LIMNOLOGY and OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 9999, 2022, 1–10

doi: 10.1002/lno.12267

Temporal variation in ecological and evolutionary contributions to phytoplankton functional shifts

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Abstract

Communities and their functioning are jointly shaped by ecological and evolutionary processes that manifest in diversity shifts of their component species and genotypes. How both processes contribute to community functional change over time is rarely studied. We here repeatedly quantified eco-evolutionary contributions to CO_2-driven total abundance and mean cell size changes after short-, mid-, and longer-term (80, 168, and > 168 d, respectively) in experimental phytoplankton communities. While the CO_2-driven changes in total abundance and mean size in the short- and mid-term could be predominantly attributed to ecological shifts, the relative contribution of evolution increased. Over the longer-term, the CO_2-effect and underlying eco-evolutionary changes disappeared, while total abundance increased, and mean size decreased significantly independently of CO_2. The latter could be presumably attributed to CO_2-independent genotype selection which fed back to species composition. In conclusion, ecological changes largely dominated the regulation of environmentally driven phytoplankton functional shifts at first. However, evolutionary changes gained importance with time, and can ultimately feedback on species composition, and thus must be considered when predicting phytoplankton change.

Phytoplankton is a diverse and globally distributed group of photoautotrophic aquatic microorganisms that constitute the base of most marine food webs. They account for approximately half of the planet's primary production (Field et al. 1998) and play an important role in biogeochemical cycling (Falkowski et al. 1998). Depending on community composition, phytoplankton can significantly affect the configuration and functioning of marine food webs (Sommer et al. 2002; Chavez et al. 2003; Stibor et al. 2004) and likely biogeochemical cycles (Spilling et al. 2018).

Climate change has been shown to change phytoplankton community structure and thus alter their ecological functioning. While ocean warming, for example, can result in reduced productivity and total biomass (Boyce et al. 2010; Hofmann et al. 2011; Lewandowska et al. 2014) arising from predictable shifts towards smaller phytoplankton species (Polovina and Woodworth 2012; Sommer et al. 2015, 2017), the effects of increasing seawater CO_2 concentrations on phytoplankton communities are more diverse. Different communities have shown varying effect sizes and directions in response to increased seawater CO_2 concentration (Eggers et al. 2014; Sommer et al. 2015; Paul et al. 2016; Schulz et al. 2017) suggesting that CO_2 effects may well depend on the species present. Using the concept of "winners and losers", some calcifying coccolithophores are negatively impacted by increased CO_2 concentration, while both larger diatoms and smaller picoplankton could benefit from the correspondingly greater supply of inorganic carbon (Kroeker et al. 2013; Bach et al. 2017). These opposing interspecific responses to increased CO_2

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

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Author Contributions Statement: G.S.I.H. and L.L. carried out lab work. Data analysis by G.S.I.H. G.S.I.H. drafted the manuscript with significant contributions of B.M. All other authors revised the manuscript and gave final approval for publication.

concentration, and thus the potential for compensatory growth of some functional groups, make predicting ecological shifts in phytoplankton structure difficult.

Predictions on the future phytoplankton community structures are additionally constrained by the largely undetected contribution of evolutionary changes, another vital mechanism by which phytoplankton keep pace with climate change (Lohbeck et al. 2012; Collins et al. 2014; Rengefors et al. 2017). Recent studies have shown pronounced intraspecific response diversity to increased CO_2 concentration among genotypes within, and between populations of the same species (Schaum et al. 2013; Hattich et al. 2017). Selection on standing intraspecific diversity can result in diversity shifts that alter a species' sensitivity. Such evolutionary responses via the selection of standing genetic diversity have been shown to take place on ecologically relevant timescales (Yoshida et al. 2007; Schoener 2011), for example in the coccolithophore *Emiliania huxleyi* in response to increased seawater CO_2 concentration (Lohbeck et al. 2012). Most experimental evolution studies so far have excluded interactions with other species (but *see* Schaum et al. 2017; Listmann et al. 2020). Given that ecological interactions such as competition between species or predator–prey dynamics can alter the direction and strength of evolutionary selection (Collins 2011; Lawrence et al. 2012; Kleynhans et al. 2016), experimental evolution studies that exclude ecological interactions might not only miss the full extent of evolution, but also its feedback upon ecological processes (Hairston et al. 2005; Fussmann et al. 2007; Becks et al. 2012; Listmann et al. 2020) and ultimately on community functioning. In particular, the relative contribution of evolutionary compared to ecological shifts for phytoplankton community change in response to climate change remain largely undiscovered.

