed in natural environments because CO_2 availability is variable in most habitats, and CO_2 concentrations can also be variable through time. Freshwater phytoplankton can face diel cycles of 100-fold differences in CO_2 availability [81], coastal habitats can face daily variations of 2.5-fold [82], and even the open sea experiences a similar level of variability over seasons [83]. Given that low CO_2 conditions are stressful, periods of low CO_2 concentration will maintain selection for organisms capable of growth at these low levels.

Traits allowing adaptation to low levels of CO_2 tend to be plastic and, thus, incur little cost at elevated CO_2 . Cyanobacteria can alter their CCM activity as a function of carbon availability [84] and chlorophytes can also switch off the CCM and reduce RubisCO content under elevated CO_2 [85]. Thus, the capacity to grow under low CO_2 concentrations might come with little or no trade-off for growth in high CO_2 conditions. This lack of trade-off reduces the potential for evolutionary change specific to elevated CO_2 .

At present, there are few examples of adaptation in response to environmental changes that increase growth, and few experiments have explored the response of organisms to such changes (Box 4). Adaptation in the context of elevated CO_2 concentrations might be more likely to occur through its effect of decreasing pH rather than through its direct effect as a carbon resource. Coccolithophores, the only phytoplankton group for which a decreased growth rate under elevated CO_2 has been reported, have been found to adapt to the acidic conditions of elevated CO_2 [86]. After long-term exposure to elevated CO_2 , adapted lines of the coccolithophores were able to regain their calcification and growth rates.

Concluding remarks

Predicting how phytoplankton will respond to global change, including rising CO₂ and increased availability of other nutrients, is essential to the accuracy of biogeochemical models and their estimates of atmospheric CO_2 , climate change, and even the productivity of fisheries. To provide accurate predictions, the current dogma of singlenutrient limitation should be reviewed in light of the possibility that nutrients can limit different aspects of productivity and that several nutrients can act in concert to control productivity. The effect of CO_2 on community composition needs to be investigated more thoroughly. Integrating current knowledge about phytoplankton physiology might provide a framework capable of predicting community composition changes in lakes and oceans. This framework is based on the expectation that the types of phytoplankton that are currently most limited by the availability of CO₂ will increase in frequency in the community. Accurate prediction might also require information about how CO₂ concentrations affect other members of the aquatic community, including macrophytes, bacteria,

and zooplankton. However, the difficulty of specific adaptation to elevated CO_2 implies that models are unlikely to require the inclusion of evolutionary change to make reliable predictions.

In this review, we have elucidated mechanisms by which the inexorable increase in the global supply of a major nutrient could drive changes in the productivity and composition of the phytoplankton communities of freshwater and marine ecosystems. Given that phytoplankton generate half of all primary production [20], it is important that we gain a clearer understanding of what these changes are likely to be and how they will come about.

Acknowledgments

This work was supported by the Natural Science and Engineering Research Council of Canada (NSERC) through grants to G.F.F. and G.B., and a scholarship to E.L-D., and by the Fonds de recherche sur la nature et les technologies du Québec (FQRNT) through a scholarship to E.L-D.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tree.2014.02.006.

