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Short- and long-term conditioning of a temperate marine diatom community to acidification and warming

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Ocean acidification and greenhouse warming will interactively influence competitive success of key phytoplankton groups such as diatoms, but how long-term responses to global change will affect community structure is unknown. We incubated a mixed natural diatom community from coastal New Zealand waters in a short-term (two-week) incubation experiment using a factorial matrix of warming and/or elevated pCO_2 and measured effects on community structure. We then isolated the dominant diatoms in clonal cultures and conditioned them for 1 year under the same temperature and pCO_2 conditions from which they were isolated, in order to allow for extended selection or acclimation by these abiotic environmental change factors in the absence of interspecific interactions. These conditioned isolates were then recombined into 'artificial' communities modelled after the original natural assemblage and allowed to compete under conditions identical to those in the short-term natural community experiment. In general, the resulting structure of both the unconditioned natural community and conditioned 'artificial' community experiments was similar, despite differences such as the loss of two species in the latter. pCO_2 and temperature had both individual and interactive effects on community structure, but temperature was more influential, as warming significantly reduced species richness. In this case, our short-term manipulative experiment with a mixed natural assemblage spanning weeks served as a reasonable proxy to predict the effects of global change forcing on diatom community structure after the component species were conditioned in isolation over an extended timescale. Future studies will be required to assess whether or not this is also the case for other types of algal communities from other marine regimes.

1. Introduction

In the present-day ocean, anthropogenic CO_2 emissions to the atmosphere are driving environmental change processes that are probably unprecedented in their rapidity and scope. These impacts include increased sea surface temperatures due to 'greenhouse' warming, and a decrease in pH due to the direct effects of CO_2 uptake on seawater chemistry [1]. It is likely that a selective advantage will be provided for those species that are best able to cope with and respond to these multiple environmental changes [2]. At present, however, the long-term responses of most marine organisms to these global change variables over years or decades are virtually unknown [2].

Diatoms within the protistan division Bacillariophyta are one of the most important groups of microalgae in terms of both abundance and ecological functionality in the ocean. Marine diatoms carry out an estimated 40–45% of marine primary production, and thus play an integral role in the global carbon budget as well as influencing the cycling of other elements such as

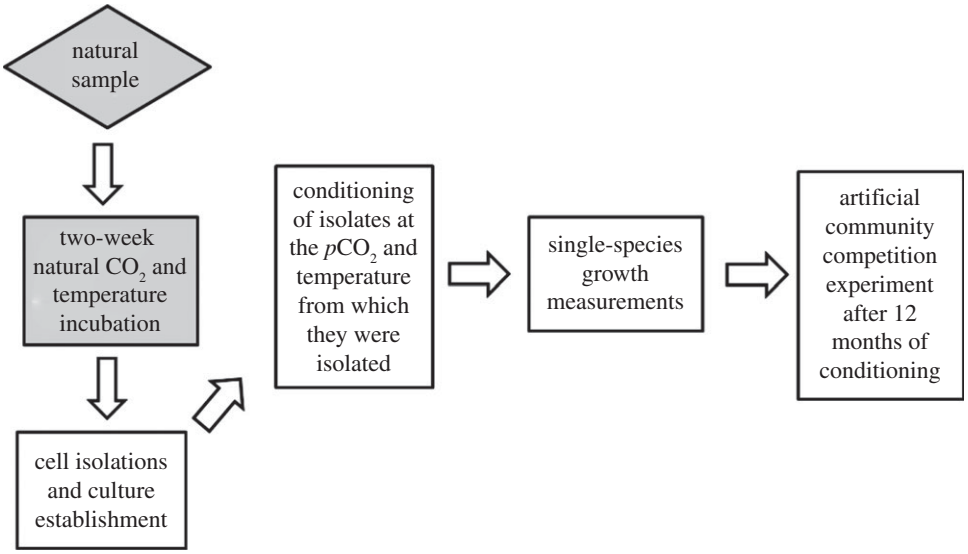

Figure 1. Flowchart depicting the entire experimental design, from the original natural community sample (shaded boxes) collection to the artificial community competition experiment after 12 months of conditioning.

silicon, nitrogen and phosphorus [3,4]. Of particular importance is the enormous amount of carbon and silicon they export to depth [4,5]. These ancient and biogeochemically critical organisms also generate a wealth of dissolved organic matter that helps to fuel the ocean's microbial food web [5]. Throughout their long evolutionary history of at least 100 million years [6], diatoms have successfully adapted to the dynamic influences of numerous natural shifts in climate and ocean conditions.

There have been a number of recent experiments that examined the effects of future pCO_2 and/or warming on natural marine diatom communities in short-term incubations, typically spanning only a few weeks [7–12]. Similarly, short-term ocean acidification studies were also conducted with single species of cultured diatoms [13–17], and a few others have used experimental designs in which isolated diatoms were subjected to altered pCO_2 conditions for longer periods (more than three months) [18–19]. Very few studies have examined longer-term responses of microalgal communities to climate change variables [20,21], and to the best of our knowledge, this has yet to be undertaken for marine diatoms. Experimental studies of freshwater green algae, coccolithophorids and dinoflagellates [20–26] have provided the evolutionary framework for these short-term investigations.

In an effort to examine effects of ocean warming and acidification on diatom assemblage structure, we implemented a novel experimental design that compared short-term natural community incubations with the results of competition in recombined 'artificial' communities after extended conditioning to altered pCO_2 and temperature combinations. The general experimental design was similar to a recent study [21] in which a two-week acidification manipulation experiment was carried out using a mixed natural dinoflagellate community. The members of that assemblage were then isolated into unialgal culture and conditioned at the experimental pCO_2 concentrations for more than 1 year, with 'artificial' community competition experiments being used to assess any changes in competitive success following long-term selection by CO_2/pH. This innovative experimental design is able to compare and contrast the ability of the same set of diatom species to compete under simulated future ocean conditions in a 'naive' mixed natural community, and after they are exposed to the same conditions for an extended period of time in the isolated clonal cultures without the context of interspecific interactions.

The principal objective of the study presented here was to use the ecologically and biogeochemically important diatoms to determine whether short-term incubations with natural communities subjected to simulated future conditions of future pCO_2 and warming yield similar outcomes with respect to changes in community structure, when compared with assemblages composed of independently conditioned populations. By individually conditioning diatom clones isolated directly from the short-term natural community experiment and then allowing them to compete in 'artificial' communities, we attempt to offer insights into the ability of conditioned cell lines to compete in a future greenhouse ocean. Our long-term goal is to determine how marine planktonic food webs will respond to climate change and ocean acidification, and to begin to distinguish short-term acclimation responses from the conditioned responses that might be expected following extended exposures to warmer temperatures and reduced pH.

2. Material and methods

(a) Experimental design

An overview of the experimental design is depicted in figure 1, and followed the general protocols for recently published dinoflagellate community experiments [21]. Sequentially, the study included a short-term two-week temperature/pCO_2 factorial matrix incubation experiment using a natural, mixed diatom assemblage, the isolation of clonal cultures from each treatment and conditioning of the clones to the pCO_2 and temperature combinations from which they were isolated for 1 year. Finally, the conditioned clones were recombined into artificial communities and allowed to compete, followed by a comparison of final community structure with that observed in the original two-week natural community experiment.

(b) Short-term natural community incubation experiment

A mixed diatom assemblage that consisted primarily of *Cylindrotheca fusiformis* Reimann and Lewin 1964, *Coscinodiscus*

spp., *Thalassiosira* spp., *Pseudo-nitzschia delicatissima* (Cleve) Heiden 1928, *Navicula* sp. and *Chaetoceros criophilus* (Castracene) *sensu* Hust 1886 was collected off the city of Dunedin on the South Island of New Zealand in January of 2011. The water was collected approximately 3 km offshore from Tairoa Head at the mouth of Otago Harbour halfway to Munida (45, 45.09° S 170, 48.6° E). The ambient sea surface temperature was 14.8°C.

