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Variation in plastic responses of a globally distributed picoplankton species to ocean acidification

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Phytoplankton are the basis of marine food webs, and affect biogeochemical cycles. As CO_2 levels increase, shifts in the frequencies and physiology of ecotypes within phytoplankton groups will affect their nutritional value and biogeochemical function. However, studies so far are based on a few representative genotypes from key species. Here, we measure changes in cellular function and growth rate at atmospheric CO_2 concentrations predicted for the year 2100 in 16 ecotypes of the marine picoplankton *Ostreococcus*. We find that variation in plastic responses among ecotypes is on par with published between-genera variation, so the responses of one or a few ecotypes cannot estimate changes to the physiology or composition of a species under CO_2 enrichment. We show that ecotypes best at taking advantage of CO_2 enrichment by changing their photosynthesis rates most should increase in relative fitness, and so in frequency in a high-CO_2 environment. Finally, information on sampling location, and not phylogenetic relatedness, is a good predictor of ecotypes likely to increase in frequency in this system.

Marine phytoplankton are the foundation of ocean ecosystems. These small but mighty microbes are responsible for roughly half of global carbon fixation, and form a fundamental part of the biological carbon pump that exports fixed carbon to the deep ocean[1,2]. Ocean acidification will affect the composition of phytoplankton communities, which will in turn affect oxygen production, the efficacy of the biological carbon pump[3,4], and air–water CO_2 exchange. Calcifiying taxa such as coccolithophores will probably be adversely affected[5], whereas other groups such as cyanobacteria may benefit from ocean acidification[6]. Higher trophic levels will be affected indirectly if their food quality deteriorates[7]. Empirical studies so far predict changes in phytoplankton communities using single or a few[8] genotypes to represent functional groups[9,10] such as silicifying diatoms, calcifying coccolithophores and N_2-fixing cyanobacteria, but variation in responses within functional groups has not been quantified.

Here, we use 16 ecotypes of *Ostreococcus tauri* from 9 habitat types (Supplementary Table S1) to quantify variation in plastic responses to elevated CO_2 for ecologically relevant traits such as photosynthesis, and characterize changes in traits affecting food quality for 5 of these ecotypes. *O. tauri* is the smallest known free-living eukaryote[11], is globally distributed, has distinct ecotypes[12,13] and is an important primary producer[14], making it ideal for eco-evolutionary studies. As phytoplankton communities will change by sorting within and between species, we compare our measured within-species variation in plastic responses to published differences in plastic responses between functional groups. An ecotype is considered more plastic if its phenotype over a few generations of exposure to CO_2 enrichment changes more. Changes in phenotype on this timescale involve sustained changes in gene expression, but exclude transient acclimation and genetic change (evolution). We link plasticity in photosynthesis rates to changes in the relative fitness of ecotypes during asexual growth, and use this link to predict which ecotypes are likely to rise in frequency in a high-CO_2 environment.

Results and discussion

Ecotypes vary in their plastic responses to CO_2 enrichment. Using representative genotypes to predict functional group responses to ocean acidification requires that variation in responses within functional groups be small relative to variation between functional groups. However, our estimate of variation within *O. tauri* ranges from 1.8 to 3.6 times variation between functional groups (assuming equal or low within- and between-ecotype variation, respectively). See Table 1 and Supplementary Table S2. Our results are conservative, because many published studies differ in their methods, which increases within-group variation, whereas our study uses consistent methods, which minimizes variation. Other estimates of within-species variation in response to carbon enrichment are lower than in *O. tauri*[8], possibly because this study measures four times more ecotypes than previous work.

Although all 16 ecotypes of *O.tauri* increase their growth rates (μ) under CO_2 enrichment ($F_{15,96} = 248.85$, $p < 0.0001$), the magnitude of response varies among ecotypes ($F_{15,96} = 1,233.63$, $p < 0.00001$), ranging from a 1.06- to a 1.9-fold increase (Fig. 1a). As variation within species and between functional groups is similar, single or a few ecotypes of a species cannot be used to estimate the response of a functional group to ocean acidification; both a mean and a variance for each functional group (or species) are needed. The relative magnitudes of within- and between-species variation show that ecological, compositional processes (species sorting within communities) and evolutionary processes (lineage sorting within species) must be considered simultaneously to understand community responses to ocean acidification in marine phytoplankton.

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Table 1 | Fold change in μ between and within functional groups in response to CO_2 enrichment.