References

- 1 Crutzen, P.J. (2002) Geology of mankind. Nature 415, 23
- 2 Tans, P. and Keeling, R. (2013) Trends in Atmospheric Carbon Dioxide, Global Greenhouse Gas Reference Network
- 3 Royer, D.L. (2006) CO2-forced climate thresholds during the Phanerozoic. *Geochim. Cosmochim. Acta* 70, 5665–5675
- 4 Pagani, M. et al. (2011) The role of carbon dioxide during the onset of Antarctic glaciation. Science 334, 1261–1264
- 5 Smil, V. (2000) Phosphorus in the environment: natural flows and human interferences. Annu. Rev. Energy Environ. 25, 53–88
- 6 Smith, V.H. (2009) Eutrophication. In *Encyclopedia of Inland Waters* (Likens, G.E., ed.), pp. 61–73, Elsevier
- 7 Fowler, D. et al. (2013) The global nitrogen cycle in the twenty-first century. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 368, 20130164
- 8 Bennett, E.M. *et al.* (2001) Human impact on erodable phosphorus and eutrophication: a global perspective. *Bioscience* 51, 227–234
- 9 Rauch, J.N. and Pacyna, J.M. (2009) Earth's global Ag, Al, Cr, Cu, Fe, Ni, Pb, and Zn cycles. *Global Biogeochem. Cycles* http://dx.doi.org/ 10.1029/2008GB003376
- 10 Vitousek, P. and Howarth, R. (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13, 87–115
- 11 Schindler, D.W. (1971) Carbon, nitrogen, and phosphorus and the eutrophication of freshwater lakes. J. Phycol. 7, 321–329
- 12 Schindler, D.W. and Fee, E.J. (1973) Diurnal variation of dissolved inorganic carbon and its use in estimating primary production and CO2 invasion in lake 227. J. Fish. Res. Board Can. 30, 1501–1510
- 13 Schindler, D.W. (1977) Evolution of phosphorus limitation in lakes. Science 195, 260–262
- 14 Conley, D.J. et al. (2009) Ecology. Controlling eutrophication: nitrogen and phosphorus. Science 323, 1014–1015
- 15 Libes, S. (2009) Introduction to Marine Biogeochemistry, Academic Press
- 16 Bertilsson, S. et al. (2003) Elemental composition of marine Prochlorococcus and Synechococcus: implications for the ecological stoichiometry of the sea. Limnol. Oceanogr. 48, 1721–1731
- 17 Klausmeier, C. et al. (2004) Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature 429, 171–174
- 18 Mills, M.M. and Arrigo, K.R. (2010) Magnitude of oceanic nitrogen fixation influenced by the nutrient uptake ratio of phytoplankton. *Nat. Geosci.* 3, 412–416
- 19 Howarth, R.W. and Cole, J.J. (1985) Molybdenum availability, nitrogen limitation, and phytoplankton growth in natural waters. *Science* 229, 653–655
- 20 Guidry, M. and Mackenzie, F. (2000) Apatite weathering and the Phanerozoic phosphorus cycle. *Geology* 28, 631–634
- 21 Tyrrell, T. (1999) The relative influences of nitrogen and phosphorus on oceanic primary production. *Nature* 400, 525–531

