

The effect of elevated CO₂ on growth and competition in experimental phytoplankton communities

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Abstract

We report an experiment designed to identify the effect of elevated CO₂ on species of phytoplankton in a simple laboratory system. Major taxa of phytoplankton differ in their ability to take up CO₂, which might lead to predictable changes in the growth rate of species and thereby shifts in the composition of phytoplankton communities in response to rising CO₂. Six species of phytoplankton belonging to three major taxa (cyanobacteria, diatoms and chlorophytes) were cultured in atmospheres whose CO₂ concentration was gradually increased from ambient levels to 1000 parts per million over about 100 generations and then maintained for a further 200 generations at elevated CO₂. The experimental design allowed us to trace a predictive sequence, from physiological features to the growth response of species to elevated CO₂ in pure culture, from the growth response in pure culture to competitive ability in pairwise mixtures and from pairwise competitive ability to shifts in the relative abundance of species in the full community of all six species. CO₂ altered the dynamics of growth in a fashion consistent with known differences among major taxa in their ability to take up and use CO₂. This pure-culture response was partly successful in predicting the outcome of competition in pairwise mixtures, especially the enhanced competitive ability of chlorophytes relative to cyanobacteria, although generally statistical support was weak. The competitive response in pairwise mixtures was a good predictor of changes in competitive ability in the full community. Hence, there is a potential for forging a logical chain of inferences for predicting how phytoplankton communities will respond to elevated CO₂. Clearly further extensive experiments will be required to validate this approach in the greater complexity found in diverse communities and environments of natural systems.

Keywords: competition coefficient, ecological response, global change, photosynthesis, taxonomic group

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Introduction

The concentration of CO₂ in the atmosphere is expected to rise from current levels of 380 parts per million (ppm) to between 700 and 1000 ppm within the next century (IPCC, 2007). This is likely to have a profound effect on photosynthetic organisms and, through them, on the entire biosphere (Attiwill, 1971). In this paper, we describe an experimental approach to predicting the effect of elevated CO₂ on the composition of phytoplankton communities.

Initially, research into the effect of rising atmospheric CO₂ focused primarily on the physiological response of land plants (Cure & Acock, 1986). It is now clear that elevated CO₂ tends to increase growth and photosynthesis, although its effect varies among functional groups (Ainsworth & Long, 2005). In contrast, the long-term effects of elevated CO₂ concentration on the composition of plant communities and on the evolution of specific species have seldom been studied. Elevated

CO₂ may alter the composition of plant communities when species have different physiological responses (Niklaus *et al.*, 2001; Kardol *et al.*, 2010). Furthermore, genotypes within a species may vary in their response to high CO₂ (Lau *et al.*, 2007), although experiments have yet to detect an evolutionary response to high CO₂ concentration in plants (Potvin & Tousignant, 1996; Rasse *et al.*, 2005). Our limited knowledge of how communities and species evolve under elevated CO₂ is largely attributable to the impracticability of conducting experiments with plants that last more than a few generations.

Less is known about how phytoplankton respond to elevated CO₂. Only recently was it realized that marine phytoplankton play a crucial role in the global carbon cycle (Falkowski, 1994), having a global primary production rivaling that of land plants (Field *et al.*, 1998). Phytoplankton production is generally not expected to respond to elevated atmospheric CO₂ because it is rarely limiting to growth. Freshwater lakes are usually in equilibrium with the atmosphere, or even super-saturated in CO₂, because of the predominance of heterotrophy, especially bacterial degradation of organ-

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ic matter in the water column (Cole & Caraco, 2001; Duarte & Prairie, 2005). Hence, an increased atmospheric supply of CO₂ may have little effect on the overall carbon budget of lakes. Marine ecosystems are net sinks of CO₂ (Sabine & Tanhua, 2010) with CO₂ concentrations much lower than their freshwater counterparts (Duarte & Prairie, 2005). However, most phytoplankton, both marine and freshwater, have active carbon concentration mechanisms (CCMs) which allow the uptake of bicarbonate and its conversion to CO₂ (Giordano *et al.*, 2005). This suggests that CO₂ is not limiting for phytoplankton growth at current atmospheric concentrations. Marine phytoplankton production is usually limited by the supply of either fixed inorganic nitrogen or iron (Falkowski, 1994). There is some experimental evidence, however, that atmospheric CO₂ can directly affect productivity (Riebesell *et al.*, 1993; Hein & Sand-Jensen, 1997; Kim *et al.*, 2006; Tortell *et al.*, 2008; Yoshimura *et al.*, 2009). The effect of a global increase in the atmospheric concentration of CO₂ on marine primary productivity and biomass yield has not yet been decisively determined.

The composition of a phytoplankton community will be at equilibrium when the net growth rates of all its constituents are zero. This equilibrium will be stable if the growth rate of any component species becomes negative when its frequency increases, as might be the case, for example, if it has high affinity for a limiting nutrient but is vulnerable to a predator. The competitive balance between species will not only respond to changes in limiting factors, but to changes of any sort that affects how these factors can be exploited and whose effect varies among species. Changes in temperature, isolation, viral density or a multitude of other factors are likely to affect species unequally and thereby cause shifts in their relative abundance. In practice, the composition of plankton communities is highly dynamic rather than being close to a steady state, but the same principle, that changes modulating the effects of limiting factors may alter the relative abundance of species, will continue to hold.