Functional group	Mean fold-change in μ	Variance fold-change in μ within group	Variance in fold-change in μ within group	References
Cyanobacteria (N_2 fixing)*	1.5	0.04	0.1	6,31–34
Diatoms (silicifying)†	1.1	0.03	0.1	23,35–37
Coccolithophores (calcifying)*	0.91	0.02	0.1	5,8,26,30,38–40
Green algae*	1.5	0.36	0.1	This study

In all studies cited here CO_2 levels were 380–440 ppm and 800–1,200 ppm CO_2. Fold-change μ bigger than 1 indicate that cell numbers increased faster, whereas fold-change μ smaller than 1 indicate that cell numbers increased more slowly than at control conditions. Note that this table is not exhaustive and that it includes both mesocosm and laboratory experiments. So far, there have been no studies that directly compared laboratory and mesocosm responses to ocean acidification. *A single species was considered for this table. †Multiple species were considered. Note that we have used a single individual from each ecotype of *O. tauri* to estimate within-species variation, which will overestimate variation within species if variation within ecotypes is large relative to variation within species. See Supplementary Table S2.

There is similar variation in plastic responses among ecotypes for traits affecting oxygen production, size, and food quality of phytoplankton (Fig. 1b–d). There is a 1.02- to a 2.18-fold increase in photosynthesis rates ($F_{15,96} = 27.59$, $p < 0.0001$) with significant variability between ecotypes ($F_{15,96} = 727.54$, $p < 0.0001$). Differences in plastic responses for C/N ratios, which partly determine the food quality[7,15] of phytoplankton, range from 1.06- to 1.56-fold increases (response to CO_2: $F_{4,59} = 69.99$, $p < 0.001$, variance: $F_{4,59} = 361.46$, $p < 0.0001$). Ecotypes also vary in how much their size increases under CO_2 enrichment with fold differences from 1.3 to 1.9 (response to CO_2: $F_{15,79} = 375.14$, $p < 0.0001$; variation: $F_{15,79} = 174.1$, $p < 0.0001$). However, there is only a weak tendency for the amount of the main light-harvesting molecule in photosynthesis, chlorophyll *a*, to increase in cells grown at high CO_2 (Supplementary Fig. S2). The ranges of phenotype values for all traits are larger at high CO_2. Although variability in dissolved inorganic carbon is higher at elevated CO_2 (ref. 16), this is unlikely to be driving our results because the same pattern holds when we correct for the absolute value of the mean (Supplementary Table S3).

In the absence of information on initial ecotype abundances, the magnitude of the change in average phenotype expected in *O. tauri* cannot be predicted, although the direction of change is consistent. As CO_2 levels increase, *O. tauri* will grow and photosynthesize faster, and have larger cells with a higher C/N ratios than contemporary cells. Higher μ values indicate that *Ostreococcus*, along with other green algae[17] and cyanobacteria[6], are likely to increase in abundance in high-CO_2 conditions. Faster photosynthesis rates would allow future *Ostreococcus* communities to produce more oxygen and fix and sequester more carbon from the atmosphere. Increases in cell size would compound this effect, because bigger cells contain more carbon and can sink faster, which will affect geochemical cycles and food webs. Modest changes in cell diameter translate into large changes in cell volume, affecting the nutrients needed to build cells, susceptibility to grazing, carbon export efficiency and a host of other traits. As high C/N ratios usually indicate a nitrogen-deplete reservoir, *O. tauri* in a high-CO_2 world may be a lower quality food source than it is now, which, coupled with similar changes in prokaryotic picoplankton[18,19], raises concerns about food quality for grazers. Changes in C/N ratios can also affect bacterial degradation[20] and the efficiency of the biological carbon pump[3].

More plastic ecotypes increase in relative fitness at high CO_2. Under CO_2 enrichment, there is variation in the change in μ among ecotypes of *O. tauri*, which is expected to drive changes in their relative fitnesses (Supplementary Table S4), and so change the genetic composition of the species under ocean acidification. Here, relative fitness is defined by which ecotype grows fastest and should therefore outcompete others in the absence of more complex interactions[21,22]. Assuming the relative fitness of ecotypes under present-day conditions (380 ppm CO_2) at least partially determines their frequencies now, an increase in the relative fitness of an ecotype under elevated CO_2 indicates that its frequency is likely to rise, and a decrease indicates that its frequency is likely to fall as the species adapts.