- 22 Downing, J.A. *et al.* (1999) Meta-analysis of marine nutrientenrichment experiments: variation in the magnitude of nutrient limitation. *Ecology* 80, 1157-1167
- 23 Moore, C.M. et al. (2013) Processes and patterns of oceanic nutrient limitation. Nat. Geosci. 6, 701–710
- 24 Liebig, J. and Playfair, L.P. (1840) Organic Chemistry in its Applications to Agriculture and Physiology by Justus Liebig; Edited From the Manuscript of the Author by Lyon Playfair, Taylor & Walton
- 25 Ryabov, A.B. and Blasius, B. (2011) A graphical theory of competition on spatial resource gradients. *Ecol. Lett.* 14, 220–228
- 26 Carpenter, S.R. *et al.* (2009) Leading indicators of phytoplankton transitions caused by resource competition. *Theor. Ecol.* 2, 139– 148
- 27 Farrior, C.E. et al. (2013) Resource limitation in a competitive context determines complex plant responses to experimental resource additions. Ecology 94, 2505–2517
- 28 Agren, G.I. et al. (2012) Nutrient limitation on terrestrial plant growth-modeling the interaction between nitrogen and phosphorus. New Phytol. 194, 953-960
- 29 Elser, J.J. (2011) Geochemistry. A world awash with nitrogen. Science 334, 1504–1505
- 30 De Baar, H.J.W. (1994) von Liebig's law of the minimum and plankton ecology (1899–1991). Prog. Oceanogr. 33, 347–386
- 31 Jansson, M. et al. (2012) Carbon dioxide supersaturation promotes primary production in lakes. Ecol. Lett. 15, 527–532
- 32 Tortell, P.D. et al. (2008) CO2 sensitivity of Southern Ocean phytoplankton. Geophys. Res. Lett. 35, L04605
- 33 Hein, M. and Sand-Jensen, K. (1997) CO2 increases oceanic primary production. *Nature* 388, 526–527
- 34 Connell, S.D. et al. (2013) The other ocean acidification problem: CO2 as a resource among competitors for ecosystem dominance. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 368, 20120442
- 35 Kaplan, A. and Reinhold, L. (1999) CO2 concentrating mechanisms in photosynthetic microorganisms. Annu. Rev. Plant Biol. 50, 539– 570
- 36 Sterner, R.W. (2008) On the phosphorus limitation paradigm for lakes. Int. Rev. Hydrobiol. 93, 433-445
- 37 O'Brien, W.J. (1972) Limiting factors in phytoplankton algae: their meaning and measurement. Science 178, 616–617
- 38 Falkowski, P. et al. (1992) Physiological limitations on phytoplankton productivity in the ocean. Oceanography 5, 84–91
- 39 Beardall, J. et al. (2001) Approaches for determining phytoplankton nutrient limitation. Aquat. Sci. 63, 44–69
- 40 Botkin, D.B. (1977) Forests, lakes, and the anthropogenic production of carbon dioxide. *BioScience* 27, 325–331
- 41 Lehman, J. et al. (1975) The assumptions and rationales of a computer model of phytoplankton population dynamics. *Limnol. Ocean.* 20, 343–364
- 42 Harpole, W.S. et al. (2011) Nutrient co-limitation of primary producer communities. Ecol. Lett. 14, 852–862
- 43 Elser, J.J. et al. (1990) Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. Can. J. Fish. Aquat. Sci. 47, 1468–1477
- 44 Lewis, W.M. and Wurtsbaugh, W. (2008) Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. *Int. Rev. Hydrobiol.* 93, 446–465
- 45 Elser, J.J. et al. (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142
- 46 Davidson, E.A. and Howarth, R.W. (2007) Environmental science: nutrients in synergy. *Nature* 449, 1000–1001
- 47 Spijkerman, E. et al. (2011) Independent colimitation for carbon dioxide and inorganic phosphorus. PLoS ONE 6, e28219
- 48 Spijkerman, E. (2010) High photosynthetic rates under a colimitation for inorganic phosphorus and carbon dioxide. J. Phycol. 46, 658– 664
- 49 Danger, M. et al. (2008) Does Liebig's law of the minimum scale up from species to communities? Oikos 117, 1741–1751
- 50 Tortell, P.D. (2000) Evolutionary and ecological perspectives on carbon acquisition in phytoplankton. *Limnol. Oceanogr.* 45, 744–750
- 51 Barcelos e Ramos, J. et al. (2007) Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer Trichodesmium. Global Biogeochem. Cycles http://dx.doi.org/10.1029/2006GB002898