As in plants, major taxa of phytoplankton differ in their ability to take up CO₂ and in their growth response to elevated CO₂ (Birmingham & Colman, 1979; Rost *et al.*, 2003; Riebesell, 2004). Species from a given major taxon (such as cyanobacteria or diatoms) have common physiological characteristics relating to CO₂ uptake, including CCMs and the related CO₂ compensation point, and differ consistently from species in other taxa (Birmingham & Colman, 1979; Tortell, 2000). Moreover, the carbon:chlorophyll ratio differs among major taxa, being higher in dinoflagellates, for example, than in diatoms. These physiological differences may lead directly to a shift in the frequency of major taxa if an

increase in CO₂ concentration stimulates growth, with species from the taxon with the largest requirement for CO₂ showing the greatest increase in growth. Species whose growth rate is increased most by elevated CO₂ in pure culture may then increase in abundance relative to species with a lower CO₂ requirement. More generally, the effects of nutrient supply, viral infection, zooplankton predation and other factors on growth are likely to be modulated by carbon supply, such that changes in CO₂ concentration will alter the competitive relations among species and thereby lead to shifts in relative abundance. Hence, fundamental physiological differences between major taxa may lead to shifts in community composition in response to changes in CO₂ supply (Riebesell *et al.*, 2007; Tortell *et al.*, 2008).

We report a serial transfer experiment extending over hundreds of generations to investigate whether elevated atmospheric CO₂ causes a shift in the competitive relations between major taxa of phytoplankton and thereby alters community composition. The experiment evaluated how the relative growth of two species of freshwater algae from each of three major taxa (cyanobacteria, diatoms and chlorophytes) responded to CO₂ supplementation in pure culture, in pairwise mixtures and in the full community of all six species. We specifically tested our experimental results against the null hypotheses that species/taxa in pure culture show no growth differences between ambient and elevated CO₂ and that the competitive ability of species/taxa in mixed cultures remains unaltered by the CO₂ environment. We also tested whether CO₂-related differences in growth in pure cultures or in competitive ability in pairwise mixtures can predict differences in competitive performance in multispecies cultures.

Our approach has three main limitations. First, the growth medium was buffered to isolate the effect of CO₂ supplementation alone, and hence the experiment does not address the indirect consequences of elevated CO₂, such as acidification. Acidification would increase the concentration of dissolved inorganic carbon and the proportion found as aqueous CO₂, potentially amplifying the direct effect of rising atmospheric CO₂ on phytoplankton. Secondly, freshwater species were used because of the ease of maintaining axenic cultures over many transfers, and extending the results to marine systems will necessarily require further experimental validation. Finally, although the design permits the treatment effect to be partitioned between major taxa and species within major taxa, the replication is minimal and more extensive experiments will be necessary to establish the generality of the conclusions. Within these limitations, the experiment can be used to evaluate the predicted causal linkage between the physiological response of growth rate in pure culture, the

competitive response of two species in mixed culture and shifts in whole-community dynamics attributable to changes in CO₂ concentration.

Materials and methods

Experimental organisms

The experimental community comprised three major taxa of freshwater phytoplankton: cyanobacteria, diatoms and chlorophytes. Each taxon was represented by two species of different growth form or size, where possible, that were available in axenic culture (Canadian Phycological Culture Centre number in brackets): the cyanobacteria *Synechococcus leopoliensis* (102) and *Anabaena variabilis* (105), the diatoms *Navicula pelliculosa* (552) and *Nitzschia palea* (160) and the chlorophytes *Pseudokirchneriella subcapitata* (37) and *Scenedesmus acutus* (10).

Long-term CO₂ enrichment experiment

Axenic lines were maintained by serial transfer every 5 days. For each line, 0.5 mL of culture was inoculated into 50 mL of Bold's basal medium (Bold, 1949) in a 150 mL flask stoppered with hydrophobic cotton batting. The medium was supplemented with a source of silicate (0.58 g L⁻¹ of Na₂SiO₃) and vitamins [2 mL L⁻¹ of the vitamin mix from F/2 medium (Guillard, 1975)] to allow diatom growth. The medium was set to pH 7 and buffered against changes in pH using HEPES buffer (4.766 g L⁻¹, Wehr *et al.*, 1986). The cultures were continuously shaken at 250 rpm with a 3 mm rotation diameter to accelerate gas diffusion from the flask airspace to the culture medium. They were maintained in growth chambers under 100 μmol m⁻² s⁻¹ of continuous light at 25 °C.

Three replicate lines of each species were propagated under increasing atmospheric CO₂ concentration, rising over 20 transfers and about 100 generations from ambient levels to 1000 ppm. They were then propagated at this concentration for a further 200 generations. A second set of three replicate lines per species was propagated at ambient CO₂ levels (375–400 ppm) throughout the experiment (Fig. 1). In the CO₂ treatment chamber (with rising CO₂ concentration), a solenoid valve controlled by an infrared gas analyser injected CO₂ into the growth chamber to maintain CO₂ concentrations at the set level. The culture vessels were allowed to equilibrate with chamber air for 5 days before inoculation. CO₂ concentration in the culture vessel airspace and in the culture medium was thus assumed to be initially in equilibrium with growth chamber air. Treatment-chamber combinations were switched every third transfer to minimize any chamber effect.