Seawater was collected for both the initial incubations and the short-term experimental dilution water. All water was combined into an approximately 500 l container and subsampled after filtering through 80 µm mesh to remove large zooplankton. Volumes (800 ml) were added to triplicate polycarbonate bottles and spiked with an f/50 nutrient derivative (10 µm $NaNO_3^-$, 0.8 µm $NaH_2PO_4^{3-}$, 10 µm Na_2SiO_3 and f/50 vitamin and trace metal concentrations [27,28]) to promote diatom growth. The bottles were incubated on a 12 L : 12 D cycle under 140 µE of cool white fluorescent illumination in free-standing laboratory incubators at 14 or 19°C. The temperatures (ambient and +5°C) were selected based on predicted sea surface warming from the IPCC [29]. Triplicate sterilized 1 l polycarbonate bottles were gently bubbled at each temperature using commercially prepared air/CO_2 mixtures (Alphagaz, Air Liquide) at three concentrations also based on IPCC scenarios (approx. 210 µatm = pre-industrial pCO_2; approx. 370 µatm = current pCO_2; and approx. 560 µatm = future, year 2050 projected pCO_2) [29]. Cellular abundances in an unbubbled control treatment did not significantly deviate from results of the current pCO_2-bubbled treatment (data not shown). This methodology has been used for other CO_2 experiments [21,30,31], including previous diatom studies [10,16,20].

The six pCO_2/temperature treatments were maintained in active growth using semicontinuous culture methods [21]. Each bottle was diluted to the original time-zero *in vivo* chlorophyll *a* fluorescence value every 2 days with nutrient-amended 0.2 µm-filtered seawater. Aliquots were removed initially and after one and two weeks for examination of carbonate system parameters and community structure using microscopic cell counts.

(c) Establishment of clonal cultures

Two to four individual cells from the six dominant diatom species were isolated from each of the short-term incubation bottles at the termination of the experiment. Inverted light microscopy was used to make taxonomic determinations based on morphological characteristics to make sure the isolates for each cell line were from the same species [32]. These monospecific clones were propagated in 24-well plates prior to being transferred to tissue culture flasks for long-term maintenance under pCO_2 and temperature conditions identical to those from which they were isolated. A set of the culture isolates were transported under controlled temperature conditions to the University of Southern California in Los Angeles, CA, USA, where conditioning of the isolates and the 12-month community recombination experiments presented here were carried out. The culture isolates were maintained unreplicated for the first few weeks until they were verified to be established and growing well, at which time they were transferred into triplicate cultures for long-term maintenance; initial growth rates were obtained from these original unreplicated cell lines. These cultures were then maintained for a period of 1 year in exponential growth phase using the same recipe of autoclave-sterilized enriched seawater growth medium, and with other environmental variables such as light, pCO_2 bubbling, temperature etc., maintained as in the two-week natural community experiment. Semicontinuous weekly dilutions were performed based on specific growth rates within each bottle, calculated as in [21]. The approximate number of generations during this time period was: *C. fusiformis* (185–212), *Coscinodiscus* sp. (169–229), *Thalassiosira* sp. (179–200), *P. delicatissima* (178–221), *Navicula* sp. (188–212) and *C. criophilus* (194–236).

(d) Artificial community competition experiments

After the 12-month pCO_2/temperature conditioning period, the cultures were recombined into artificial communities in the same relative proportions and abundance as in the original natural assemblage collected from Otago Harbour. The incubations of these artificial communities were performed under experimental conditions, duration and dilution frequencies identical to those of the original short-term natural community experiment.

(e) Cell counts and growth rates

Samples for cell counts were obtained at the time of collection, before and after dilution and upon termination of the natural and artificial community incubations to determine abundances of each species. Cell-specific growth rates for each clonal culture were determined in individual culture flasks at the beginning of the 1 year conditioning period and in triplicate replicates after approximately 10 months of conditioning. These were calculated from samples taken 3 days apart using the growth rate equation $\mu = \ln(N_t/N_o)/t_1 - t_0$ (where N is the number of cells at time t_1 and t_0 (in days)) and represent a long-term steady-exponential state of growth. Algal cells were collected in 30 ml borosilicate glass scintillation vials, preserved with acidified Lugol's solution and enumerated using an Accu-Scope v. 3032 inverted microscope using the Utermöhl method [33].

(f) Carbonate system characterization

Samples for carbonate system parameter analysis were taken at the time of the natural sample collection and at the termination of the short- and long-term experiments. Spectrophotometric pH for the initial community incubations was measured after [34] as described in [35] using a UV–vis spectrophotometer (Ocean Optics USB4000). For samples from the 12-month community incubations, spectrophotometric pH was determined using a Shimadzu 1800UV spectrophotometer according to a similar method [36]. Temperature was monitored using standard laboratory incubator thermometers and salinity by conductivity with an interchangeable probe using an Orion 5-star plus pH meter. For pH measurements, temperature and salinity values for the initial experiment were 23.6°C and 35, respectively. For the conditioned experiment, the temperature was 25°C and salinity 35. Dissolved inorganic carbon was analysed using a CM5230 CO_2 coulometer (UIC) [37]. Experimental pCO_2 was calculated using CO_2SYS software [22] with dissociation constants from Dickson & Millero [38] using the combined data of [39,40] and KSO_4 from [41] (table 1).

(g) Statistics

Multivariate analyses were conducted using the PRIMER v6 statistics package [42] with the PERMANOVA add-on [43]. Bray–Curtis similarities were computed following square-root transformation of final cell abundances (cells m^{-1}) for all six diatom species from replicate bottles. PERMANOVA was used to test for significant differences among and within predefined groups in response to differing pCO_2 competition levels and differing temperature. Data from the original natural community experiment and from the artificial community competition trials 12 months later were analysed. Pseudo-*F* values of 1 are typical of a large overlap among sample groups that are being compared (confirmation of H0 hypothesis), whereas pseudo-*F* values greater than 1 indicate little or no overlap between the compared groups [43]. Observed interactions between pCO_2 and temperature were interrogated using PERMANOVA as well as pairwise comparisons (one-way ANOSIM [38]). *R*-values close to zero were indicative of no difference among groups, whereas *R*-values close to 1 meant that dissimilarities among groups were larger than any dissimilarity within groups [42].

Table 1. Measured and calculated seawater carbonate system values in the natural community experiment (initial) and the final conditioned artificial community experiment after 12 months (conditioned) at 14°C and 19°C. Measured values include pH (spectrophotometric) and total dissolved inorganic carbon (DIC, μmol kg^{-1}), and calculated values including pCO_2 (μatm), total alkalinity (TA, μmol kg^{-1}), bicarbonate ion (HCO_3^-, μmol kg^{-1}), carbonate ion (CO_3^{2-}, μmol kg^{-1}), and the saturation states for calcite (Ω_{cal}) and aragonite (Ω_{ara}) were obtained from these measured parameters. The temperature and salinity values for the initial experiment were 23.6°C and 35, respectively. For the conditioned experiment, the temperature was 25°C and salinity 35. Throughout the text the pCO_2 values of 196–229 are referred to as 'pre-industrial', values of 333–401 are referred to as 'current', and values of 519–573 are referred to as 'future'. Values shown are averages of triplicates, with standard deviations in parentheses.

community	pH	total DIC (μmol kg^{-1})	calculated pCO_2	TA (μmol kg^{-1})	$[HCO_3^-]$ (μmol kg^{-1})	$[CO_3^{2-}]$ (μmol kg^{-1})	Ω_{cal}	Ω_{ara}
initial-14°C								
pre-industrial	8.25 (0.02)	1956 (14)	229 (15)	2427 (12)	1617 (25)	333 (13)	8.0 (0.3)	5.3 (0.2)
current	8.02 (0.01)	2092 (3)	401 (4)	2401 (3)	1855 (3)	225 (1)	5.4 (0.1)	3.6 (0.1)
future	7.89 (0.02	2172 (9)	573 (15)	2407 (11)	1977 (9)	178 (4)	4.3 (0.1)	2.8 (0.1)
initial-19°C								
pre-industrial	8.26 (0.02)	1960 (15)	224 (16)	2432 (4)	1609 (28)	338 (13)	8.2 (0.3)	5.4 (0.2)
current	8.08 (0.02)	2080 (38)	383 (35)	2404 (5)	1802 (37)	248 (13)	5.9 (0.2)	4.0 (0.2)
future	7.95 (0.02)	2154 (12)	556 (23)	2420 (6)	1940 (16)	198 (5)	4.8 (0.1)	3.2 (0.1)
conditioned-14°C								
pre-industrial	8.31 (0.01)	1961 (19)	199 (9)	2441 (12)	1615 (23)	339 (6)	8.1 (0.1)	5.3 (0.1)
current	8.10 (0.02)	2028 (24)	355 (22)	2355 (17)	1785 (29)	233 (8)	5.6 (0.1)	3.7 (0.1)
future	7.97 (0.02)	2148 (24)	519 (30)	2403 (20)	1944 (28)	189 (7)	4.5 (0.1)	3.0 (0.1)
conditioned-19°C								
pre-industrial	8.31 (0.01)	1963 (4)	196 (3)	2448 (9)	1614 (4)	343 (4)	8.2 (0.1)	5.4 (0.1)
current	8.13 (0.02)	2020 (12)	333 (21)	2361 (10)	1767 (19)	244 (10)	5.8 (0.2)	3.8 (0.2)
future	7.95 (0.02)	2159 (10)	553 (35)	2400 (4)	1961 (17)	181 (8)	4.3 (0.1)	2.8 (0.1)

We used a two-way crossed design for the ANOSIM routine to examine the comparative effects of differing pCO_2 competition levels and differing temperature on algal assemblages. This approach tests the average effect of pCO_2 levels during competition removing differences in temperature and the average effect of temperature levels removing differences in competition pCO_2 [42]. Cell abundance information for these analyses was taken from the final time points of our initial natural community experiment and the artificial community competition trial after 12 months.