When the genetic composition of a species changes adaptively, the mean values of traits correlated with fitness are expected to systematically change when there is variation in those traits. Changes in photosynthesis rates affect functions such as primary productivity and oxygen production, so we test how we expect this trait to change as *O. tauri* evolves. We confirm that photosynthesis rate is positively correlated with μ, as expected[17,23] ($r^2 = 0.62$ and $r^2 = 0.86$, $p < 0.05$ and $p < 0.0001$ for 380 ppm and 1,000 ppm CO_2, respectively).

There is variation in both absolute photosynthesis rates and plasticity in photosynthesis rates, but which one best predicts the change in relative fitness of ecotypes under CO_2 enrichment? If the absolute rate of photosynthesis does, then ecotypes that photosynthesise the fastest at high CO_2 will become more frequent, regardless of whether they respond to changes in CO_2. If the magnitude of the plastic response in photosynthesis rates is the best predictor, we instead expect the most responsive ecotypes to increase in frequency, even if they do not have the highest rates of photosynthesis when growing in high CO_2. Although photosynthesis rate at high CO_2 predicts changes in relative fitness under CO_2 enrichment ($F_{15,29} = 46.61$, $p < 0.01$), plasticity in photosynthesis rate is a far better predictor of it here ($F_{15,29} = 106.45$, $p < 0.0001$; Supplementary Table S4).

Surprisingly, we do not expect ecotypes with the highest absolute photosynthesis rates in high-CO_2 environments to increase in frequency when CO_2 levels rise, even though photosynthesis rate is positively correlated with μ. Instead, we expect ecotypes that are most plastic, and so best able to take advantage of an environmental change to increase in frequency. For example, deep-sea ecotype rcc809 has only a modest plastic response to CO_2 enrichment. This ecotype drops in rank fitness and is expected to decrease in frequency despite its high photosynthesis rate. In contrast, the plastic ecotypes oth95 and rcc1108 increase in rank fitness under CO_2 enrichment, and are expected to rise in frequency under future conditions, although their photosynthesis rates are not unusually high. If more plastic ecotypes become more frequent, changes in community function should be larger than the average response of the species now. For example, the average response to carbon enrichment in *O. tauri* is to increase photosynthesis rates by about 1.4-fold, but will be higher after lineage sorting (closer to 1.7-fold).

Plastic responses correlate with geography at large scales. More plastic ecotypes are likely to increase in frequency in response to CO_2 enrichment, but it is not always possible to measure plasticity in a large number of ecotypes for all species, so 'more easily obtainable' indicators of the rank plasticities of ecotypes within species are useful. To this end, we test whether differences in