Assay procedure

The lines were assayed five times during the period of CO₂ increase. For an assay, a small inoculum of phytoplankton culture was removed from each experimental flask (ambient and rising CO₂) and used to measure growth and competitive

ability of species and taxa at their current atmospheric CO₂ concentration in the experiment. Pure cultures of each species were inoculated with a sample containing 2.5 μg of chlorophyll *a*. Likewise, mixed cultures were inoculated with a total of 5 μg of chlorophyll *a* (2.5 μg from each species) for all pairwise combinations of species from different major taxa. In the pure cultures growth was measured as yield after 5-day growth. In the pairwise cultures, the competition coefficient was determined from measurements of the shift in composition over one complete growth cycle (method given below). About 200 generations after reaching peak CO₂ concentrations, a sixth assay of the complete community of six species was performed, again with an inoculum of 2.5 μg of chlorophyll *a* for each species, in addition to monitoring the pure cultures and all pairwise combinations of species (Fig. 1).

Cell density was measured over the complete growth cycle in the final assay. Measurements throughout the growth cycle indicated that all cultures were in log phase growth at or shortly before 5-day transfer and that growth measurements from 5-day yield was an adequate metric of growth.

Phytoplankton quantification

A FluoroProbe (bbe Moldaenke, Kiel-Kronshagen, Germany) was used to measure the chlorophyll *a* of each taxonomic phytoplankton group and the total chlorophyll *a* in pure cultures and pairwise mixtures of species from different major taxa. Each taxon has distinct accessory pigments that are excited by different wavelengths and transfer energy to chlorophyll *a*. The FluoroProbe emits excitation wavelengths and measures re-emission after each excitation to assign chlorophyll *a* concentration to major taxa. We used the chlorophyte fingerprint created by bbe Moldaenke and created fingerprints for diatoms and cyanobacteria. These fingerprints provided good discrimination between taxa, with an overlap of <1% in most cases. For all species-treatment combinations in pure culture there was a fixed relation between estimates of chlorophyll concentration using fluorescence and estimates using optical absorbance of chlorophyll extracted with ethanol, both for the inocula and for the cultures after 5 days of growth. Cell density over the complete growth cycle was estimated from optical density at 660 nm measured with a Synergy HT microplate reader (BioTek, Winooski, VT, USA).

Cell density was estimated by microscopy for cultures containing more than one species from the same major taxon. The sample was fixed using Lugol's solution (5% of sample volume) and photographed on a haemocytometer. Cells were then enumerated manually using the IMAGEJ software (Wayne Rasband, NIH, Bethesda, MD, USA).

Growth and competition

Growth (*r*) in doublings per day was measured from the ratio of 5-day yield in chlorophyll concentration (*Chl*₅) and the initial chlorophyll concentration at inoculation (*Chl*₀):

$$r = \frac{\log_2 \left(\frac{Chl_5}{Chl_0} \right)}{\Delta \text{time}} \quad (1)$$

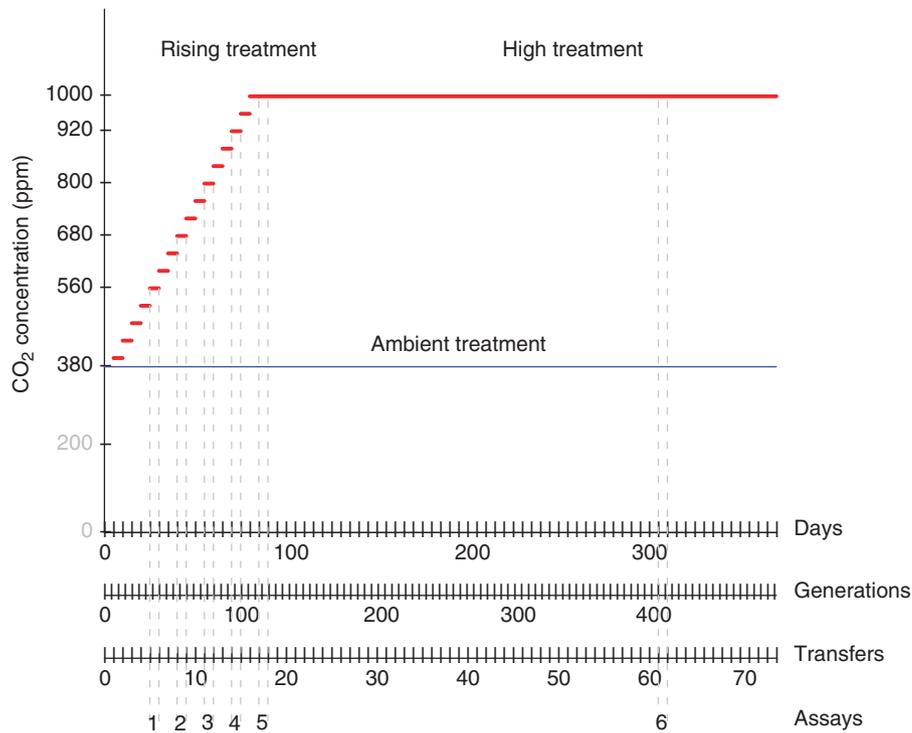


Fig. 1 Experiment timeline. Three replicate high CO₂ treatment lines of each species were propagated by serial transfer in atmospheres with increasing CO₂ concentration, rising by 40 ppm each transfer over 20 transfers and about 100 generations from ambient levels to 1000 ppm (rising treatment). The peak CO₂ concentration of 1000 ppm was then maintained for the remainder of the experiment (high treatment). A second set of three control replicate lines per species was maintained at ambient CO₂ levels (375–400 ppm) throughout the experiment (ambient treatment). During the increasing CO₂ phase of our experiment, five assays were performed, each including pure-culture growth assays and competitions between species of different taxa. In addition to the measurements done in the five previous assays, a sixth assay in which we investigated the full community dynamics and the response in competitions between species of the same taxa was performed after 200 generations of treatment at the highest CO₂ concentration (1000 ppm).