Differences between specific growth rates after 10 months of conditioning in addition to cell abundances from the original natural community and the final artificial community experiments under the four temperature and pCO_2 combinations were tested using one-way ANOVA using Microsoft Excel 2013.

3. Results

(a) Collected natural community composition

The natural assemblage at the time of collection contained a diverse diatom community, consisting of both centric and pennate forms. Relative abundances of the six dominant species are plotted in figure 2*a*, and their absolute abundances are depicted in figure 2*b*. The most abundant species was *C. fusiformis* (32.7%), followed by *P. delicatissima* (19.4%), *Coscinodiscus* sp. (16.2%), *Navicula* sp. (14.0%), *C. criophilus* (9.4%) and *Thalassiosira* sp. (8.3%). Various other diatom species composed less than 1% of the sample, and phytoplankton taxa other than diatoms were also rare and made an insignificant contribution to total cell abundance.

(b) Short-term natural community incubation experiments

(i) Overall trends in community structure

Each temperature and pCO_2 combination yielded a different diatom assemblage at the end of the initial two-week natural community incubation, indicating that community structure was influenced by both of these variables (figure 3). Within each of the temperature treatments, trends in community structure were relatively consistent in all three experimental bottles growing at each pCO_2 level. This is demonstrated by the clustering of Bray–Curtis similarities for the three replicates at each pCO_2 in non-parametric, multi-dimensional plots at both 14 (figure 4*a*) and 19°C (figure 4*b*). Likewise, the community was also strongly structured by temperature as depicted by Bray–Curtis similarity plots at each pCO_2: pre-industrial (figure 5*a*), current (figure 5*b*) and future (figure 5*c*).

(ii) Effects of pCO_2 at low temperature

Following two weeks of incubation at 14°C the final relative abundance of the dominant organism from the natural sample collection, *C. fusiformis*, was closest to its relative abundance in the original community at current pCO_2 (figure 3*a*). Across the three pCO_2 treatments at this temperature, its final relative abundance was highest in the pre-industrial pCO_2 treatment (79.4%) and declined at current (49.4%) as well as at future pCO_2 (66.7%), but none of these differences were significant owing to large standard deviations. The final relative

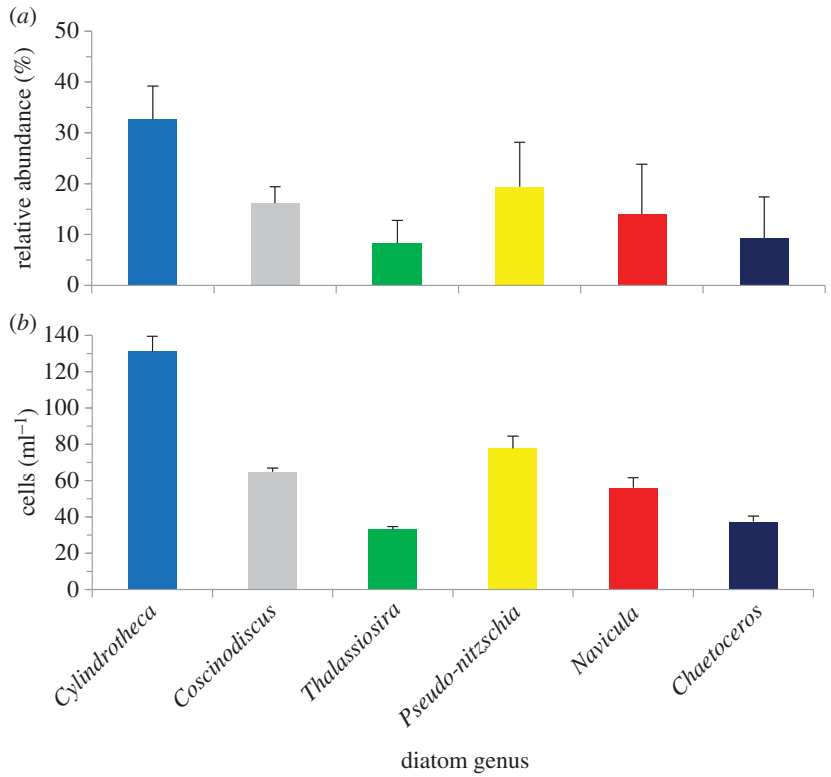

Figure 2. (*a*) Initial relative abundance and (*b*) absolute abundance of *Cylindrotheca fusiformis*, *Coscinodiscus* sp., *Thalassiosira* sp., *Pseudo-nitzschia delicatissima*, *Navicula* sp. and *Chaetoceros criophilus* in the collected natural sample.

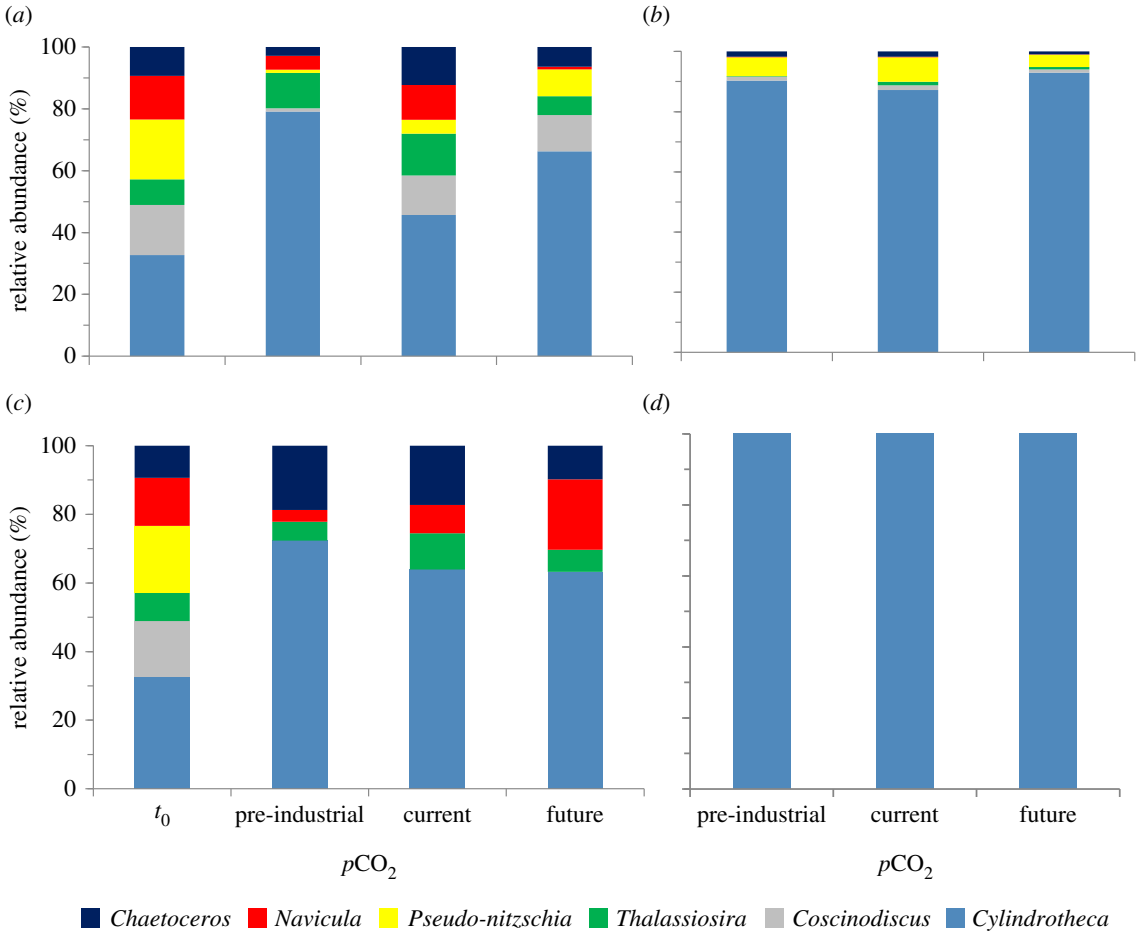

Figure 3. Relative abundance graphs of diatom community structure at pre-industrial, current and future pCO_2 at the end of the natural community short-term experiment at (*a*) 14°C and (*b*) 19°C as well as in the final 12-month conditioned artificial community experiment at (*c*) 14°C and (*d*) 19°C.