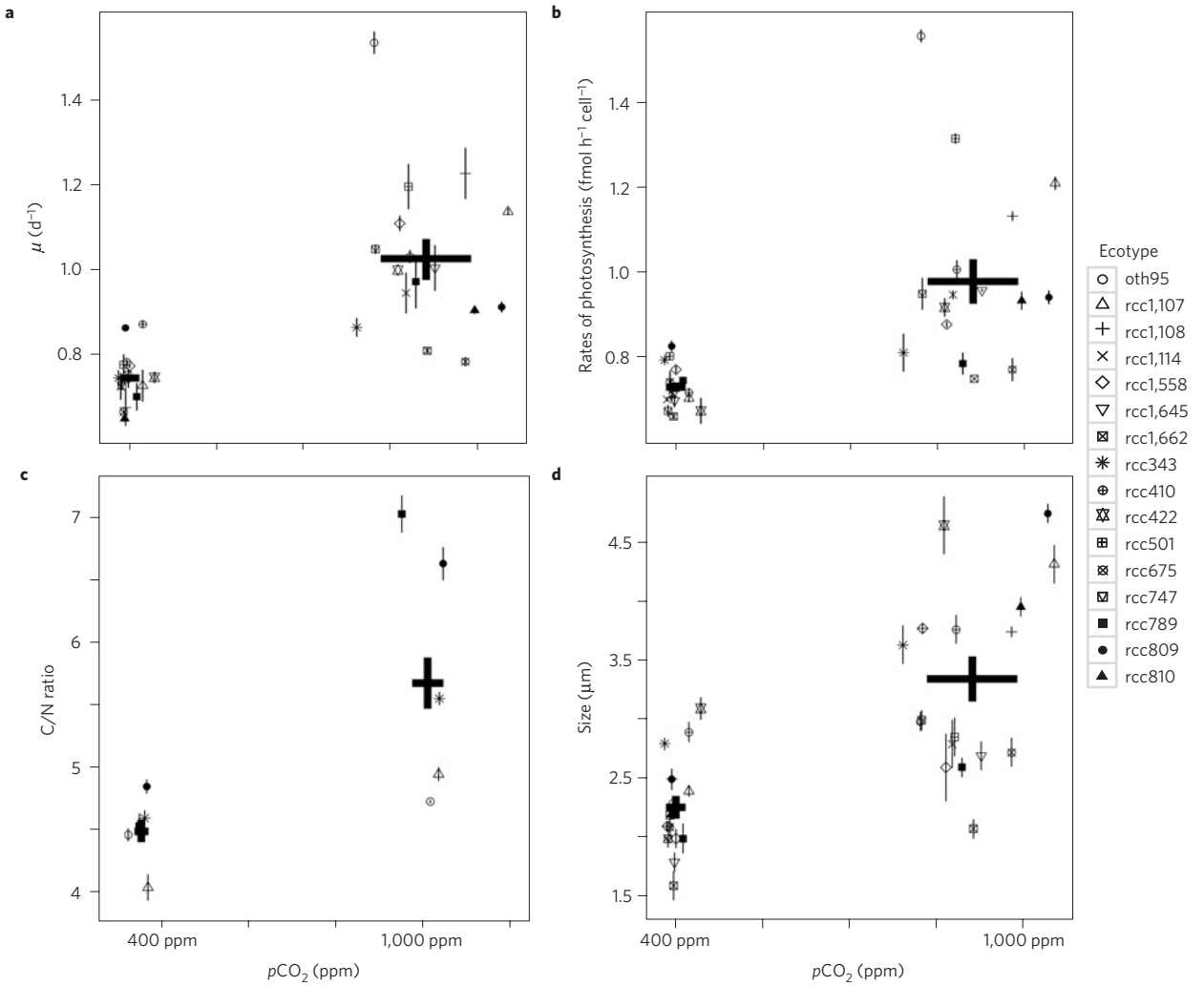

Figure 1 | Physiological changes in *O. tauri* in response to elevated CO_2 levels. a, All 16 ecotypes divided more rapidly at 1,000 ppm CO_2 than at 380 ppm CO_2 (1.48 ± 0.42-fold increase in growth at high relative to ambient CO_2 levels (ANOVA $F_{15,96} = 248.85$, $p < 0.0001$); mean daily μ at 380 ppm is $0.76 \pm 0.02\ d^{-1}$ and at 1,000 ppm is $1.04 \pm 0.05\ d^{-1}$). The magnitude of the response varied between ecotypes (ANOVA $F_{15,96} = 1,233.63$, $p < 0.00001$). **b**, Net photosynthesis rates are faster at elevated CO_2 (ANOVA $F_{1,96} = 27.59$, $p = 0.0001$) and increase by 1.4 ± 0.3-fold for photosynthesis and 1.2 ± 0.2-fold for respiration (oxygen evolution is 7.42 ± 0.29 fmol O_2 per cell and hour at 380 ppm and 9.61 ± 0.4 fmol O_2 per cell and hour at 1,000 ppm). Although responses are consistent in direction, they vary significantly between ecotypes (ANOVA $F_{15,96} = 727.54$, $p < 0.0001$ for photosynthesis, $F_{1,596} = 514.21$ and $p < 0.001$ for respiration). **c**, The C/N ratio of cells is significantly higher in cells grown at 1,000 ppm CO_2 than in cells grown at 380 ppm CO_2 (ANOVA $F_{4,59} = 59.99$, $p < 0.001$, C/N mass ratio 4.49 ± 0.09 at 380 ppm CO_2 and 5.68 ± 0.2 at 1,000 ppm CO_2). The increase in C/N ratio is due to increases in carbon content (fold increase in particulate organic carbon 2.12 ± 0.8), as well as decreases in nitrogen content of cells (fold change in particulate organic nitrogen is 3.16 ± 1.18). Variation between ecotypes is significant (ANOVA $F_{4,59} = 361.46$, $p < 0.0001$). These changes are consistent with the increase in cell size seen (**c**) and are caused by carbon enrichment rather than nitrogen limitation, as the medium used was nitrogen-rich. On the basis of these changes, future *Ostreococcus* populations could more than double their intracellular carbon quota, with some ecotypes decreasing their intracellular nitrogen quota by a third. **d**, Cells also increase in size in response to CO_2 enrichment (1.48 ± 0.21 fold increase in size, $p < 0.0001$; mean size at 380 ppm is 2.26 ± 0.29, at 1,000 ppm is 3.35 ± 0.6) and the magnitude of the response varies between ecotypes (ANOVA $F_{4,79} = 375.14$, $p < 0.0001$). Future *Ostreococcus* cells have the capacity to be nearly 1.5 times bigger than contemporary *Ostreococcus* cells. All ecotypes are shown as means ($n = 3$–5) and standard error. Black bars indicate overall means and standard errors for all ecotypes at either 380 or 1,000 ppm CO_2.