To address this question, we here quantified how total community changes in phytoplankton mean trait (cell size) and community property (total abundance) under a changing environment were mediated by ecological shifts in species diversity and evolutionary shifts in genotype diversity over 180 generations. We established phytoplankton model communities comprising two coexisting species (*E. huxleyi* and *Chaetoceros affinis*), each with an initial intraspecific diversity of nine genotypes exposed to ambient and high CO_2 conditions and observed diversity shifts of species and genotypes over time (data also used in Listmann et al. 2020 assessing the effect of competition on adaptation potential). Moreover, we repeatedly assessed the relative contributions of ecological and evolutionary shifts to total community changes in abundance and mean size over $\sim$180 generations. These assessments were realized by experimental assays in which species, genotype and both species and genotype diversities were manipulated based on changes we observed in the longer-term sorting experiment and their effects quantified. A previous method-oriented paper served to establish and validate the experimental assay used here to quantify the relative eco-evolutionary contributions to a total community change (Hattich et al. 2022). For that purpose, a small portion of the data included here was already used, precisely data on total abundance changes in the short term until 80 d. Publishing the method-oriented paper separately from the rest of the collected data was chosen to focus on a detailed discussion on methodological issues and potential extended applications and thus to foster future use of the new experimental approach. In the present manuscript we extend the univariate short-term response with longer-term data and cell size as a second response variable, allowing for the novel assessment of (1) temporally changing eco-evolutionary dynamics and (2) their community functional interpretation. Following the premise that community structure and functioning are jointly determined by ecological and evolutionary change, we expected that both ecological and evolutionary changes contribute to the total abundance and mean cell size changes in response to CO_2. However, based on observed timescales over which phytoplankton genotype diversity shifted in response to environmental change elsewhere (Lohbeck et al. 2012; Wolf et al. 2019), we assumed that even evolutionary changes in the form of selection of standing intraspecific diversity would require more time than shifts in species diversity. This assumption is further supported by the observation of low evolutionary contributions to community change after short-term exposure to CO_2, as described in the method-oriented paper by Hattich et al. (2022). Hence, we hypothesize that total phytoplankton community changes in response to CO_2 are dominated by ecological changes in the short-term, while evolutionary changes increase in importance with time.

Methods

This study aimed to advance the understanding of phytoplankton community change in response to environmental factors by experimentally quantifying the relative contributions of ecological and evolutionary processes to community property and mean trait changes from the short- to longer-term.

Phytoplankton community

The experimental community studied consisted of two coexisting species, the diatom *C. affinis*, and the coccolithophore *E. huxleyi*. Each species initially represented an assemblage of nine genotypes that showed response diversity when exposed to elevated CO_2 (Hattich et al. 2017). All genotypes used in this study were isolated from nearshore waters of Gran Canaria in 2014 and 2015 (27°59′N, 15°22′W; for detailed information for genotypes and dates of isolation, *see* Hattich et al. 2017).

Experimental set-up

The experimental set-up comprised a long-term selection experiment of 288 d (corresponding to approximately 180 generations) in which both sorting of species and selection for genotypes in response to a control and an increased CO_2 treatment took place. During the time course of this sorting phase, repeated eco-evo assays allowed us to quantify the relative contributions of species and genotype diversity shifts to the observed changes in response to CO_2 in total abundance and mean cell size over time.

Both sorting phase and eco-evo assays were carried out at 21.0 ± 1.2°C and a $17L : 7D$ cycle reaching a maximum light intensity of 350 μmol m^{-2} s^{-1} and minimum intensity of 0 μmol m^{-2} s^{-1} 3 h after artificial dusk and dawn, respectively. To avoid sedimentation the cells were held in suspension by constant rotation (0.75 min^{-1}) on a plankton wheel. The communities were cultured in 0.5 L polycarbonate bottles serving as experimental units filled with sterile filtered (0.2 μm pore size) medium. This medium consisted of artificial seawater with a salinity of 35 PSU, aerated for 24 h with CO_2-enriched air set to 400 and 1250 ppm to obtain an ambient (control) and high level of CO_2 concentrations, respectively. Macronutrients were added to a final concentration of 1.00 ± 0.1 μmol L^{-1} phosphate, 19.6 ± 0.5 μmol L^{-1} nitrate, and 4.4 ± 0.8 μmol L^{-1} silicate mimicking rather natural conditions allowing bloom formations in laboratory settings but are slightly higher than prevailing nutrient concentrations in the oligotrophic water around Gran Canary (Taucher et al. 2017). Micronutrients and trace metals were added according to f/8 concentration (Guillard 1975).