The competition coefficient used in this study is a measure of the difference in growth rate of two species estimated from the observed change in frequency per generation. The competition coefficient for species 1 (c_1) in competition with species 2 (c_2) is given by

$$c_1 = \frac{r_1 - r_2}{r_{\text{population}}} \ln(2) = \frac{1}{g} \ln \left(\frac{f_1^{\text{final}}}{f_1^{\text{initial}}} \frac{f_2^{\text{initial}}}{f_2^{\text{final}}} \right) = -c_2, \quad (2)$$

where r is the growth rate in doubling per day of either of the two species or the total population; g is the number of generations (doublings) of the total population; f is the relative frequency of species at inoculation and at harvest; and c_2 is the competition coefficient of species 2. The measure is based on the classical method for the calculation of a selection coefficient (Bell, 2008, p. 62) when measuring competition between two genotypes or species. In full communities, $r_{\text{population}}$ is the mean growth of all species and $f_2 = 1 - f_1$ is the frequency of all species bar the focal species.

The growth response to CO₂ was calculated as the difference between the mean yield of three lines grown at ambient CO₂ and the mean yield of three lines grown in elevated CO₂. The competition response was calculated as the difference between the mean competition coefficient for a focal species in three

cultures grown at ambient CO₂ and the mean competition coefficient for the same focal species in three cultures grown at elevated CO₂.

Statistical analysis

A summary of the statistical analysis is given in Table 1 and tables summarizing all the analyses are provided as supporting information.

(a) *Physiological response.* To identify the effect of CO₂ treatment on growth, growth rate was measured in pure culture in both ambient and elevated CO₂ lines. A two-way factorial ANOVA with treatment (CO₂ concentration) and assay as factors was used to estimate the main effect of treatment CO₂ (Table 1, #1; supporting information Analysis 1).

To identify differences in the response of major taxa to CO₂ treatment, a two-way factorial ANOVA was conducted on growth rates averaged by species for each assay with major taxon and CO₂ treatment as factors (Table 1, #2; supporting information Analysis 2).

This analysis was repeated without averaging and with 'species' in the place of 'major taxon' to identify any species-specific responses (Table 1, #3; supporting information Analysis 3).

Table 1 Summary of analysis: the effect of rising CO₂ on growth rate in pure culture, competition between species pairs and competition in the full community was analysed

Analysis	Model: response ~ factors	Data separation and (averaging)	Number of analysis data blocks	Data per analysis block	Critical <i>P</i> value using Bonferroni correction
Growth response					
1	Growth rate ~ CO ₂ treatment × assay	By species	6	36	0.0028
2	Growth rate ~ CO ₂ treatment × taxon	By assay (averaged by species)	6	12	0.0028
3	Growth rate ~ CO ₂ treatment × species	By assay	6	36	0.0028
Competition response in pair competition between species from different major taxa					
4	Competition coefficient ~ CO ₂ treatment × species from taxon 1 × species from taxon 2	By assay By taxonomic competition	18	24	0.0028
Competition response in pair competition between species from same major taxa					
5	Competition coefficient ~ CO ₂ treatment	By taxonomic group	3	6	0.0169
Competition response in full community					
6	Competition coefficient of six species ~ CO ₂ treatment		1	18	0.0500
Prediction of full community competition response from response in pair culture					
7	Response in full community ~ response in pair culture		1	6	0.0500

To offer the best test of the hypothesis of interest, data were separated and individual analyses were conducted on each stratum of the data. The most conservative corrected critical *P* value using a Bonferroni correction is presented for analyses of growth rate in pure culture, pairwise competition between species from different major taxa, pairwise competition between species of the same major taxon and competition in the full community. Tables providing details of each these analyses are provided in the supporting information.

(b) *Competition response.* A separate analysis was conducted for each pairwise mixture of species from different major taxa: cyanobacteria against chlorophytes, cyanobacteria against diatoms and chlorophytes against diatoms. These analyses were performed separately for each assay. To identify the effect of CO₂ treatment on competition a three-way factorial ANOVA was performed on the competition coefficient with CO₂ treatment, the focal species and the competitor species as fixed factors (Table 1, #4; supporting information Analysis 4). The data on which this analysis is based are shown in Fig. 2.

Pairwise competition between species from the same major taxon was evaluated only at the sixth assay, and analysed for each major taxon separately using ANOVA with the competition coefficient as the response variable and CO₂ treatment as the only factor (Table 1, #5; supporting information Analysis 5).

The combined six-species mixture was likewise evaluated only at the sixth assay, and analysed species by species in a similar manner. To test whether full community dynamics changed under elevated CO₂, a MANOVA was conducted on the effect of CO₂ treatment on the competition coefficient of each species in the mixture (Table 1, #6; supporting information Analysis 6).

To test whether the response of growth in pure culture or of competition in pairwise culture could predict the full community response, a linear model was fitted to relate the mean

species response in either pure culture or pairwise competition to the response of competitive ability in the full community (Table 1, #7; supporting information Analysis 7).