plasticity among ecotypes are explained best by their location or by phylogeny. Our reasoning is that plasticity could be maintained by natural selection because different regions of surface ocean differ in the magnitude, tempo and predictability of environmental fluctuations. Alternatively, differences in plasticity may mainly reflect phylogenetic similarity.

Here, sampling location explained most of the variation in plasticity in photosynthesis. We find that most of the variability in physiology among ecotypes (93%) is explained by how plastic they are, rather than by absolute trait values or μ. Ecotypes cluster on the basis of the depth and location that they were collected at (mixed-effect analysis of variance (ANOVA) for variation in photosynthetic rates: sampling depth $F_{15,37} = 2$–195.21, $p < 0.0001$, ocean of origin $F_{15,34} = 224.79$, $p < 0.001$, Fig. 2). Ecotypes from the ocean surface cluster together, and are more plastic, which is consistent with these ecotypes experiencing variable conditions of sea surface pCO_2 (ref. 24,25; $r^2 = 0.71$, Supplementary Fig. S3 and Table S5). Ecotypes collected from the surface of the Mediterranean are more plastic than ecotypes collected elsewhere, suggesting that plasticity may be selected for and that although the magnitude of CO_2 fluctuations in some regions of the Mediterranean is relatively low, some other aspect of variation in CO_2 may maintain plasticity

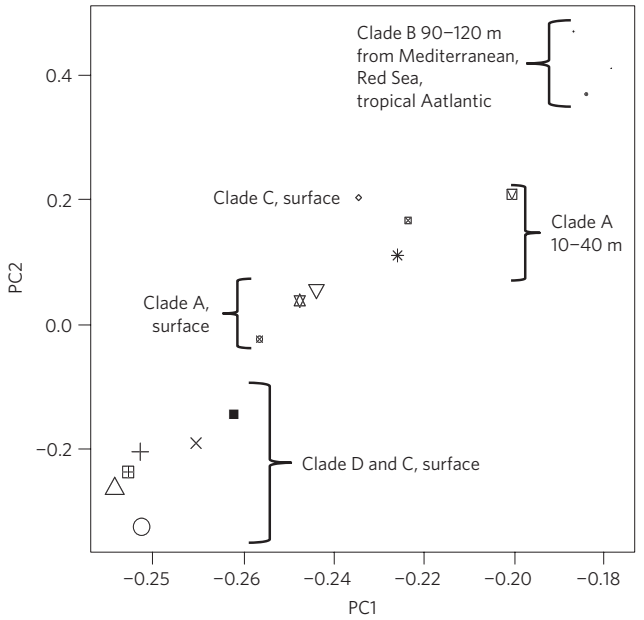

Figure 2 | Variability of *O. tauri* ecotypes in photosynthesis and μ after acclimation to 1,000 ppm CO_2. Ecotypes cluster in a principal component analysis on the basis of sampling depth and location. Symbol size scales with level of plasticity, with larger symbols indicating more plastic ecotypes. Surface ecotypes are more plastic than deep-sea ecotypes, and ecotypes from similar environments usually cluster together regardless of clade. If not categorized as either surface or deep sea, ecotypes were collected between 10 and 40 m. Ecotype legend is identical to Fig. 1.

there. All but one Mediterranean ecotype form a distinct cluster regardless of belonging to different clades. Surface ecotypes from other regions form a second cluster, and deep-sea ecotypes form a third. The patterns we see are not explained by previously published relatedness[12] (Supplementary Fig. S4). This suggests that environment is more important than phylogenetic similarity for explaining variation in plasticity among ecotypes of *O. tauri*, which opens the possibility of identifying locations where marine phytoplankton communities are likely to be the most and least stable over the coming decades. Whereas other studies show that physico-chemical parameters predict aspects of phytoplankton physiology[13], we show that location, which is simpler to measure, and which does not require an understanding of whether and how plasticity is selected in any given environment, explains variation in plasticity in a marine picoplankton.