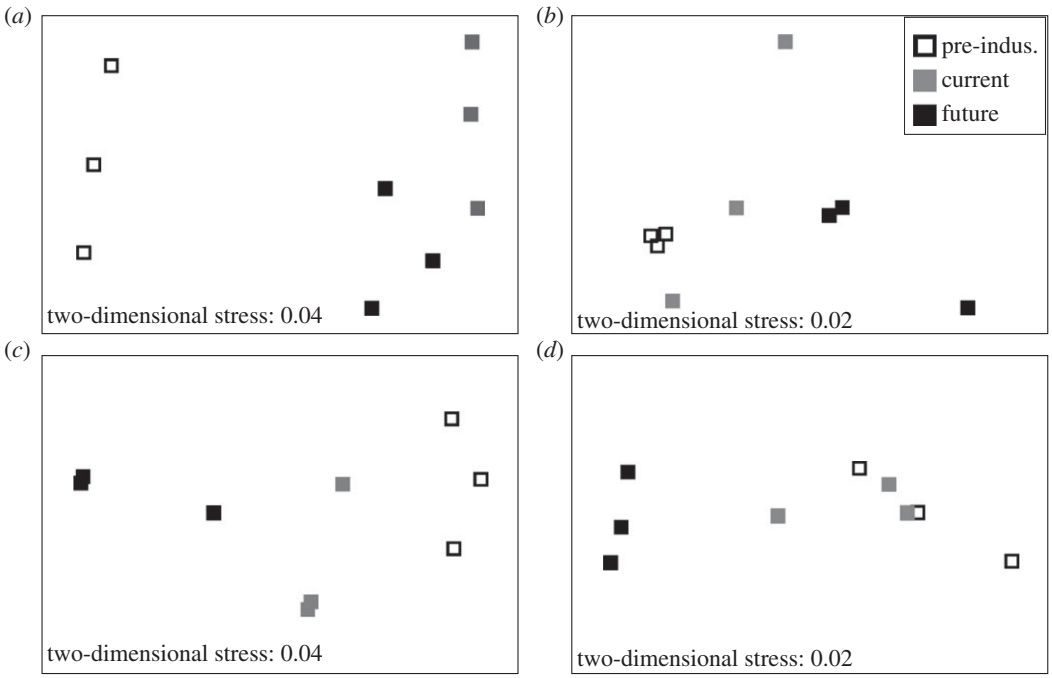

Figure 4. Bray–Curtis non-parametric MDS plots showing similarities between community structure in triplicate bottles for each pCO_2 treatment in the original natural community short-term experiment at (*a*) 14°C and (*b*) 19°C and in the final conditioned artificial community experiment at (*c*) 14°C and (*d*) 19°C.

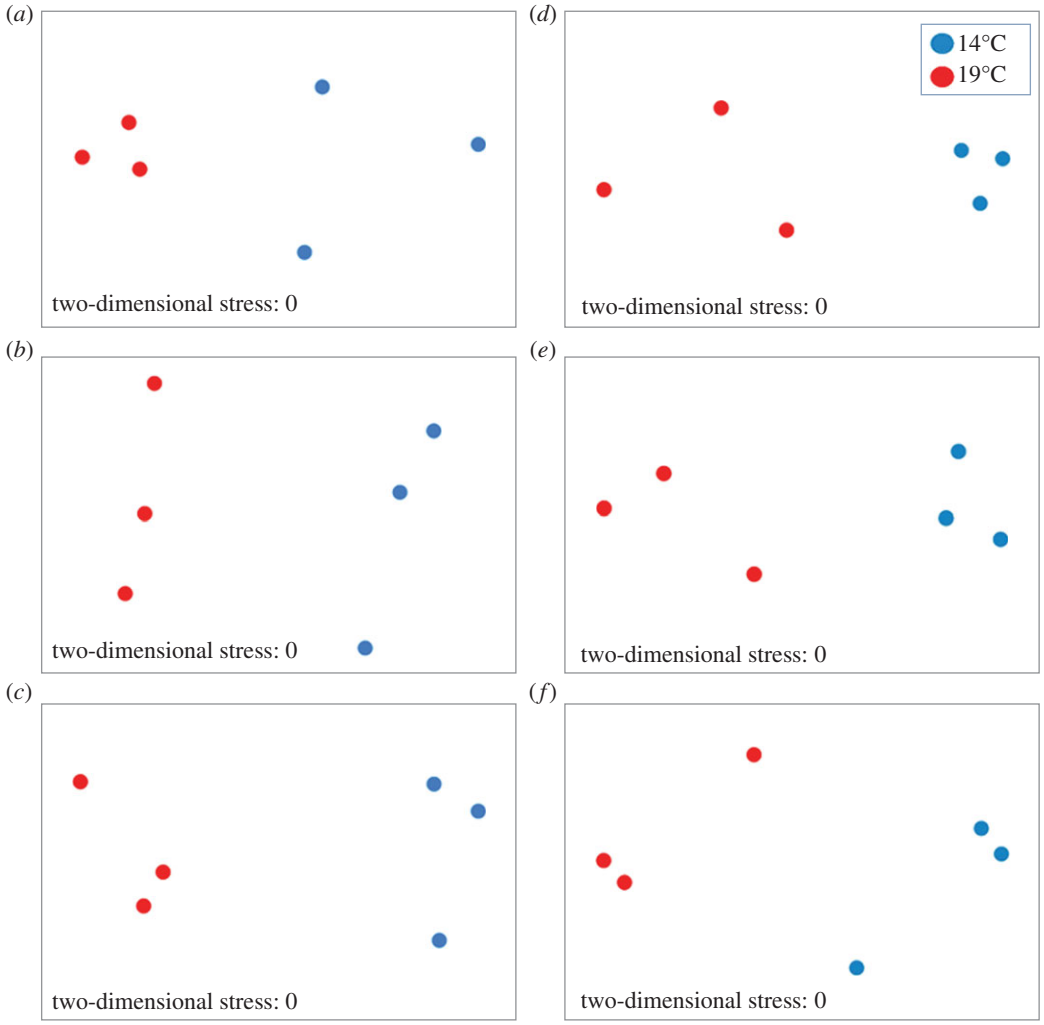

Figure 5. Bray–Curtis non-parametric MDS plots showing similarities between community structure in triplicate bottles for both temperature treatments in the original natural community short-term experiment at (*a*) pre-industrial pCO_2, (*b*) current pCO_2, (*c*) future pCO_2 and in the final conditioned artificial community experiment at (*d*) pre-industrial pCO_2, (*e*) current pCO_2 and (*f*) future pCO_2. (Online version in colour.)

abundance of *Coscinodiscus* sp. in the pre-industrial pCO_2 communities was only 1%, but this increased significantly in the current treatment to 13.4% and in the future CO_2 treatment to 11.8% ($F_1 = 31.74$, $p = 0.005$; $F_1 = 891.49$, $p = 0.00049$). After the two-week incubation, the relative abundance of *Thalassiosira* sp. was 11.5% in the pre-industrial pCO_2 treatment, 12.7% at current pCO_2 and 6.2% at future pCO_2, yet none of the differences was significant. For *P. delicatissima*, there was a trend of increasing relative abundance (1%, 4.3% and 8%) as pCO_2 increased. The difference from pre-industrial to future was significant ($F_1 = 8.45$, $p = 0.044$). At 14°C, *Navicula* sp. represented 4.4%, 7.6% and 1% of the community from pre-industrial to future pCO_2 ($F_1 = 11.17$, $p = 0.028$) and the difference between current and future pCO_2 was significant ($F_1 = 14.96$, $p = 0.018$). *C. criophilus* accounted for only 2.9% of the community at pre-industrial pCO_2, which increased to 12.3% at current pCO_2 and 6.4% in the future pCO_2 treatments (figure 3*a*). For this species, the pairwise comparisons of pre-industrial with current ($F_1 = 197.56$, $p = 0.000149$) and current with future ($F_1 = 16.07$, $p = 0.016$) were significantly different.