Test values were interpreted using critical *P* values corrected for the inflation of type I error from multiple testing using the very conservative Bonferroni correction (Cabin & Mitchell, 2000). Test values considered significant at the 5% level but not with the corrected critical *P* value were deemed marginally significant (Table 1, supporting information). All analyses were conducted using the R statistical package (R Development Core Team, Vienna, Austria).

Results

Physiological response

All six species showed an increase in growth in pure culture in the high CO₂ treatment across all assays, with the exception of *Anabaena* in the sixth assay. The difference in growth between ambient and high treatments was significant across CO₂ concentrations for all species ($F_{1,24} > 32.82$, $P < 0.0001$) and this growth response to CO₂ treatment varied with CO₂ concentration of the

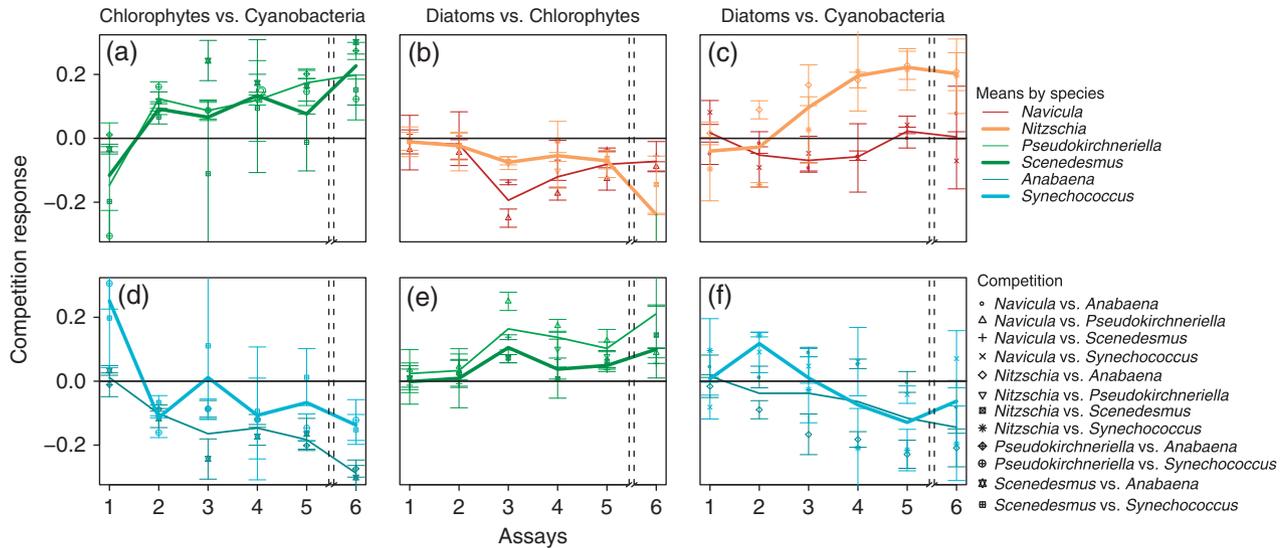


Fig. 2 Competition response for competitions between major taxa. Each focal species competed with two other species, one from each of the other taxa. The mean competition coefficient of the focal species over these two competitions is shown with full lines. Chlorophytes (a) gain from CO₂ increase at the expense of their cyanobacteria competitors (d). Diatoms (b) lose competitive ability relative to their chlorophyte competitors (e). The competitive ability of *Navicula* (c) is not affected by elevated CO₂, but *Nitzschia* increases in its ability to compete at the expense of both cyanobacteria (f).

elevated treatment in a given assay for all species ($F_{5,24} > 4.74$, $P < 0.0038$), except *Synechococcus*, which had a particularly large variance in growth rate ($F < 1$, supporting information Analysis 1).

The greatest increase of growth of cyanobacteria occurred at intermediate concentrations of CO₂ (<800 ppm) with the response leveling off at higher concentrations (Fig. 3a). The response of diatoms and chlorophytes, in contrast, continued to increase up to concentrations of 1000 ppm (Fig. 3b and c). These trends are not formally significant, however, as variance in growth response to CO₂ treatment between species could not be detected (major taxon × CO₂ interaction, $F_{2,6} < 2.41$, $P > 0.1706$, supporting information Analysis 2). In three assays there was likewise no significant overall variation in response among species ($F_{5,24} < 1.31$, $P > 0.2949$) and in the remaining three assays (2, 3 and 6) species differed only marginally in their growth response to CO₂ treatment ($F_{5,24} > 2.955$, $P < 0.032$, only the last assay being significant with the conservative Bonferroni correction for 18 tests, $F_{5,24} > 16.979$, $P < 0.0001$), with the decrease in growth of *Anabaena* under elevated CO₂ involving the only formally significant species × CO₂ treatment interaction in the last assay.

Competition response

Elevated CO₂ altered the amount, and sometimes the direction, of change in the frequency of species in mixed

cultures (Fig. 3 and supporting information Fig. S1). Specifically, chlorophytes benefited from rising CO₂ at the expense of cyanobacteria (Fig. 3d and f). The average competitive ability of diatoms did not change significantly under rising CO₂: instead, the two species diverged in their competitive response to elevated CO₂, with *Navicula* having a slightly depressed capacity to compete whereas *Nitzschia* had an enhanced capacity to compete under elevated CO₂ (Figs 2 and 3e).

At the lowest level of the rising CO₂ treatment (560 ppm), no significant response to CO₂ elevation was detected in the competitive ability of taxa or species, except *Synechococcus*, which benefited from the slight increase in CO₂ when in competition with chlorophytes (CO₂ treatment × competitor species interaction, $F_{1,16} = 13.977$, $P = 0.0018$, Fig. 2d, supporting information Analysis 4).