- 52 Fu, F.X. et al. (2007) Effects of increased temperature and CO2 on photosynthesis, growth, and elemental ratios in marine Synechococcus and Prochlorococcus (cyanobacteria). J. Phycol. 43, 485–496
- 53 Reinfelder, J.R. (2010) Carbon concentrating mechanisms in eukaryotic marine phytoplankton. Annu. Rev. Mar. Sci. 3, 291–315
- 54 Raven, J.A. et al. (2013) Interactions of photosynthesis with genome size and function. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 368, 20120264
- 55 Liu, J. et al. (2010) Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning. Aquat. Microb. Ecol. 61, 1–15
- 56 Yoshimura, T. et al. (2009) Impacts of elevated CO2 on phytoplankton community composition and organic carbon dynamics in nutrient-depleted Okhotsk Sea surface waters. Biogeosci. Discuss. 6, 4143–4163
- 57 Engel, A. et al. (2007) Effects of CO2 on particle size distribution and phytoplankton abundance during a mesocosm bloom experiment (PeECE II). Biogeosci. Discuss. 4, 4101–4133
- 58 Riebesell, U. *et al.* (2007) Enhanced biological carbon consumption in a high CO2 ocean. *Nature* 450, 545–548
- 59 De Kluijver, A. et al. (2010) Carbon fluxes in natural plankton communities under elevated CO2 levels: a stable isotope labeling study. Biogeosci. Discuss. 7, 3257–3295
- 60 Kluijver, de A. (2012) PhD thesis, University of Utrecht
- 61 Finkel, Z.V. et al. (2009) Phytoplankton in a changing world: cell size and elemental stoichiometry. J. Plankton Res. 32, 119–137
- 62 Paulino, A.I. *et al.* (2007) Effects of increased atmospheric CO2 on small and intermediate sized osmotrophs during a nutrient induced phytoplankton bloom. *Biogeosci. Discuss.* 4, 4173–4195
- 63 Shapiro, J. (1997) The role of carbon dioxide in the initiation and maintenance of blue-green dominance in lakes. *Freshw. Biol.* 37, 307-323
- 64 Caraco, N.F. and Miller, R. (1998) Effects of CO2 on competition between a cyanobacterium and eukaryotic phytoplankton. Can. J. Fish. Aquat. Sci. 55, 54–62
- 65 Low-Décarie, E. et al. (2011) The effect of elevated CO2 on growth and competition in experimental phytoplankton communities. Global Change Biol. 17, 2525–2535
- 66 Philibert, A. and Prairie, Y. (2002) Diatom-based transfer functions for western Quebec lakes (Abitibi and Haute Mauricie): the possible role of epilimnetic CO2 concentration in influencing diatom assemblages. J. Paleolimnol. 27, 465–480
- 67 Wolfe, A.P. and Siver, P.A. (2013) A hypothesis linking chrysophyte microfossils to lake carbon dynamics on ecological and evolutionary time scales. *Global Planet. Change* 111, 189–198
- 68 Orr, J.C. et al. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437, 681–686
- 69 Bell, G. and Collins, S. (2008) Adaptation, extinction and global change. Evol. Appl. 1, 3
- 70 Collins, S. et al. (2014) Evolutionary potential of marine phytoplankton under ocean acidification. Evol. Appl. 7, 140–155
- 71 Schaum, E. et al. (2012) Variation in plastic responses of a globally distributed picoplankton species to ocean acidification. Nat. Clim. Change 2, 1–5
- 72 Price, G.D. and Badger, M.R. (1989) Isolation and characterization of high CO2-requiring-mutants of the cyanobacterium Synechococcus pcc7942: two phenotypes that accumulate inorganic carbon but are apparently unable to generate CO2 within the carboxysome. *Plant Physiol.* 91, 514–525
- 73 Moroney, J.V. et al. (1989) Isolation and characterization of a mutant of Chlamydomonas reinhardtii deficient in the CO2 concentrating mechanism. Plant Physiol. 89, 897–903
- 74 Collins, S.S. and Bell, G. (2004) Phenotypic consequences of 1,000 generations of selection at elevated CO2 in a green alga. *Nature* 431, 566–569
- 75 Collins, S. et al. (2006) Changes in C uptake in populations of Chlamydomonas reinhardtii selected at high CO2. Plant Cell Environ. 29, 1812–1819
- 76 Collins, S. and Bell, G. (2006) Evolution of natural algal populations at elevated CO2. *Ecol. Lett.* 9, 129–135
- 77 Low-Décarie, E. et al. (2013) Long-term culture at elevated atmospheric CO2 fails to evoke specific adaptation in seven freshwater phytoplankton species. Proc. Biol. Sci. 280, 20122598