Sorting phase

In the sorting phase that started on 10 January 2017 the experimental communities were exposed to the above-described ambient and high CO_2 conditions, replicated five times. Specifically, the starting communities in the first batch cycle were inoculated with the same biovolume (2.75×10^6 μm^3) of *E. huxleyi* and *C. affinis* to adjust for substantial differences in size. The nine genotypes of each species were added in equal cell contributions (11%). Every subsequent batch cycle was initiated by transferring a total of 5.5×10^6 μm^3 of the whole community from the previous batch to new media. Thereby, species and genotype frequency shifts in response to the CO_2 environment were not manipulated but transferred to the following batch cycle. Each batch cycle lasted for 8 d, which corresponded to approximately five generations, and ran into the stationary phase, where competition for nutrients was assumed to become stronger. As a measure of total community change at the end of each batch cycle total abundance and mean cell size (measured as cell volume) of the communities were monitored. Both abundance and size were analyzed from Lugol's iodine-fixed samples using an inverted light microscope (Zeiss, Axiovert 200 and Observer A1; ×20 and ×40 magnification). Cell size was calculated from the diameter/width and length measurements (Hillebrand et al. 1999) of five randomly chosen *E. huxleyi*/*C. affinis* cells per experimental replicate. The "master" trait cell size affects many processes including nutrient uptake, edibility, package effect and sinking rates and thus, is functionally important (Marañón 2015). Mean community cell size was calculated as the sum of each species' cell abundances multiplied with their respective size, divided by the total cell abundance. The combination of size and cell abundance determines total community biomass, for which we did not assess the relative eco-evo contributions over time, considering that opposing effect signs in the responses of size and abundance would be hidden. Nevertheless, the total biomass was calculated as the sum of both species' cell abundance multiplied with size to allow the calculation of the specific transfer volume of each replicate containing a total biovolume of 5.5×10^6 μm^3. Genotype frequency shifts were analyzed by isolating 20 individuals per replicate after 8, 32, 64, 80, 168, and 288 d (corresponding to about 5, 20, 40, 50, 105, 180 generations), respectively. Isolated cells were grown for 2 weeks to reach a sufficient density for subsequent microsatellite analysis (for details *see* Hattich et al. 2017 and Listmann et al. 2020).

Eco-evo assay

The eco-evo assay is an experimental protocol developed to quantify ecological and evolutionary contributions to total phytoplankton community changes (*see* Hattich et al. 2022). In short, the assay allows to separate and quantify the contributions of ecological and evolutionary shifts to total community changes by measuring the effects of different combinations of species and genotype diversity manipulations that mimic diversity changes observed in the sorting phase. Here, the eco-evo assays were carried out at different time points using communities of the sorting phase, which had been exposed to ambient and high CO_2 concentration for 80, 168, 288 d (hereafter referred to as short-, mid-, and longer-term), respectively. Temporal repetition of the assay allowed us to ask whether ecological and/or evolutionary changes dominate a phytoplankton community response to CO_2 concentration at different points in time. To practically carry out the assay, first, species composition of the communities in the sorting phase were assessed. Second, the two species from the sorting phase were physically separated over a 20 μm sieve. Third, species were artificially reassembled and partly manipulated regarding the genotype and/or species diversity to obtain the following assay communities: $Control_{ambient}$, $Effect_{novel}$, Eco, Evo, and EcoEvo communities. In detail, the $Control_{ambient}$ and $Effect_{novel}$ communities reflected all species and genotype diversity changes of the communities sorted in response to the ambient and high CO_2 environment,

respectively, and during the assay continued to grow in their original CO_2 conditions. The Eco, Evo, and EcoEvo communities were reassembled to include either species, genotype, or both species and genotype diversity changes as observed in the high CO_2 environment in the sorting phase. Apart from the specific diversity manipulations these communities otherwise displayed the diversity that was observed in ambient CO_2 concentration and continued to grow in the ambient CO_2 environment. While the resulting species diversity shifts could be observed microscopically and directly manipulated, genotype diversity changes remained invisible and were indirectly manipulated by using inoculates from those populations that were selected by the required environment. That means, cells from the communities exposed to high CO_2 in the sorting phase were used to assemble the Evo and EcoEvo communities. For the Evo community, the species composition, however, was assembled as observed under ambient sorting, while species composition in the EcoEvo community was assembled as found under high CO_2 sorting. Because of these indirect manipulations of genotype diversity, the Evo and Eco-Evo responses might, additionally to the effect of differences in genotype sorting between environments, also include plasticity and, with time de novo mutations. However, the appearance of fitness improvements due to de novo mutations generally take longer than selection on standing genetic variation. Therefore, we assume that the effects caused by the Evo communities should mainly reflect the selection of genotype variation. For example, in coccolithophores such beneficial mutations occurred only after ca. 500 asexual generations (Lohbeck et al. 2012). For further details on potential problems and possible extensions of the here used eco-evo assay *see* Hattich et al. (2022). The assay communities were grown for one batch cycle and as a community response again the resulting cell abundance and mean size responses were measured (as described for the sorting phase above).

Statistical analysis and visualization

Total community abundance and mean cell size changes that happened in the sorting phase over time and in response to the CO_2 environment were analyzed with a generalized least square model (gls[size/total abundance/relative *E. huxeli* contribution to biovolume ~ CO_2 × time]). The GLS was parameterized to account for an autocorrelation with time (correlation = corAR1 [form = ~1 |Time]). In addition, we accounted for heterogeneity between CO_2 conditions and time points (weights = varIdent [form = ~1 | CO_2 × time]). Genotype sorting was described qualitatively.