(iii) Effects of pCO_2 at high temperature

At 19°C, the final relative abundance of *C. fusiformis* was 90% in the pre-industrial pCO_2 treatment, 87% at current pCO_2 and 93% at future pCO_2 (figure 3*b*). Final relative abundances of *Coscinodiscus* sp. were less than 2% at all pCO_2 levels, with no clear treatment-related trends (pre-industrial to future pCO_2 respectively, 1.3%, 1.4%, 1.8%). Similar trends were obtained for *Thalassiosira* sp. for all pCO_2 treatments (0.1%, 1.2%, 0.8%). At the end of the two-week natural community experiment, *P. delicatissima* composed 6% of the community at pre-industrial pCO_2, 8% at current pCO_2 and 4% at future pCO_2, with the difference between current and future pCO_2 being significantly different ($F_1 = 13.41$, $p = 0.022$). At the warmer temperature, the presence of *Navicula* sp. in the community was always less than 0.2%. At 19°C, *C. criophilus* also competed poorly, with final relative abundances of 1.7%, 1.7% and 1% across the three pCO_2 levels (figure 3*b*).

(iv) Low versus high temperature

The relative abundance within each treatment changed significantly over the course of the two-week experiments (figure 3; absolute abundance data are presented in the electronic supplementary material, table S1). The originally dominant species from the natural sample collection, *C. fusiformis*, increased in all temperature and pCO_2 treatments (figure 3*a*,*b*) relative to the original community (figure 2). The relative dominance of this species at the end of the two-week natural community experiment was also higher in all pCO_2 treatments at 19°C (figure 3*b*) relative to 14°C (figure 3*a*), but owing to standard deviation, there were no significant differences between treatments. The final relative abundance of *Coscinodiscus* sp. was lower in all treatments (figure 3*a*,*b*) than in the original community and was 89% and 91% higher at 14°C than at 19°C in the current and future pCO_2 treatments ($F_1 = 30.18$, $p = 0.005$; $F_1 = 736.15$, $p = 0.00011$). At pre-industrial pCO_2, this organism competed identically, regardless of temperature. *P. delicatissima* declined substantially in all treatments (figure 3*a*,*b*) relative to the original community. At the end of the two-week natural community experiment, while not numerically dominant, *P. delicatissima* was able to maintain a substantial presence in all treatments except the lowest pCO_2 at 14°C. In this pre-industrial pCO_2 treatment, *P. delicatissima* was 86% more abundant at 19°C, compared with 14°C ($F_1 = 169.59$, $p = 0.000201$), and 47% more abundant at the higher temperature at current pCO_2, but this difference was not significant. At future pCO_2, however, this trend reversed as their final relative abundance was 49% higher at 14°C than at 19°C, but again the difference was not significant. In general, the relative abundance of *Thalassiosira* sp., *Navicula* sp. and *C. criophilus* was lower in most treatments after the two-week natural community experiment than in the original community, and this was especially pronounced at the higher temperature (figure 3*a*,*b*). *Thalassiosira* sp. always competed better at lower temperature, regardless of pCO_2 level, with final cell concentrations that were 98, 90 and 87% higher across the pCO_2 range ($F_1 = 7.97$, $p = 0.048$). At the lower temperature, *Navicula* sp. was 94, 97 and 82% more abundant with successively increasing pCO_2 concentrations ($F_1 = 8.23$, $p = 0.045$; $F_1 = 16.55$, $p = 0.015$; $F_1 = 86.20$, $p = 0.007$). *C. criophilus* also competed better at the lower temperature with final relative abundance differences of 86% at current pCO_2 being significant ($F_1 = 304.62$, $p = 0.00063$).

(c) Steady-state-specific growth rates initially and during the 12-month conditioning period

(i) Initial rates immediately following the short-term experiment

Specific growth rates of the clonal cultures immediately after being isolated from the short-term natural community incubation experiment ranged from 0.32 to 0.47 d^{-1}, and were similar between all six species ('initial 14 and 19°C' columns; table 2). Because these initial growth rates were obtained from unreplicated isolation bottles, shortly before they were divided into triplicate long-term cultures, statistical comparison between treatments is not possible. However, in most cases, growth rates were higher at 19°C than at 14°C, whereas there were no clearly defined common trends with pCO_2 treatment (table 2). These initial growth rates were also similar to those observed after 10 months of conditioning (see below), although again no significance tests were possible with the data from these unreplicated initial isolates.

(ii) Effects of pCO_2 at low temperature after 10 months of conditioning

After 10 months of conditioning, the mean specific growth rate of semicontinuously cultured *C. fusiformis* at 14°C was 11% higher ($F_1 = 14.84$, $p = 0.018$; $F_1 = 15.75$, $p = 0.017$) at pre-industrial pCO_2 compared with current and future pCO_2 ('conditioned 14°C' column, table 2). The increases in the specific growth rate of *Coscinodiscus* sp. from pre-industrial pCO_2 to current pCO_2 (19%) and future pCO_2 (13%) were both significant ($F_1 = 46.12$, $p = 0.002$; $F_1 = 37.83$, $p = 0.035$, respectively). Differences in the growth rates of *Thalassiosira* sp. across the pCO_2 range at 14°C were not significant. *P. delicatissima* exhibited significantly faster growth rates at pre-industrial pCO_2 than at current and future pCO_2 (18%, $F_1 = 112.36$, $p = 0.005$). The mean-specific growth rates for *Navicula* sp. grown at 14°C were identical across all pCO_2 levels. For *C. criophilus*, growth rate slightly decreased as pCO_2 increased, but these values were not significantly different (table 2).

Table 2. Cell-specific growth rates (day^{-1}) at 14°C and 19°C obtained initially for freshly isolated clones directly after the two-week natural community experiment (initial columns) and for the same cell lines after 10 months of conditioning (conditioned columns). Shown are growth rates for *Cylindrotheca fusiformis*, *Coscinodiscus* sp., *Thalassiosira* sp., *Pseudo-nitzschia delicatissima*, *Navicula* sp. and *Chaetoceros criophilus* at pre-industrial, current and future pCO_2. Initial values reported represent results from a single unreplicated bottle, whereas conditioned rates are averages of triplicates, with standard deviations in parentheses.

diatom genus	pCO_2	initial 14°C specific growth rate (day^{-1})	initial 19°C specific growth rate (day^{-1})	conditioned 14°C specific growth rate (day^{-1})	conditioned 19°C specific growth rate (day^{-1})
Cylindrotheca	pre-indus.	0.38	0.42	0.39 (0.01)	0.40 (0.01)
	current	0.35	0.37	0.35 (0.01)	0.33 (0.04)
	future	0.32	0.35	0.35 (0.01)	0.33 (0.01)
Coscinodiscus	pre-indus.	0.37	0.38	0.32 (0.02)	0.43 (0.02)
	current	0.36	0.40	0.43 (0.02)	0.41 (0.02)
	future	0.40	0.42	0.36 (0.01)	0.43 (0.02)
Thalassiosira	pre-indus.	0.36	0.39	0.37 (0.02)	0.36 (0.05)
	current	0.37	0.40	0.35 (0.02)	0.38 (0.02)
	future	0.33	0.42	0.34 (0.01)	0.36 (0.02)
Pseudo-nitzschia	pre-indus.	0.36	0.45	0.40 (0.01)	0.41 (0.02)
	current	0.33	0.40	0.34 (0.004)	0.43 (0.01)
	future	0.32	0.43	0.34 (0.01)	0.38 (0.01)
Navicula	pre-indus.	0.41	0.34	0.36 (0.02)	0.36 (0.01)
	current	0.39	0.39	0.36 (0.01)	0.37 (0.01)
	future	0.36	0.42	0.36 (0.02)	0.40 (0.01)
Chaetoceros	pre-indus.	0.37	0.43	0.40 (0.02)	0.42 (0.01)
	current	0.37	0.45	0.39 (0.02)	0.45 (0.02)
	future	0.43	0.46	0.37 (0.01)	0.43 (0.01)

(iii) Effects of pCO_2 at high temperature after 10 months of conditioning

As was observed at the lower temperature, following 10 months of conditioning at 19°C, the mean specific growth rate of *C. fusiformis* at pre-industrial pCO_2 was significantly higher at current and future pCO_2 (21%; $F_1 = 61.00$, $p = 0.0015$; 'conditioned 19°C' column, table 2). The specific growth rates of *Coscinodiscus*, *Thalassiosira* sp., *P. delicatissima* and *C. criophilus* were not significantly different between the three pCO_2 treatments at the warmer temperature. Growth rates of *Navicula* sp. were significantly higher at future pCO_2 than at pre-industrial pCO_2 (11%, $F_1 = 17.81$, $p = 0.013$; table 2), but neither were significantly different from the current pCO_2 growth rates.