In assays 2–6, with CO₂ concentration in the treatment lines rising from 680 to 1000 ppm, chlorophytes benefited from the increase in CO₂ when in competition with cyanobacteria, with the exception of assay 3 in which a significant effect is detected only after the removal of the idiosyncratic response of the competition between *Scenedesmus* and *Synechococcus* (marginal significance of CO₂ treatment for assay 4 with $F_{1,16} = 5.540$, $P = 0.0317$, significant for assays 2, 5 and 6 with $F_{1,16} > 36.498$, $P < 0.0001$, Fig. 2a.). In assays 3–6, with elevated CO₂ from 800 to 1000 ppm, chlorophytes also benefited from the increase in CO₂ when in competition with diatoms ($F_{1,16} > 14.284$, $P < 0.0020$, Fig. 2e). On

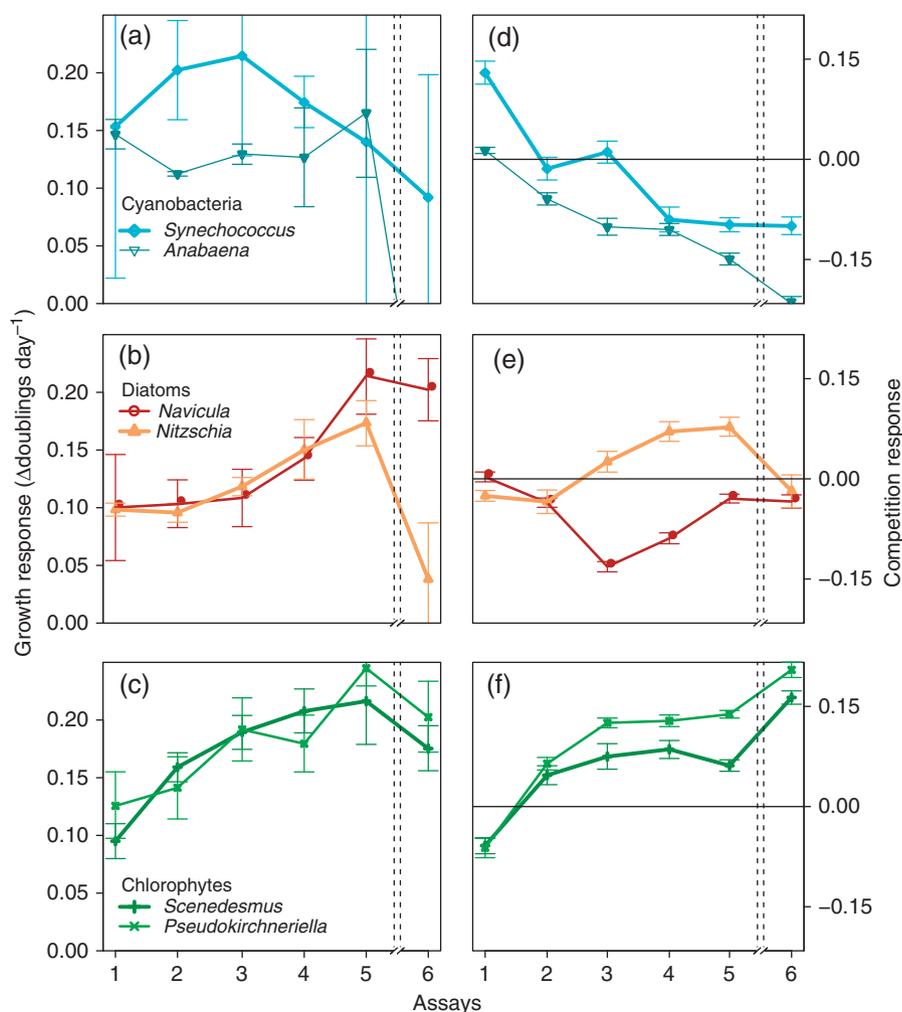


Fig. 3 Response to elevated CO₂. (a–c) Growth response. (a) Cyanobacteria reach their highest growth response at intermediate CO₂ concentrations (<800 ppm). (b, c) The response of both diatoms and chlorophytes increases with CO₂ concentration, with the exception of *Nitzschia* in assay 6. Species differed in their growth response at moderate CO₂ concentrations (assays 2 and 3) and after long-term exposure (assay 6). However, major taxa do not differ in their response to CO₂. Error bars are the 95% confidence intervals of the mean calculated with 1000 iterations of weighted bootstrapping. (d–f) Mean competition response for a species. Each species is competed separately with four other species. The competitive response of the species is the mean value of the competition coefficient across all four competitions. (d) Cyanobacteria have a marked decrease in their ability to compete with an increase in CO₂. (e) Diatom species diverged in their competitive response, resulting in no effect of CO₂ treatment on the average competitive ability of diatoms. (f) Chlorophytes show a marked increase in their ability to compete under elevated CO₂. Error bars are the 95% confidence intervals of the mean calculated with 1000 iterations of weighted bootstrapping.

average, competition between diatoms and cyanobacteria was not affected by CO₂ treatment, although elevated CO₂ enhanced the capacity of *Nitzschia* to compete with cyanobacteria for assays 3–6 (CO₂ treatment \times focal species interaction, $F_{1,16} > 6.299$, $P < 0.023$ for assays 3 and 6 and $F_{1,16} > 15.975$, $P < 0.001$ for assays 4 and 5, Fig. 2c).