In each assay experiment, the differences between $Effect_{novel}$, Eco, Evo, or EcoEvo communities and the $Control_{ambient}$ communities were tested using an ANOVA, with $Control_{ambient}$ set as control (i.e., intercept). Significant responses due to species or genotype diversity changes (Eco and Evo communities) or significant total changes justified the subsequent calculation of the relative ecological and evolutionary contributions to total community changes by dividing the respective absolute values of $Effect_{eco}$, $Effect_{evo}$, $Effect_{eco\times evo}$ and $Effect_U$ by their sum (Eqs. 1–4):

$$\%\text{Ecology} = \frac{|\text{Effect}_{eco}|}{|\text{Effect}_{eco}| + |\text{Effect}_{evo}| + |\text{Effect}_{eco\times evo}| + |\text{Effect}_U|}, \quad (1)$$

$$\%\text{Evolution} = \frac{|\text{Effect}_{evo}|}{|\text{Effect}_{eco}| + |\text{Effect}_{evo}| + |\text{Effect}_{eco\times evo}| + |\text{Effect}_U|}, \quad (2)$$

$$\%\text{Eco} \times \text{Evo} = \frac{|\text{Effect}_{eco X evo}|}{|\text{Effect}_{eco}| + |\text{Effect}_{evo}| + |\text{Effect}_{eco\times evo}| + |\text{Effect}_U|}, \quad (3)$$

$$\%U = \frac{|\text{Effect}_U|}{|\text{Effect}_{eco}| + |\text{Effect}_{evo}| + |\text{Effect}_{eco\times evo}| + |\text{Effect}_U|}. \quad (4)$$

To achieve these calculations of the $Effect_{eco}$ and $Effect_{evo}$ the response of the $Control_{ambient}$ community was subtracted from the response of Eco, and Evo communities, respectively (Eqs. 5, 6):

$$\text{Effect}_{eco} = \text{Eco} - \text{Control}_{ambient}, \quad (5)$$

$$\text{Effect}_{evo} = \text{Evo} - \text{Control}_{ambient}. \quad (6)$$

The $Effect_{eco\times evo}$ constitutes the difference between the measured combined effect of both processes and the sum of the single ecological and evolutionary effects (Eq. 7):

$$\text{Effect}_{eco\times evo} = (\text{EcoEvo} - \text{Control}_{ambient}) - [(\text{Eco} - \text{Control}_{ambient}) + (\text{Evo} - \text{Control}_{ambient})]. \quad (7)$$

The *unexplained* variance (Eq. 8) was expressed as the difference between the sum of the ecological and evolutionary changes and their interaction to the total community change between $Effect_{novel}$ and $Control_{ambient}$ communities:

$$\text{Effect}_U = (\text{Effect}_{novel} - \text{Control}_{ambient}) - (\text{Effect}_{eco} + \text{Effect}_{evo} + \text{Effect}_{eco\times evo}). \quad (8)$$

To standardize the visualization, the potential importance of eco-evolutionary changes are shown in relation to the temporarily dynamic effect size of CO_2 on the total change (calculated as mean difference observed between $Control_{ambient}$ and $Effect_{novel}$ communities, Borenstein et al. 2009).

All data analyses, an inspection of normality and heterogeneity of variances, and plotting were undertaken in R (R Development Core Team 2016) using the packages "ggplot2" (Wickham 2009) and "nlme" (Pinheiro et al. 2018).

Results

Over the experimental sorting phase of 288 d, corresponding to at least 180 *E. huxleyi* and *C. affinis* generations, total cell abundance gradually increased in both CO_2 environments and was, in the end, 10 times higher than at the onset (Fig. 1A; Time: $F_{1,342} = 208.88$, $p < 0.001$). Over the same time mean cell size (measured as cell volume) declined to a fifth of the initial cell size (Fig. 1B; time: $F_{1,342} = 353.04$, $p < 0.001$). Furthermore, the time course revealed two distinct phases of CO_2 responses over time (Fig. 1A,B; time × CO_2 for abundance: $F_{1,342} = 326.29$, $p < 0.001$; time × CO_2 for mean size: $F_{1,342} = 327.91$, $p < 0.001$), with CO_2 being a strong driver for total cell abundance and mean cell size in the short- to mid-term (Fig. 1A,B; i.e., until 80 and 168 d, respectively), but not in the longer-term (Fig. 1A,B; i.e., > 168 d).