(iv) Low temperature versus high temperature after 10 months of conditioning

Pairwise temperature comparisons of steady-state growth rates of the isolates at identical pCO_2 conditions after 10 months of long-term conditioning revealed a number of significant differences, which demonstrated increases at the higher temperature ('conditioned 14 and 19°C' columns, table 2). For *C. fusiformis*, there were no significant differences between treatments. By contrast, for *Coscinodiscus* sp. growth rates increased at the higher temperature by 25.3% at pre-industrial pCO_2 ($F_1 = 47.55$, $p = 0.002$) and by 15.3% at future pCO_2 ($F_1 = 53.95$, $p = 0.002$). Similar to *C. fusiformis*, the specific growth rates for *Thalassiosira* sp. were also not significantly different across treatments. At current pCO_2, *P. delicatissima* grew 26.5% faster at the high temperature than at the low temperature ($F_1 = 335.46$, $p = 0.00005$) and 11.7% faster in the warmer temperature at future pCO_2 ($F_1 = 25.05$, $p = 0.007$). *Navicula* sp. grew 10% faster at future pCO_2 ($F_1 = 18.41$, $p = 0.01281$), but the effects of temperature on growth of this species in the other pCO_2 treatments were not significant. For *C. criophilus*, the increase in specific growth rates from low to high temperature was significant at current and future pCO_2 (11.5%, $F_1 = 18.49$, $p = 0.0126$) and (12.8%; $F_1 = 45.24$, $p = 0.003$; table 2).

(d) Artificial community experiments

(i) Overall community structure trends

As in the two-week natural community experiment, final overall community structure in the 12-month artificial community was strongly affected by both temperature and pCO_2. This was apparent from the Bray–Curtis non-parametric, multi-dimensional plots showing clustering of the triplicate bottles in each of the three pCO_2 treatments at 14°C (figure 4*c*) and 19°C (figure 4*d*) as well as in both temperature treatments at pre-industrial pCO_2 (figure 5*d*), current pCO_2 (figure 5*e*) and future pCO_2 (figure 5*f*).

(ii) Effects of pCO_2 at low temperature on individual species

After the 12-month artificial community experiment at 14°C, *C. fusiformis* had the highest final relative abundance at pre-industrial pCO_2 (72.3%) but occurred at a similar relative abundance in the current (63.9%) and future pCO_2 (63.2%) treatments (figure 3*c*). The differences from pre-industrial to both current and future were significant ($F_1 = 9.21$, $p = 0.038$;

Table 3. Statistical results from PERMANOVA (type 1, sequential, 9999 permutations) for algal community structure comparisons in response to competition pCO_2 level (pre-industrial, current and future) and temperature (14 and 19°C) and their interactive effect during (*a*) our initial natural community experiment and (*b*) in artificial communities after a 12-month conditioning period. d.f., degrees of freedom; SS, sums of squares; MS, mean of squares; *p* (perm), permutation significance level.

	d.f.	SS	MS	pseudo-*F**	*p* (perm)	unique permutations
(*a*)						
pCO_2	2	755	378	5	0.003	9948
temperature	1	2886	2886	37	0.0001	9952
pCO_2 × temperature	2	1544	772	10	0.0001	9958
res	12	931	78			
total	17	6117				
(*b*)						
pCO_2	2	647	324	15	0.0002	9952
temperature	1	11 799	11 799	562	0.0002	9807
pCO_2 × temperature	2	712	356	17	0.0002	9949
res	12	252	21			
total	17	13 410				

$F_1 = 9.44$, $p = 0.037$). At 14°C, *Thalassiosira* sp. composed 5.5% of the community at pre-industrial pCO_2, 10.5% at current pCO_2 and 6.5% at future pCO_2 (figure 3*c*), with no significant differences. *Navicula* sp. responded significantly to higher pCO_2, being least abundant at pre-industrial pCO_2 (3.4%), and increasing at current (8.3%) and future pCO_2 (20.5%). The increases from pre-industrial to current, pre-industrial to future and future to current pCO_2 were all significant ($F_1 = 11.55$, $p = 0.027$; $F_1 = 23.22$, $p = 0.008$; $F_1 = 10.73$, $p = 0.031$). The highest final relative abundance for *C. criophilus* was 18.7% in the pre-industrial pCO_2 treatment, followed by 17.2% at current pCO_2 and finally 9.8% at future pCO_2, with the difference from both pre-industrial and current to future being significant ($F_1 = 20.54$, $p = 0.011$). No cells of *Coscinodiscus* sp. or *P. delicatissima* were detected after the two-week artificial community incubation at 14°C in any pCO_2 treatments (figure 3*c*).

(iii) Effects of pCO_2 at high temperature on individual species

At 19°C, *C. fusiformis* was extremely dominant at all pCO_2 levels and virtually excluded the other species. Its relative abundance was 99.9% at pre-industrial pCO_2, 100% at current pCO_2 and 99.8% at future pCO_2 (figure 3*d*). *Navicula* sp. was the only other diatom present after the two-week incubation, with miniscule final relative abundance values of 0.02% (pre-industrial pCO_2) and 0.2% (future pCO_2). No cells of *Thalassiosira* sp., *Coscinodiscus* sp., *P. delicatissima* or *C. criophilus* were detected after the two-week incubation period at the high temperature (figure 3*d*).

(iv) Low versus high temperature effects on individual species

At the end of the artificial community competition experiments after 12 months of conditioning, *C. fusiformis* was significantly more abundant at 19°C (figure 3*d*) versus 14°C (figure 3*c*), with increases of 27.6% at pre-industrial pCO_2, 50.5% at current pCO_2 and 36.7% at future pCO_2 (figure 3, $F_1 = 26.78$, $p = 0.006$; $F_1 = 42.74$, $p = 0.003$; $F_1 = 140.26$, $p = 0.0003$). *Navicula* sp., *C. criophilus* and *Thalassiosira* sp. all maintained a significant presence at 14°C (figure 3*c*), but of these three species, *Navicula* sp. was represented by only a few remaining cells at 19°C at the end of the two-week experiment, so the values for 14°C for this species were significantly higher at all pCO_2 levels (figure 3*c*; $F_1 = 38.53$, $p = 0.003$; $F_1 = 33.53$, $p = 0.004$). *Coscinodiscus* sp. and *P. delicatissima* virtually disappeared from all treatments in the artificial community competition experiment.

(e) Statistical evaluation of diatom community structure

Multivariate analyses indicated that community structure varied significantly in response to different CO_2 levels and temperatures during the initial natural community experiment (pseudo-$F = 5$ and 37, respectively at $p \leq 0.003$; table 3*a*) and during the artificial community competition trial 12 months later (pseudo-$F = 15$ and 562, respectively at $p = 0.0002$; table 3*b*). pCO_2 levels and temperature also interacted in affecting community structure during both trials (pseudo-$F = 10$ and 17, respectively at $p = 0.0002$; table 3*a*,*b*). One-way ANOSIM indicated that the pCO_2 treatments at increased temperature (19° C) resulted in slightly more similar assemblages compared with pCO_2 treatments at ambient temperature (14°C) (global *R*-values of 0.56 and 0.67 at 19°C compared with 0.87 and 0.95 at 14°C, respectively; $p \leq 0.03$). Average *R*-values resulting from a two-way ANOSIM test (crossed design at 9999 permutations) showed that both pCO_2 level and temperature were forcing factors on overall community structure, but that temperature had a stronger effect during both the initial natural community experiment (global *R* of 0.96 compared with 0.71 at $p \leq 0.001$) and the 12 months artificial community competition trial (global *R*-value of 1 compared with 0.81 at $p \leq 0.001$). This trend was further illustrated when Bray–Curtis similarities were plotted as non-parametric multi-dimensional plots (MDS) for each of the experiments (figures 4 and 5).