- 78 Tatters, A.O. et al. (2013) Short- and long-term conditioning of a temperate marine diatom community to acidification and warming. *Philos. Trans. R. Soc. Lond. B: Biol. Sci.* 368, 20120437
- 79 Chevin, L-M. et al. (2013) Phenotypic plasticity and evolutionary demographic responses to climate change: taking theory out to the field. Funct. Ecol. 27, 967–979
- 80 Collins, S. (2011) Competition limits adaptation and productivity in a photosynthetic alga at elevated CO2. Proc. R. Soc. B: Biol. Sci. 278, 247–255
- 81 Bowes, G. (1996) Photosynthetic responses to changing atmospheric carbon dioxide concentration. *Photosynth. Environ.* 5, 387–407
- 82 Kayanne, H. et al. (1995) Diurnal changes in the partial pressure of carbon dioxide in coral reef water. Science 269, 214–216
- 83 Wanninkhof, R. et al. (2007) Air–sea CO2 fluxes in the Caribbean Sea from 2002–2004. J. Mar. Syst. 66, 272–284
- 84 Badger, M.R. et al. (2006) The environmental plasticity and ecological genomics of the cyanobacterial CO2 concentrating mechanism. J. Exp. Bot. 57, 249–265
- 85 Spijkerman, E. (2008) What physiological acclimation supports increased growth at high CO2 conditions? *Physiol. Plant.* 133, 41-48
- 86 Lohbeck, K.T. et al. (2012) Adaptive evolution of a key phytoplankton species to ocean acidification. Nat. Geosci. 5, 1–6
- 87 Cole, J.J. and Prairie, Y.T. (2009) Dissolved CO2. In *Encyclopedia of Inland Waters* (Gene, E.L., ed.), pp. 30–34, Elsevier
- 88 Talling, A.J.F. (2010) pH, the CO2 system and freshwater science. BioOne 3, 133–146
- 89 Emerson, S. and Hedges, J. (2008) Carbonate chemistry. In *Chemical Oceanography* (Emerson, S. and Hedges, J., eds), pp. 101–133, Cambridge University Press
- 90 Dickson, A.G. (2010) Part 1: Seawater carbonate chemistry. The carbon dioxide system in seawater: equilibrium chemistry and measurements. In *Guide to Best Practices in Ocean Acidification Research and Data Reporting* (Riebesell, U. et al., eds), pp. 17–40, Luxembourg, Publications Office of the European Union
- 91 Reinfelder, J.R. (2011) Carbon concentrating mechanisms in eukaryotic marine phytoplankton. Ann. Rev. Mar. Sci. 3, 291–315
- 92 Sabine, C.L. and Tanhua, T. (2010) Estimation of anthropogenic CO2 inventories in the ocean. Ann. Rev. Mar. Sci. 2, 175–198
- 93 Duarte, C.M. and Prairie, Y.T. (2005) Prevalence of heterotrophy and atmospheric CO2 emissions from aquatic ecosystems. *Ecosystems* 8, 862–870
- 94 Cai, W-J. (2011) Estuarine and coastal ocean carbon paradox: CO2 sinks or sites of terrestrial carbon incineration. Ann. Rev. Mar. Sci. 3, 123–145
- 95 Sobek, S. et al. (2005) Temperature independence of carbon dioxide supersaturation in global lakes. Global Biogeochem. Cycles 19, 1-10
- 96 Knoll, L.B. et al. (2013) Temperate reservoirs are large carbon sinks and small CO 2 sources: results from high-resolution carbon budgets. *Global Biogeochem. Cycles* 27, 52–64
- 97 Maberly, S.C. et al. (2012) Catchment productivity controls CO2 emissions from lakes. Nat. Clim. Change 3, 391–394
- 98 Tranvik, L.J. et al. (2009) Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol. Oceanogr.* 54, 2298–2314
- 99 McGillis, W. et al. (2007) Air-water flux reconciliation between the atmospheric CO2 profile and mass balance techniques. In *Transport* at the Air-Sea Interface: Measurements, Models and Parametrizations (Garbe, C.S. et al., eds), pp. 1–12, Springer-Verlag
- 100 Gudasz, C. et al. (2010) Temperature-controlled organic carbon mineralization in lake sediments. Nature 466, 478–481
- 101 Beardall, J. et al. (2009) Living in a high CO2 world: impacts of global climate change on marine phytoplankton. Plant Ecol. Divers. 2, 191–205
- 102 Ainsworth, E.A. and Long, S.P. (2005) What have we learned from 15 years of free-air CO2 enrichment (FACE)?. A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2. New Phytol. 165, 351–372
- 103 Raven, J.a. et al. (2008) The evolution of inorganic carbon concentrating mechanisms in photosynthesis. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 363, 2641–2650
- 104 Leliaert, F. et al. (2012) Phylogeny and molecular evolution of the green algae. CRC Crit. Rev. Plant Sci. 31, 1–46