Until mid-term the total cell abundance in high CO_2 conditions was reduced to a third of that in ambient CO_2 conditions (Fig. 1A). At the same time, mean cell size doubled in response to high CO_2 concentration compared to the control (Fig. 1B, CO_2: $F_{1,342} = 142.55$, $p < 0.001$). The underlying species diversity was significantly affected by the CO_2 environment, while genotype selection was not (Fig. 2). Thus, the observed changes in total abundance and mean size were likely driven by the underlying species diversity changes (Figs. 1A,B, 2B, S1–S3). Both species and genotype diversity, however, changed with time. More precisely, until mid-term *C. affinis* dominated the community and *E. huxleyi* had a minor contribution (Fig. 2B). This pattern was more pronounced under high CO_2 conditions (7% and 21% *E. huxleyi* contribution under high and ambient CO_2 conditions, respectively; Fig. 2B, CO_2: $F_{1,342} = 478.8$, $p < 0.001$). This was an expected result given that the short-term growth rates (Rokitta and Rost 2012; Krumhardt et al. 2017) and relative abundances (Eggers et al. 2014; Riebesell et al. 2017) of calcifying phytoplankton have been elsewhere shown to decrease in response to elevated CO_2 concentrations. Likewise, an increase in community cell size observed in response to CO_2 concentration has previously been attributed to a shift towards a higher proportion of larger diatoms (Sommer et al. 2015). In the longer-term we here, however, observed a dominance switch and *E. huxleyi* increased to a relative contribution of 56% and 63% to total biovolume under ambient and high CO_2 conditions, respectively (Fig. 2B, time × CO_2: $F_{1,342} = 29.4$, $p < 0.001$). Genotype sorting of both species was characterized by competitive exclusion. Changes of *E. huxleyi* genotype diversity were reflected in strong increases of the relative abundances of some genotypes and reduction to near detection limits of others already in the first batch cycle (Fig. 2C). With ongoing time rapid genotype exclusion resulted in the same three *E. huxleyi* genotypes that dominated

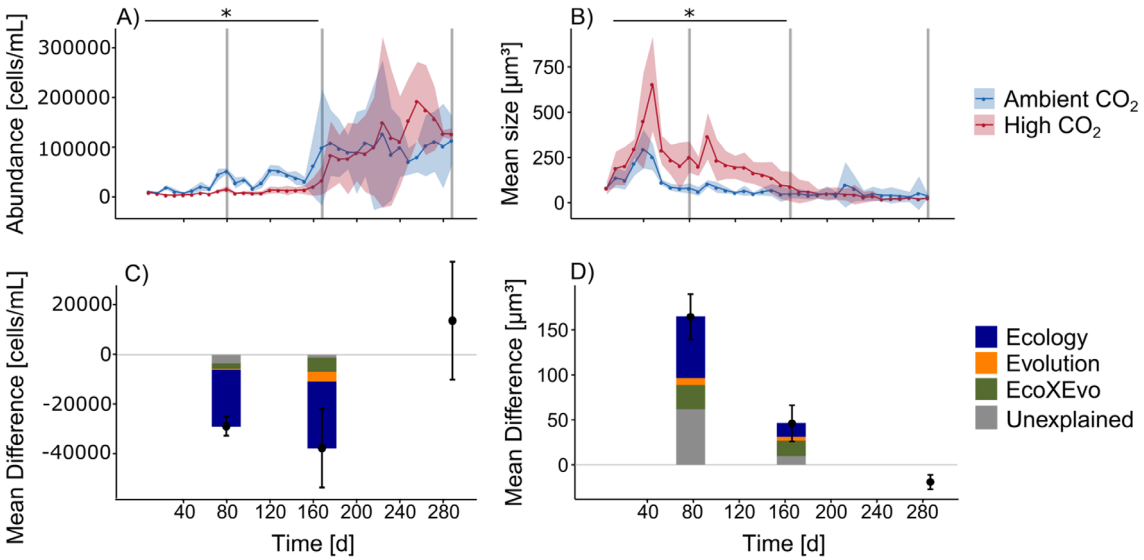

Fig. 1. Upper panel; community total cell abundance (**A**) and mean size (**B**) changes in response to ambient (400 ppm) and high (1250 ppm) CO_2 condition in the sorting phase (mean and 95% CI, $n = 5$ until 216 d in high and 264 d in ambient then $n = 4$). Here, short- to mid-term responses (until 80 and 168 d, respectively) were significantly different between CO_2 treatments (indicated by upper black line with asterisks) and vanished in the longer-term (> 168 d). Lower panel; outcomes of the eco-evo assays undertaken using communities at different time points in the sorting phase (depicted by the gray lines in the upper figures) to quantify the ecological and evolutionary contributions to observed total changes in response to CO_2 condition. (**C**) Calculated mean difference (effect size) and the standard error of total cell abundance, and (**D**) of mean size between the $Effect_{novel}$ and $Control_{ambient}$ community in the eco-evo assay. Bar charts show the fraction of the total change explained by ecological and evolutionary changes, respectively. Relative contributions were calculated from assay results (Figs. S4A,B, S5A,B), which were not valid at 288 d as no significant total change or significant effects of species or genotype changes were found (Figs. S4C, S5C).