In the sixth assay, we were not able to detect an effect of elevated CO₂ on competition between species of the same taxon for chlorophytes ($F_{1,4} = 0.84$, $P = 0.4118$) and diatoms ($F_{1,4} = 0.49$, $P = 0.5229$), while CO₂ treatment

may have slightly altered the competitive dynamics between cyanobacteria, with *Synechococcus* gaining a marginally significant advantage over *Anabaena* under elevated CO₂ ($F_{1,3} = 0.24$, $P = 0.0308$).

Community response

The changes in the capacity of species from different taxa to compete with each other under elevated CO₂ altered the competitive dynamics in the full community (MANOVA, CO₂ treatment, $F_{6,11} = 22.2$, $P < 0.001$). More-

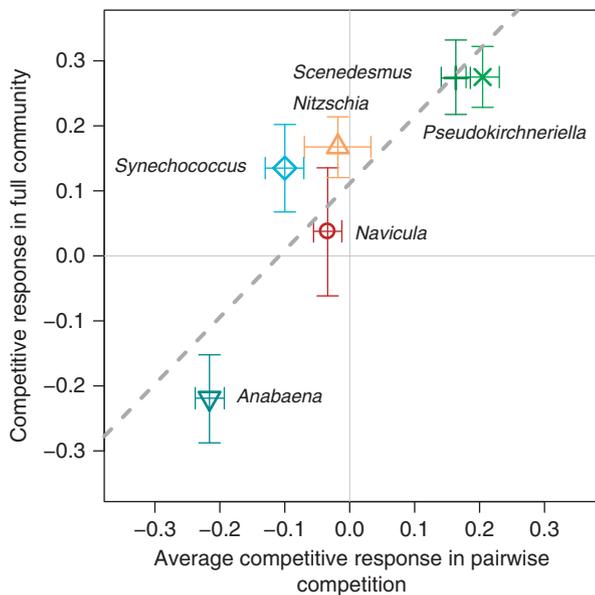


Fig. 4 Predicting the competitive response to elevated CO_2 in the full community from competitive response in pairwise competition. The shifts in competitive ability in pairwise mixtures predict the changes in the composition of the complete community of six species ($R^2 = 0.78$, $P = 0.02$). Furthermore, removing values from competition between *Nitzschia* and *Pseudokirchneriella* (the only competition for which the values of the sixth assay are inconsistent with the five previous assays) and the values from *Synechococcus* (which displays a more idiosyncratic response to CO_2 than any other species), improves the fit to an $R^2 = 0.998$, $P = 4.7 \times 10^{-5}$. Details of the linear regression are provided in the supporting information.

over, the shifts in competitive ability that we observed in pairwise mixtures predicted changes in competition within the complete community of six species (linear regression of observed on expected values, $R^2 = 0.78$, $P = 0.019$, Fig. 4). Both chlorophytes increased in frequency significantly (*Scenedesmus* $F_{1,16} = 22.5$, $P < 0.0001$, and *Pseudokirchneriella* $F_{1,16} = 24.6$, $P < 0.0001$). Diatoms showed a weaker response, with only *Nitzschia* increasing significantly in competitive ability ($F_{1,16} = 7.9$, $P = 0.012$). *Anabaena* responded with a sharp decline in its ability to compete ($F_{1,16} = 9.8$, $P = 0.006$), whereas the response of *Synechococcus* was more idiosyncratic and was not significant ($F_{1,16} = 3.67$, $P = 0.0733$).

Discussion

Response of average growth to CO_2

The physiological effect of elevated CO_2 is expected to be an increase in growth. All species and major taxa had increased growth under elevated CO_2 , suggesting that

in our system CO_2 is a limiting resource. Our findings are consistent with reports that elevated CO_2 can stimulate phytoplankton productivity in a wide range of conditions including nutrient-replete freshwater cultures (Schippers *et al.*, 2004), artificial freshwater blooms (Ibelings & Maberly, 1998), high-productivity marine cultures (Levitan *et al.*, 2007; Riebesell *et al.*, 2007) and nutrient-poor oceans (Hein & Sand-Jensen, 1997).

The linear decrease in the absolute value of the competition coefficient with increasing concentration of CO_2 in the elevated treatment is consistent with the system being limited by CO_2 supply.

Predicting the response of species to elevated CO_2

Prior knowledge of physiological mechanisms should allow the understanding of how CO_2 concentration alters the growth of species belonging to different major taxa. Cyanobacteria are more efficient than chlorophytes at concentrating carbon, and consequently have a lower photosynthetic CO_2 compensation point (Birmingham & Colman, 1979). This efficiency allows cyanobacteria to grow faster than chlorophytes at low CO_2 concentrations, but this advantage diminishes as CO_2 concentration increases. The response of cyanobacteria resembles most physiological responses to elevated CO_2 in plants, where growth response to increasing CO_2 rapidly saturates (Neales & Nicholls, 1978; Poorter & Navas, 2003).

This is consistent with our estimates of growth in pure culture, where chlorophytes had a lower growth response at modestly elevated CO_2 but a greater response at the highest levels of CO_2 . This trend has only weak support, however, as the major taxon $\times \text{CO}_2$ treatment interaction is not significant. This appears to result from the large variance among replicate lines of the same species, so that a larger experiment would be necessary to confirm the link between the physiology of carbon uptake and growth.