Review

- 105 Arrigo, K.R. (2005) Marine microorganisms and global nutrient cycles. *Nature* 437, 349–355
- 106 Saito, M.a. et al. (2008) Some thoughts on the concept of colimitation: three definitions and the importance of bioavailability. Limnol. Oceanogr. 53, 276–290
- 107 Price, N.M. and Morel, F.M.M. (1990) Cadmium and cobalt substitution for zinc in a marine diatom. *Nature* 344, 658–660
- 108 Park, H. et al. (2007) Diversity of the cadmium-containing carbonic anhydrase in marine diatoms and natural waters. Environ. Microbiol. 9, 403–413
- 109 Peers, G. et al. (2005) Copper requirements for iron acquisition and growth of coastal and oceanic diatoms. Limnol. Oceanogr. 50, 1149–1158
- 110 Morel, F.M.M. et al. (1994) Zinc and carbon co-limitation of marine phytoplankton. Nature 369, 740–742
- 111 Grover, J.P. (1997) Resource Competition, Springer
- 112 Tilman, D. (1982) Resource Competition and Community Structure, Princeton University Press
- 113 Miller, T.E. *et al.* (2005) A critical review of twenty years' use of the resource-ratio theory. *Am. Nat.* 165, 439–448
- 114 Miller, T. *et al.* (2007) Evaluating support for the resource-ratio hypothesis: a reply to Wilson *et al. Am. Nat.* 169, 707–708

- 115 Wilson, J. et al. (2007) Is there really insufficient support for Tilman's R* concept?. A comment on Miller et al.. Am. Nat. 169, 700–706
- 116 Cardinale, B.J. et al. (2009) Separating the influence of resource 'availability' from resource 'imbalance' on productivity-diversity relationships. Ecol. Lett. 12, 475–487
- 117 Tilman, D. (1985) The resource-ratio hypothesis of plant succession. Am. Nat. 125, 827–852
- 118 Vitousek, P. et al. (2002) Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57-58, 1-45
- 119 Zackrisson, O. *et al.* (2004) Nitrogen fixation increases with successional age in boreal forests. *Ecology* 85, 3327-3334
- 120 DeLuca, T.H. *et al.* (2008) Ecosystem feedbacks and nitrogen fixation in boreal forests. *Science* 320, 1181
- 121 Owens, B. (2013) Long-term research: slow science. Nature 495, 300–303
- 122 Davies, M.S. and Snaydon, R.W. (1973) Physiological differences among populations of Anthoxanthum odoratum L. collected from the park grass experiment, Rothamsted. I. Response to calcium. J. Appl. Ecol. 10, 33–45
- 123 Rasse, D.P. et al. (2005) Seventeen years of elevated CO2 exposure in a Chesapeake Bay wetland: sustained but contrasting responses of plant growth and CO2 uptake. Global Change Biol. 11, 369–377