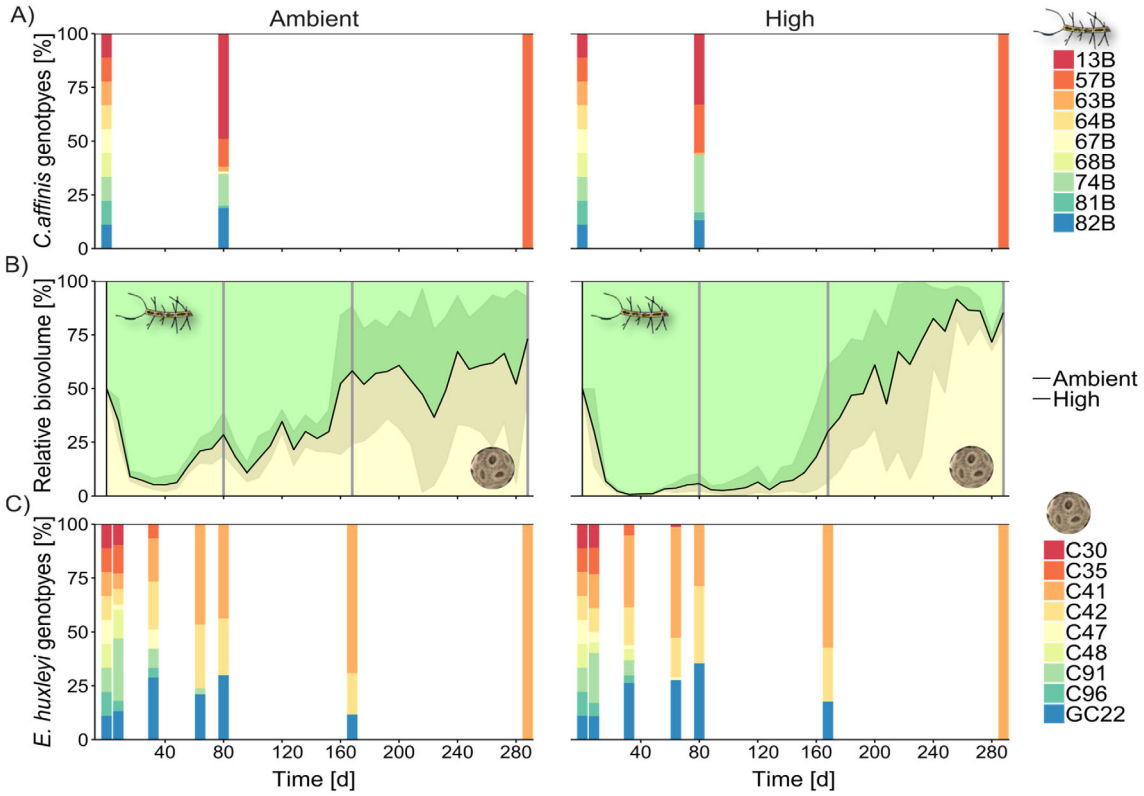

Fig. 2. Community shifts over time in response to ambient and high seawater CO_2 concentration in the sorting phase (plots on left- and right-hand side, respectively). Shifts in relative biovolume contributions of the two species in the community are shown in the middle (**B**; green: *Chaetoceros affinis*, light-yellow: *Emiliania huxleyi*; mean and 95% CI), top and bottom plots show sorting of *C. affinis* (**A**) and *E. huxleyi* (**C**) genotypes, respectively. $n = 5$ experimental units until day 216 in high and day 264 in ambient then $n = 4$. Genotype sorting are expressed as the mean of an N ranged between 33 and 83 isolated individuals (*see* Table S1 for details).

both CO_2 treatments by mid-term, but with slight compositional differences. Genotype diversity was further reduced to the same *E. huxleyi* genotype in ambient and high CO_2 in the longer-term (Fig. 2C). Genotype sorting of *C. affinis* showed comparable patterns, with strong selection over time leaving the same genotype under both CO_2 environments in the long-term (Fig. 2A).

The eco-evo assay revealed, that in the short-term, ecological species shifts largely explained changes in total abundance and mean size under high CO_2, with the contribution of evolutionary changes to total change slightly increasing in the mid-term (Fig. 1C,D). Specifically, in the short-term 80% of the total abundance decline in response to high CO_2 concentration could be attributed to ecological changes (Fig. 1C). In the mid-term, 72% of the total abundance decline was attributable to ecological changes, 10% to evolutionary changes, and 15% to the interaction between ecology and evolution (Fig. 1C). Similarly, in the short-term, 42% of the increased mean cell size in the high compared to ambient CO_2 treatment could be explained by ecological changes and only 5% by evolution and 16% by their interaction. In the mid-term, the relative contributions to mean cell size shifts of ecological changes declined to 33%, while evolutionary changes and their interaction increased to 9% and 37%, respectively (Fig. 1D). By 288 d the CO_2 treatment did not affect total abundance and mean size. The eco-evo assay could not attribute significant compensatory effects to either ecological or evolutionary restructuring (Figs. 1C,D, S4C, S5C).