Conclusions

To predict how future phytoplankton communities may differ from contemporary ones, we need to take plastic, ecological and evolutionary responses into account. Here, we have characterized plastic responses to carbon enrichment in 16 ecotypes of a cosmopolitan green alga, *O. tauri*. In general, surface picoplankton under CO_2 enrichment are expected to grow faster, be larger and store more carbon and less nitrogen per cell, with more plastic ecotypes expected to increase in frequency under future ocean conditions. This can change their abundance and quality as a food source and the magnitude of their roles in biogeochemical cycles. Most surprisingly, the variation in responses to CO_2 enrichment among ecotypes of a single species is similar in magnitude to the variation in responses among functional groups in published studies, suggesting that ignoring within-species sorting underestimates how much food webs and biogeochemical cycles will change. Although extrapolating from laboratory to natural conditions must be done cautiously[22], variation seen in the laboratory is heritable, consistent and affects μ, so it has the potential to affect relative fitness in natural populations.

One challenge to predicting community-level responses to changing environments is that we must either measure responses in a large number of the organisms present, or extrapolate from a few genotypes. Differences in basic biology can predict changes in community composition at the level of functional groups[26]. However, shifts within groups also affect ecosystem function—frequencies of toxic versus non-toxic cyanobacteria is one example[27]. At the scale of our study, sampling location explains variation in plastic responses to CO_2 enrichment in a species, and may be a useful proxy when measuring reaction norms is not feasible, as is the case for most species on the planet. Although environmental genomics can tell us the composition of the present community, we suggest that the potential for changes in community composition can be partly predicted using geography.

Methods

Culture conditions. *O. tauri* ecotypes were obtained from the Roscoff Culture Collection (rcc) and Plymouth Marine Laboratory, grown in Keller medium[28] and made clonal by dilution, so that each culture originated from single cells. Light intensity, salinity and incubator temperature reflected a compromise between environments experienced by different ecotypes. Samples were pre-acclimated and acclimated for 5–7 asexual generations to 380 ppm CO_2 or 1,000 ppm CO_2 in a closed-system and grown in semi-continuous batch cultures at low densities. Details and effects of ecotype history and isolation date (all non-significant) are in Supplementary Tables S1 and S7.

Growth rate (μ). Cell density was determined as attenuance at 650 nm (EL 808 Biotek) twice a day for a seven-day period. μ was calculated as

$$\mu(\mathrm{d}^{-1}) = \frac{\ln(N_1) - \ln(N_0)}{\Delta t}$$

with N_1 and N_0 being cell numbers at t_1 and t_0, and Δt the time in days between sampling intervals.

Net photosynthetic oxygen evolution. Cultures were centrifuged to concentrate cells. Oxygen evolution in the light was measured in a Clark-type oxygen electrode illuminated at 180 µmol photons m^{-2} s^{-1}, with stirring.

Plasticity and fitness responses. Plasticity responses were calculated from oxygen evolution as follows,

$$\frac{|PS_{\text{high } CO_2} - PS_{\text{ambient } CO_2}|}{PS_{\text{ambient } CO_2}}$$

where *PS* is net photosynthetic oxygen evolution (or respiration) in femtomoles per cell per hour. For fitness responses, μ replaces *PS*.

Carbonate chemistry. Seawater carbonate chemistry was calculated from pH and alkalinity using the CO2sys software[29]. Dissolved inorganic carbon was measured colorimetrically Total alkalinity was inferred from linear Gran-titration plots. For detailed methods see Supplementary Table S7.

Chlorophyll and C/N ratio. Details on chlorophyll *a* content determination through acetone extraction and measurements of particulate organic carbon and particulate organic nitrogen content as described in ref. 30 for 5 of the 16 ecotypes, chosen to span the range of responses to CO_2 enrichment, are in the Supplementary Information and Figs S5–S7.

Data analysis. Data were analysed in the *R* environment, using mixed models within the nlme and lme4 packages. Data were tested for normality before performing mixed linear models with pCO_2 as a fixed effect. Depending on the test performed there were up to four random effects that were all treated as not nested within each other. Degrees of freedom were calculated manually.

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Author contributions

E.S. designed and performed the experiments, analysed data and wrote the manuscript. S.C. designed the experiments, analysed data, wrote the manuscript and supervised laboratory work. B.R. supervised the laboratory work at the Alfred-Wegener-Institute and contributed to the manuscript. A.J.M. contributed to the manuscript.

Additional information

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Competing financial interests

The authors declare no competing financial interests.