(f) Taxon-specific statistical analyses

(i) Natural community experiment

During the original natural community experiment, for *C. fusiformis*, there was a global *R* of 0.26 and 0.36 for pCO_2 and temperature, respectively (table 4). This indicated only

Table 4. Global *R*-values from one-way ANOSIM analysis of taxon-specific responses to temperature and pCO_2 combinations from the initial short-term natural community experiment and from the 12-month artificial community experiments using conditioned strains.

	initial natural community experiment		12-month conditioned artificial community experiment	
diatom genus	competing pCO_2	temperature	competing pCO_2	temperature
Cylindrotheca	0.26	0.36	0.51	1
Coscinodiscus	0.34	0.48	—	—
Thalassiosira	0.16	0.96	0	1
Pseudo-nitzschia	0.48	0.37	—	—
Navicula	0.44	0.96	0.34	0.64
Chaetoceros	0.71	0.96	0	1

limited but still significant forcing by these two variables ($p = 0.049$, $p = 0.040$, respectively; ANOSIM). For *Coscinodiscus* sp., there was a global *R* of 0.34 and 0.48 for pCO_2 and temperature, showing limited, but nonetheless significant, forcing effects ($p = 0.006$, $p = 0.40$, respectively; ANOSIM). *Thalassiosira* sp. had a global *R*-value of 0.16 for pCO_2 and 0.96 for temperature, indicative of no effect at the different pCO_2 levels ($p > 0.05$) but a strong effect for temperature ($p = 0.001$; ANOSIM). Moderate forcing for pCO_2 and a limited temperature effect ($p = 0.008$, $p = 0.37$, respectively; ANOSIM) were seen for *P. delicatissima* (global *R*-values of 0.48 and 0.37 for pCO_2 and temperature, respectively). *Navicula* sp. had global *R*-values of 0.44 and 0.96 (pCO_2 and temperature). There was only a significant pairwise effect between ambient and future pCO_2 (indicating moderate forcing), but a strong temperature effect ($p = 0.006$, $p = 0.001$; ANOSIM). Finally, for *C. criophilus*, global *R*-values of 0.71 and 0.96 indicated strong significance among all pCO_2 and temperature levels (table 4, $p = 0.0002$ and $p = 0.001$, respectively; ANOSIM).

(ii) Twelve-month artificial community experiment

In the 12-month artificial community competition experiment, *C. fusiformis* had global *R*-values of 0.51 and 1 for pCO_2 and temperature (table 4, $p = 0.003$, $p = 0.001$; ANOSIM). Despite the high *R* for pCO_2 treatments, there was only one pairwise significance (pre-industrial to future), indicating limited forcing by different CO_2 levels. The *R*-value of 1 designates a very strong effect for temperature. For *Thalassiosira* sp., global *R*-values were 0 and 1 for pCO_2 and temperature (table 4, $p > 0.05$, $p = 0.001$; ANOSIM). Essentially, there was no effect for pCO_2, but a very strong effect indicated for temperature (this species disappeared entirely from the high-temperature treatment). For *Navicula* sp., global *R*-values were 0.34 and 0.64 for pCO_2 and temperature, respectively ($p = 0.011$, $p = 0.001$; ANOSIM), indicative of limited forcing due to pCO_2 treatment and a moderate effect of temperature. *C. criophilus* had global *R*-values of 0 and 1 for pCO_2 and temperature (table 4, $p > 0.05$, $p = 0.001$; ANOSIM). Like *Thalassiosira* sp., there was no effect for pCO_2 but a very strong effect for temperature (no cells of this species remained after the competition at high temperature). There were no cells of *Coscinodiscus* sp. or *P. delicatissima* detected at the end of the 12-month artificial community experiments.

4. Discussion

To examine the potential interactive effects of ocean warming and acidification on diatom community structure, we used an experimental design that compared community structure resulting from short-term incubations with the outcomes of recombined assemblages after extended conditioning to altered pCO_2 and temperature combinations. This strategy allowed us to examine responses to abiotic factors without the effect of assemblage interactions during the conditioning process. The principal objective of our study was to determine whether the communities that emerge during short-term global change simulations are reliable proxies for community structure following long-term conditioning of their component diatom taxa. As in recent experiments with dinoflagellates [21], we used relative diatom cell abundance at the conclusion of both sets of mixed community experiments as an indicator of competitive success.

Our work indicates that community structure varied significantly in response to both pCO_2 and temperature as individual influences during the initial natural community experiment and during the 'artificial' community competition trial 12 months later. pCO_2 and temperature levels also had interactive effects on community structure during both trials. Furthermore, increased temperature (19°C) resulted in, while still significantly different, more similar assemblages compared with pCO_2 treatments at ambient temperature (14°C).

Although both pCO_2 concentration and temperature were forcing factors on overall community structure, temperature had a substantially stronger effect during both the initial natural community experiment and the 'artificial' community competition trial after 12 months of conditioning. These results are in accordance with observations from short-term natural diatom community experiments [10,12], where temperature exerted a stronger influence than pCO_2, but statistically pCO_2 was still a contributor to observed effects. These overall community structure effects were paralleled by the effects of the two global change variables on the performance of individual species; in most cases, temperature was more influential than CO_2 in determining their competitive success in both natural and 'artificial' communities.

In theory, rising atmospheric pCO_2 could benefit contemporary plants, with aquatic autotrophs being no exception. In evolutionary terms, present-day microalgae are generally living in relatively low pCO_2 environments, and it has been

suggested that the relatively high half-saturation constant of the carbon-fixing enzyme Rubisco for CO_2 may make phytoplankton growth at least occasionally vulnerable to inorganic carbon limitation [44]. However, virtually all phytoplankton, including diatoms, possess various inorganic carbon concentrating mechanisms to help them to overcome potential pCO_2 limitation during photosynthesis [44–47]. Collective uncertainties underscore the need for global change experimentation with these important organisms.

Our experiments provide little evidence that these diatom species obtained a growth advantage at higher CO_2 concentrations. Indeed, growth rates at both experimental temperatures of the dominant species *C. fusiformis* in unialgal cultures, and its final relative abundance in mixed communities, were invariably highest in the pre-industrial pCO_2 treatment rather than the current and future pCO_2 treatments. Growth rates and competitive success of the other diatom species were either unaffected by pCO_2 differences or were only marginally affected. Only *Coscinodiscus* sp. at low temperature and *Navicula* sp. at high temperature exhibited significantly increased growth rates as pCO_2 increased. The final natural and 'artificial' communities were significantly structured by pCO_2 (although less so than by temperature), but this effect was due largely to the negative effects of future pCO_2 on the abundance of *C. fusiformis*. These observations suggest the possibility that although 'carbon fertilization' may have theoretical benefits, the accompanying decrease in pH may also pose a challenge to particular groups of marine microalgae.

Our experiments suggested that warming was a dominant forcing factor on the outcome of interspecific competition between diatoms. In every case, except for *P. delicatissima* in the initial experiment, temperature was a much stronger influence on the final abundance of each species than pCO_2 (table 4). This trend was similar for most species in the original experiment and in the conditioned competition experiment, although in several cases (*C. fusiformis*, *Thalassiosira* sp. and *C. criophilus*) temperature was an even stronger forcing factor relative to pCO_2 after the conditioning period.

Temperature has long been recognized as a fundamental driver of phytoplankton biochemistry, biogeography and community composition [48,49]. A culture study on the effects of temperature demonstrated that *Phaeodactylum tricornutum* was unable to grow at 30°C, whereas other diatoms including *Navicula* sp. and *Nitzschia* (=*Cylindrotheca*) *closterium* (all isolated from Spanish coastal waters) were not impeded [50]. In a 50-year dataset, it was determined that the ratio of diatoms to dinoflagellates in the Northeast Atlantic and North Sea has steadily increased, which was attributed at least partially to increasing sea surface temperatures [51]. A recent study [52] suggested that contemporary marine phytoplankton are generally well adapted to their current thermal environment, and that in some instances a warmer mean temperature could result in a negative impact on these organisms, especially in tropical and polar regimes. Interspecific differences in cell size, metabolism, potential growth rates and the ability to physiologically acclimate or adapt will certainly play a role in phytoplankton responses to both warming and ocean acidification [50,53].