Discussion

In line with our predictions, we found that (1) phytoplankton community changes in response to projected future climate change have both an ecological and evolutionary component, and (2) these changes are dominated in the short- to mid-term by ecological dynamics (i.e., species compositional shifts), while (3) evolutionary dynamics increase in importance over time. The study further and unexpectedly showed that the effects of elevated CO_2 concentration on community property and mean trait changes vanished in the longer-term, for which no underlying ecological or evolutionary compensatory responses could be detected.

Comparable significant ecological contributions to total community change in response to a constant external driver in the short-term, with increasing evolutionary contribution over longer time periods, have been described elsewhere (Govaert et al. 2016). It was shown that evolutionary change was too slow to become apparent in the short- to mid-term (Govaert et al. 2016). Unlike the above study, however, we found genotype selection in both *E. huxleyi* and *C. affinis* from the onset of the experiment, albeit largely independent of CO_2 concentration (Fig. 2A,C; Listmann et al. 2020). As such, the genotype selection was not captured in the evolution term by the eco-evo assay, which exclusively partitioned the total change in response to CO_2. The increase in contributions of evolution and the interaction between ecology and evolution to total community changes in response to CO_2 in the mid-term was possibly caused by slight compositional shifts of the remaining genotypes of *E. huxleyi* between CO_2 concentrations (Fig. 2C). It has been demonstrated that not only richness, but compositional shifts can affect ecosystem functioning at the species level (Hillebrand et al. 2008; Hillebrand and Matthiessen 2009). In addition, the increased evolutionary contributions to the CO_2-driven total changes in the mid-term, especially in mean cell size, might further depend on *C. affinis* evolutionary changes (Figs. S1B, S3). We, however, cannot provide a solid underpinning of evolutionary changes due to a data gap in *C. affinis* genotype composition at mid-term (Fig. 2A). This points nonetheless to the potential importance of genotype diversity shifts of both species present in a community for the evolutionary contribution to community change and underscores the significance of shifting away from evaluating evolutionary responses of single focal species to the simultaneous impacts of the evolution of multiple species (De Meester et al. 2019).

The weak selection exerted by high CO_2 concentration was contrary to expectations, especially on *E. huxleyi* given that the genotypes used here were known to display a diversity of phenotypic responses to different CO_2 concentrations (Hattich et al. 2017). The response diversity of genotypes used in this study was greater than that among strains (Zhang et al. 2018) used in a study demonstrating strong genotype selection in *E. huxleyi* in response to enhanced CO_2 concentration (Lohbeck et al. 2012). One possible explanation for the deviation in responses could be the presence of a co-occurring species in this study. The diatom could have altered the selection environment by alleviating CO_2 stress, for example. Moreover, lower evolutionary rates and more species compositional changes are predicted to take place when pre-adapted species are present in a community (de Mazancourt et al. 2008). Of the two species used here, the diatom *C. affinis* was potentially favored by enhanced CO_2 concentration, since the mean response to enhanced CO_2 conditions expressed by a mix of *C. affinis* genotypes was positive compared to that of a *E. huxleyi* genotype mix (Hattich et al. 2017). This argument is countered, however, by the fact that selection for the same genotypes occurred not only independent of the CO_2 environment in the two-species communities but also when *E. huxleyi* grew in isolation (Monocultures in Listmann et al. 2020). An alternative explanation for the low evolutionary contribution to the total community changes is that the selective pressure of high CO_2 concentration was overridden by nutrient conditions, producing always only one "winning" genotype of *E. huxleyi* (Fig. 2C; Listmann et al. 2020). The nutrient regime in this experimental system was characterized by fluctuations with alternating repletion at the onsets and depletion in the stationary phases (Fig. S6). While this likely allowed for the stable coexistence of the two species with their diverging nutrient strategies (affinity- vs. velocity- adapted; Sommer 1984), it did not allow for stable coexistence of several genotypes of one species. Data from a reciprocal exposure experiment testing for evolutionary adaptation to CO_2 in the presence and absence of a second species in the same two-species communities (Listmann et al. 2020), also led to the hypothesis that local experimental conditions with their specific nutrient regime was the predominant selection factor on genotypes in this model system. The possibility of strong nutrient-related selection is further supported by the observed shift towards the smaller species *E. huxleyi* (Fig. 2B) and the decline in *C. affinis* cell size (Fig. S3). Smaller cells are characterized by higher surface:volume ratios, and hence lower K_n (and V_{max}), which can constitute a competitive advantage at low nutrient concentrations (Finkel et al. 2010; Marañón 2015), that is, here at the end of batch cycles. It has been shown elsewhere that CO_2 effects on species can be modulated or weakened by interactions with other drivers such as nutrient regimes (Eggers et al. 2014; Paul et al. 2016; Alvarez-Fernandez et al. 2018). A highly possible explanation for our suggestion that nutrient regime ultimately led to genotype exclusion, while simultaneously allowing the species to coexist, is that intraspecific competition for nutrients was stronger than interspecific competition. According to modern coexistence theory (Chesson 2000) higher intraspecific than interspecific competition is the stabilizing factor in this system allowing the two species to stably coexist. The consequence of the higher similarity among genotypes than between species in nutrient uptake-related traits is, however, that the applied environmental factor plays a lower role intra- than interspecifically. This finding raises the intriguing question of whether species and genotype sorting in communities are regularly driven by different selection factors, or if this finding is specific to CO_2 concentration and nutrient regime.