The growth response in pure culture should predict the response of pairwise competition between species from different major taxa. An increase in a resource is expected to increase the relative competitive ability of the taxa for which the resource is more limiting at the expense of species or taxa better adapted to lower levels of the resource (Tilman, 1982). This is also consistent with our experiments, although the statistical support is weak. The competitive response of cyanobacteria consistently falls as CO_2 concentration increases, while that of chlorophytes consistently increases, while diatoms are intermediate and show little change. The regression of competitive response (in pairwise mixture) on growth response (in pure culture) is positive in all assays, and has a $R^2 = 0.62$ ($P = 0.06$) in the final assay

(supporting information, Fig. S1). The chlorophytes had a large growth response and a correspondingly large competitive response in all assays, with both responses being low in *Anabaena*, while the diatoms were intermediate. The relationship was weakened by the idiosyncratic behaviour of *Synechococcus*, which varied widely between assays. The regression of species response in the full community on growth response in pure culture was also positive with $R^2 = 0.65$, $P = 0.05$.

The outcome of pairwise competition should likewise predict the response of the whole community. At elevated CO₂ chlorophytes were more successful, cyanophytes were less successful and diatoms were intermediate. The regression of competitive response in the whole community on competitive response in pairwise mixture is positive and significant ($R^2 = 0.78$, $P = 0.02$, Fig. 4). This predictive link is strong despite the fact that species quantification methodologies differed between pairwise competitions, for which we used the Fluoroprobe, and the full community, for which we counted cells under the microscope.

Hence, in our experiments, the causal sequence from physiology to pure-culture growth, from pure-culture growth to pairwise competition and from pairwise competition to whole-community response to elevated CO₂ can be traced, albeit with differing degrees of confidence. The evidence for some links is weaker than for others; in particular, we were unable to establish firmly that the outcome of pairwise competition is accurately predicted by the growth response in pure culture. Nevertheless, the experiment demonstrated the potential for forging a logical chain of inferences for predicting the response of phytoplankton to elevated CO₂ through long-term laboratory experiments.

Extending the laboratory results to the field

Although elevated CO₂ increases growth in cyanobacteria, our experiments show that it is likely to reduce the fitness of cyanobacteria relative to other taxa and hence to reduce their relative frequency in the community. These results are consistent with similar but less extensive studies (Richmond *et al.*, 1982; Shapiro, 1997). They suggest that most of the change in community dynamics will follow from differences between major taxa, although species within each taxon differed to a lesser degree in their competitive response to elevated CO₂. In particular, the competitive response of diatom species diverged at high CO₂. Comparable differences between species have been reported from land plants (Reich *et al.*, 2001). Hence, shifts in community composition under elevated CO₂ can be partitioned into two components, one of which (among major taxa) may be predictable from a general knowledge of physiological

mechanisms, whereas the other (among species within taxa) cannot be predicted from general principles so far as we understand. It remains to be shown, however, that the causal chain that can be traced in the laboratory will operate in the field, where the manifold chemical and biological side-effects of elevated CO₂ may also affect species abundance.

In the first place, many natural communities are not carbon limited, so competition for carbon may be weak or nonexistent. Even in situations where CO₂ may play a lesser role in competition, however, CO₂ enrichment is expected to affect community dynamics, as we have argued above. There is some experimental support for the conclusion that community dynamics are affected by a rise in CO₂ even when the dynamics in a natural plankton community are governed by factors other than CO₂ limitation, such as nutrient and light availability. In brief pulse experiments, CO₂ elevation caused a shift in the relative abundance of species within a major taxon (Tortell *et al.*, 2008) and a shift of major taxa within a community (Tortell *et al.*, 2002; Paulino *et al.*, 2008). Our long-term press experiment showed that the outcome of competition (win or lose) in pairwise mixtures rarely changed in response to elevated CO₂, but CO₂ did alter the dynamics of the competition in ways consistent with known differences among taxa in their ability to take up and use CO₂ (Tortell, 2000).

Secondly, elevated atmospheric CO₂ is expected to drive a decrease in pH, an increase in temperature and a host of indirect changes in natural aquatic systems (Orr *et al.*, 2005; IPCC, 2007). Furthermore, at any given CO₂ concentration many factors can influence the availability of CO₂ to phytoplankton, such as temperature and biological activity, and the ability of phytoplankton to take up the CO₂, such as nutrient availability and light limitation (Beardall *et al.*, 1998). The experiment we report was designed to manipulate CO₂ alone in order to isolate the effect of elevated CO₂ on species and communities. Much more extensive experiments to evaluate phytoplankton growth and competition under varying levels and combinations of global change factors (Rost *et al.*, 2008) and experiments long enough to allow for evolutionary adaptation and its interaction with ecological responses (Collins & Bell, 2004; Collins & Gardner, 2009) will be necessary to predict with more confidence the state of phytoplankton communities of the future. Moreover, the experiment explored only a small fraction of either the major taxa or (especially) the species diversity within major taxa that are present in natural phytoplankton assemblages. The importance of major taxa in predicting CO₂ response and of the predictive chain from physiological to competitive response to elevated CO₂ needs to be tested in a larger diversity of species and major taxa. We believe that the

experimental approach is the most powerful way of inferring the response of ecological communities to environmental change, but sound inference will require far more extensive experiments than have yet been attempted.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Detailed tables of all analysis of variance (ANOVA) conducted and summarized in Table 1 are provided as supplemental material.

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