Community structure resulting from competition trials after 12 months of conditioning showed trends that were generally similar to those seen in the short-term natural community experiment. Most notably in the 'artificial' communities comprising conditioned diatom isolates, *C. fusiformis* consistently dominated all treatments, just as it did in the original collected water sample as well as the final time points of all temperature and pCO_2 conditions in the natural community experiment. Moreover, there were other parallels relating to community structure between the two sets of mixed assemblage experiments. Both before and after 12 months of conditioning, species other than *C. fusiformis* were able to maintain significantly higher relative abundances in the 14°C treatments than at the warmer temperature. Incubation at 19°C thus resulted in a greater degree of competitive exclusion by the single dominant species, and warming consequently caused a significant reduction of overall community diversity and species richness. Many of the original short-term trends in individual species relative abundance across the pCO_2 gradient were also preserved after long-term conditioning, although as noted above these effects were relatively weak compared with those of temperature.

Clearly, there were also differences in community structure between our two end-member experiments. Especially notable was that *C. fusiformis* competitively excluded all other species (with the exception of a few *Navicula* cells) in every 19°C treatment during the 'artificial' community experiments with conditioned clones. In addition, after the 12-month unialgal conditioning period, *P. delicatissima* and *Coscinodiscus* sp. seemingly lost the ability to compete successfully because they were absent from all treatments at the final time point of the 'artificial' community experiments. It is evident that short-term simulations using naive communities cannot completely predict the outcomes of competition in communities after long-term conditioning. Nonetheless, the community structure similarities discussed above suggest our short-term 'greenhouse ocean' experiment with this diatom assemblage was a better predictor of long-term trends than was the case for a dinoflagellate community [21]. This previous study found relatively little resemblance between the original natural community and the final outcome of experiments using conditioned 'artificial' communities, because different species dominated in the two sets of experiments. These differences were attributed to competition for resources other than the major experimental variable pCO_2 as well as potentially to complex interspecies interactions including mixotrophy [21].

It is important to note both the parallels and differences between the outcomes of our initial short-term natural community experiment and the subsequent long-term artificial community experiments when interpreting and comparing the two. Both sets of experiments examined community structure outcomes under identical conditions of pCO_2, temperature and other environmental variables. However, the short-term experiment began with presumably genetically diverse natural populations of each species, allowing natural selection to select for the fittest genotypes over the course of the two-week incubation. We then isolated these dominant genotypes for long-term conditioning; during this process, further evolution could occur only through de novo mutations, because clonal cultures lack the genetic variance of the original natural populations. This experimental design was adopted as major containment artefacts are inevitable when conditioning an enclosed mixed phytoplankton community over extended periods (discussed in Tatters *et al.* [21]), but it does suggest that there may have been an inherent bias towards similar outcomes in both sets of experiments. It is interesting however that the outcomes of the

short- and long-term dinoflagellate community experiments of the same basic design presented in Tatters *et al.* [21] and discussed above did not have similar outcomes, despite having presumably the same bias. As discussed below though, specialized interspecific interactions such as mixotrophy may have been a significant destabilizing influence on community structure in the dinoflagellate assemblage used by Tatters *et al.* [21], but these could not have been a factor with the diatom community used here.

The specific growth rates of our unialgal cultures during the conditioning period in the various temperature and pCO_2 treatments were not always indicative of competitive success under the same conditions in the subsequent 'artificial' community incubations. For instance, the dominant species in all the mixed culture competition experiments, *C. fusiformis*, had growth rates that were comparable with (or even lower than) the other diatom species when growing alone in unialgal culture (table 2). We took great care to provide identical exposure to accessory environmental variables during all phases of this study, but competition in mixed communities obviously does not depend only on temperature and pCO_2. The species in our experiments undoubtedly had differing affinities for nutrients, light, trace elements, vitamins, etc. which would have affected the outcome of the competition experiments in unknown ways. Recent work [21] similarly found that dinoflagellate growth rates during conditioning in isolated cultures were poor predictors of competitive success in mixed communities, and indirect evidence in these previous experiments pointed to the possibility of mixotrophic interactions among the dinoflagellate species (e.g. some species were able to consume the others in addition to growing autotrophically). This cannot be the case for our diatoms, a group which is incapable of phagotrophy. However, other biotic interactions, for instance allelopathy, could have also been influential in our mixed culture experiments. Diatoms can produce metabolites and other exudates that can inhibit the growth of other algae [54]. These 'biochemical warfare' chemicals and their effects have occasionally been examined in single species and co-culture experiments [55–57], with one study notably reporting the accumulation of aldehydes by a *Navicula* species in mixed culture that were absent when cultivated alone [58]. Additionally, as we made no attempt to render our cultures axenic, differences in naturally co-occurring bacterial metacommunities associated with particular species or clones may have exerted some influence on the diatom assemblages.

A variety of incubation experiments have now been performed at a range of environmentally relevant pCO_2 levels with clonal cultures or natural communities of marine diatoms [8,15,44,46,59–61]. Some previous experiments with this key group of phytoplankton have used arguably more realistic multivariate global change experimental designs, including studies incorporating pCO_2 and nutrient limitation [16,20], pCO_2 and irradiance [14], pCO_2 and temperature permutations [10], pCO_2 and nitrogen source [62], pCO_2, irradiance and iron manipulations [12] as well as pCO_2, temperature and irradiance [17]. Short-term manipulative studies have demonstrated shifts in Antarctic diatom assemblages from small pennates such as *Pseudo-nitzschia* spp. or *Cylindrotheca closterium* to larger centric forms such as *Chaetoceros* spp. under future pCO_2 conditions [8,12], and even more dramatic shifts in subarctic communities away from diatoms and towards smaller nanophytoplankton under combined future pCO_2/temperature 'greenhouse' conditions [10]. Such shifts between larger chain-forming centric diatoms and smaller pennate forms or other minute phytoplankton taxa could have major implications for carbon and biogenic silica export, because larger cells are disproportionately responsible for sinking fluxes of these elements [12]. In this study, we found the highest ratio of larger centrics to smaller pennate diatoms was under current pCO_2 and 14°C in both the natural and 'artificial' community experiments. At elevated temperatures or pCO_2 concentrations, pennate forms were always relatively more abundant.

5. Conclusions

The large amount of stochastic variability in the seawater carbonate buffering system and the wide thermal tolerance ranges of many phytoplankton today suggest that some groups may be capable of coping with climate-change-mediated alterations in these factors [63]. Our experiments demonstrate that the composition of a temperate diatom assemblage was more affected by warming than by changing pCO_2, although both variables had individual and interactive effects. We also found that the results of our natural diatom community experiment were relatively good predictors of the outcome of 'artificial' community experiments using conditioned cell lines, in contrast to the results of a study of similar design using a temperate dinoflagellate community [21]. As the interactive effects of multiple global change variables are largely uncertain [6,49], future experiments are required that incorporate parameters beyond pCO_2 and temperature, including alterations in nutrient supplies and irradiance shifts [21,64]. These experiments also need to be conducted using different functional groups over timescales long enough to allow for a significant degree of conditioning to the changed conditions. It is important to also realize that in addition to 'bottom-up' factors such as these, changing 'top-down' controls by climate change-influenced grazing assemblages will also play an important role in structuring future phytoplankton communities, and this issue requires further experimental investigation as well [65]. An area that is just beginning to be explored is the potential for intraspecific shifts between strains or ecotypes with differential abilities to adapt or acclimate to environmental changes [66,67]. Variability in competitive abilities based on the fine-scale diversity within phytoplankton lineages could have implications for community structure and biogeochemistry that are at least as profound as those of competitive interactions between broad taxonomic groups. Increasing our understanding of marine microalgal acclimatization and adaptation in response to climate change drivers at all taxonomic levels will provide insights into potential diversity and abundance shifts that will affect community structure and function in these key organisms that form the base of virtually all ocean food webs.

Data accessibility. All data to be archived at the US National Science Foundation Biological and Chemical Oceanography Data Management Office (BCO-DMO, http://bcodmo.org/).

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