Despite the unexpected disappearance of an initial CO_2 effect and the absence of underlying CO_2-driven eco-evolutionary changes in the longer term, evolutionary changes have the potential to alter ecological interactions (Hairston et al. 2005; Schoener 2011). We here show that such evo-eco feedback even further translates to community functioning. This evo-eco functional feedback was reflected in the simultaneous sorting towards one *E. huxleyi* genotype and the

dominance shift from *C. affinis* to *E. huxleyi* (Fig. 2B; Listmann et al. 2020) that in turn translated to the temporal and CO_2 independent increase in total abundance and decline in mean cell size. The proportional increase of *E. huxleyi* was observed in all communities from mid-term to longer-term and is possibly caused by modulating- or overriding- the selective pressure of CO_2 concentration by nutrients (as explained above). Consequently, the consistent decline of calcifying coccolithophores in communities under increased CO_2 concentration observed elsewhere (Eggers et al. 2014; Riebesell et al. 2017; Schulz et al. 2017), and its effects on community mean traits and properties, was not found to hold in the longer-term when both ecological and evolutionary changes were allowed to take place. In the longer-term, a feedback from evolution to ecology was reflected in a dominance shift, propagating to total abundance and mean size changes. More precisely, the short- to mid-term increase in mean cell size in response to high CO_2 concentration, which is in line with earlier work (Sommer et al. 2017; Bach and Taucher 2019) was reversed in the longer-term. This longer-term size reduction was caused by an increased proportion of *E. huxleyi* (Fig. S1B), likely a result of the effect of evolutionary changes on species sorting. In a rapidly changing ocean ecosystem, reliable predictions of phytoplankton mean trait and property changes are essential as such diverging effects on size structure observed over short- compared to longer-terms can have strong implications for predicting future pelagic ecosystem function. For example, phytoplankton food webs are mainly size-structured (Boyce et al. 2015) and decreasing mean size can increase food chain length (Stibor et al. 2004), which in turn decreases transfer efficiency to higher trophic levels (Barnes et al. 2010).

In conclusion, this study placed evolutionary changes of multiple species into an ecological context and enhanced our understanding of the relative eco-evolutionary contributions to total community changes. We demonstrated that the relative importance of ecological and evolutionary changes exhibits temporal variation and that shifts of both genotype and species diversity have the potential to alter community mean traits and properties. We show that short-term predictions of phytoplankton changes do not require evolutionary components. Long-term projections, however, heavily rely upon both ecological and evolutionary changes which can feedback on each other and together alter community functioning.

Data availability statement

The data of the long term community selection phase to ambient and high CO2 (400 and 1250 ppm, respectively) can be found in DOI:10.1594/PANGAEA.887780. The data of the Eco-Evo assays carried out after 80, 168 and 288 d of selection are assessable under: https://doi.org/10.1594/PANGAEA.950758.

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Acknowledgments

We thank Thomas Hansen, Bente Gardeler, Cordula Meyer, Jens Wemhöner, Kastriot Qelai for their laboratory assistance. As well as our student assistants Nele Rex, Anna Lechtenbörger, Florian Webers, Julia Raab, Dorthe Ozod-Seradj, Julia Romberg, Miriam Beck, Sophia Antoniella and Gabriela Escobar for their support. We also thank KIMOCC for technical support and quality management of the Cluster's Kiel CO_2 manipulation experimental facility ($KICO_2$) at GEOMAR. LL, GSIH and this project were funded by the DFG priority program 1704 Dynatrait: Thorsten Reusch RE1708/17-1 and Birte Matthiessen MA5058/2-1. Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

None declared.

Submitted 06 April 2022
Revised 18 August 2022
Accepted 04 November 2022

Associate editor: Maren Striebel