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RESEARCH ARTICLE

Experimentally decomposing phytoplankton community change into ecological and evolutionary contributions

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Abstract

1. Shifts in microbial communities and their functioning in response to environmental change result from contemporary interspecific and intraspecific diversity changes. Interspecific changes are driven by ecological shifts in species composition, while intraspecific changes are here assumed to be dominated by evolutionary shifts in genotype frequency. Quantifying the relative contributions of interspecific and intraspecific diversity shifts to community change thus addresses the essential, yet understudied question as to how important ecological and evolutionary contributions are to total community changes. This debate is to date practically constrained by (a) a lack of studies integrating across organizational levels and (b) a mismatch between data requirements of existing partitioning metrics and the feasibility to collect such data, especially in microscopic organisms like phytoplankton.
2. We experimentally assessed the relative ecological and evolutionary contributions to total phytoplankton community changes using a new design and validated its functionality by comparisons to established partitioning metrics. We used a community of coexisting *Emiliania huxleyi* and *Chaetoceros affinis* with initially nine genotypes each. First, we exposed the community to elevated CO_2 concentration for 80 days (~50 generations) to induce interspecific and intraspecific diversity changes and a total abundance change. Second, we independently manipulated the induced interspecific and intraspecific diversity changes in an assay to quantify the corresponding ecological and evolutionary contributions to the total change. Third, we applied existing partitioning metrics to our experimental data and compared the outcomes.
3. Total phytoplankton abundance declined to one-fifth in the high CO_2 exposed community compared to ambient conditions. Consistently across all applied partitioning metrics, the abundance decline could predominantly be explained by ecological shifts and to a low extent by evolutionary changes.
4. We discuss potential consequences of the observed community changes on ecosystem functioning. Furthermore, we explain that the low evolutionary

contributions likely resulted of intraspecific diversity changes that occurred irrespectively of CO_2. We discuss how the assay could be upscaled to more realistic settings, including more species and drivers. Overall, the presented calculations of eco-evolutionary contributions to phytoplankton community changes constitute another important step towards understanding future phytoplankton shifts, and eco-evolutionary dynamics in general.

KEYWORDS

community change, eco-evolutionary shifts, environmental change, interspecific diversity, intraspecific diversity, ocean acidification, partitioning metrics, relative contribution

1 | INTRODUCTION

While the relevance of interspecific diversity changes to affect ecosystem functioning was long recognized and explained by selection and niche partitioning (Hooper et al., 2005, 2012; Loreau & Hector., 2001), the potential importance of intraspecific diversity changes was only recently described (Bolnick et al., 2011; Prieto et al., 2015). Interspecific diversity shifts are manifested in altered species composition and thus reflect ecological changes. Intraspecific diversity shifts can reflect evolutionary changes, if these shifts are manifested in altered allele frequencies. Allele frequency shifts can be the result of altered genotype composition (i.e. standing genetic diversity) or new mutations acquired by particular genotypes. However, interspecific and intraspecific trait changes are not only the result of diversity changes but may additionally depend on organisms' phenotypic plasticity. While one could expect that the scope for diversity effects is far greater between species than within species, a recent meta-analysis showed that intraspecific diversity effects can be of similar magnitude to those driven by species diversity shifts across trophic levels, and across a variety of ecosystems (Des Roches et al., 2017). Analogue to effects of interspecific diversity, significant effects of intraspecific diversity on stability (Prieto et al., 2015; Reusch et al., 2005) and productivity (Crutsinger et al., 2006; Sjöqvist & Kremp, 2016; Wolfe, 1985) have been described. In line with modern coexistence theory based on niche partitioning, competition among several phenotypes of threespine sticklebacks resulted in coexistence by character displacement (Svanbäck & Bolnick, 2007). This example indicates that the same mechanisms maintaining interspecific diversity and its consequent effects on community functioning are also at play among individuals of one species. In communities of organisms in which evolution happens contemporary to species compositional shifts and which are subject to environmental change, the study of interspecific and intraspecific diversity shifts allows a glimpse into the consequences of both ecological and evolutionary processes. As ecological and evolutionary processes can occur on similar time-scales and thus may influence one another (Carroll et al., 2007; Fussmann et al., 2007; Hairston et al., 2005; Schoener, 2011), it is important considering both interspecific and intraspecific diversity shifts in communities responding to environmental change.

Within the exponentially increasing numbers of studies published in the field of Eco-Evolution (Bassar et al., 2021), the number of studies quantifying the relative contributions of both aspects of diversity change for community properties or mean trait changes are still scarce. Existing partitioning metrics, which allow quantifying the ecological and evolutionary contributions, could be a valuable tool to assess the relative importance of intraspecific diversity effects for community-level responses. However, most existing partitioning metrics require trait measurements at the population level, and this for multiple species within the community, often resulting in high data requirements. For example, partitioning metrics based on the Price equation require information on genotype frequencies and their trait values for each species (Collins & Gardner, 2009; Govaert et al., 2016; Price, 1970; Table 1). Metrics based on reaction norms such as the reaction norm approach (Govaert et al., 2016; Stoks et al., 2016) and the Geber method (Ellner et al., 2011; Hairston et al., 2005; Pantel et al., 2015; terHorst et al., 2014) require information on species composition and their trait values collected from common garden or transplant experiments (Table 1). Last, metrics based on variance partitioning (VP) after Lepš (Lepš et al., 2011) require measurements of species frequencies and trait values (Table 1) and are widely used in terrestrial plant systems (e.g. Lajoie & Vellend, 2015, 2018; Tusifujiang et al., 2021). The above-mentioned metrics are often applied on traits that can be measured for single individuals; however, this is not easily applicable to microscopic organisms, as for example phytoplankton.

Phytoplankton constitutes the base of most aquatic food webs and play a key role for global biogeochemical cycles (Cassar et al., 2015; Field et al., 1998). Biodiversity shifts driven by environmental changes result in altered phytoplankton mean functional traits and/or community properties (Boyce et al., 2010, 2015; Filstrup et al., 2014; Stibor et al., 2004; Vallina et al., 2017) and thus can influence these major ecosystem functions. Under persisting increase in seawater CO_2 concentration for example, significant compositional shifts are described (Eggers et al., 2014; Schulz et al., 2017; Tortell et al., 2008) that are likely the result of unique and in parts opposing environmental sensitivities exhibited by major phytoplankton groups. One-third of the anthropogenically emitted CO_2 since pre-industrialization has been

TABLE 1 Comparison of existing eco-evolutionary partitioning metrics applied at the community level, the Price equation, two approaches based on reaction norms (Geber method following Ellner et al., 2011; Hairston et al., 2005; reaction norm approach following Govaert et al., 2016; Stoks et al., 2016), Variance partitioning (Lepš et al., 2011), and the here introduced Eco-Evo assay, with regard to requirements and the ability to also partition mean trait changes and community properties

		Required data:				Applicable to:	
		Trait values	Reaction norms	Genotype composition	Species composition	Mean trait changes	Community properties (e.g. abundance, biomass)
Price equation		Yes (genotypes)	No	Yes	Yes	Yes	No (except biomass as in Fox & Kerr, 2012)
Based on reaction norms	Geber method	Yes (populations)	Yes	No	Yes	Yes	Yes (as state variable as in Pantel et al., 2015 or if measured experimentally)
	Reaction norm approach	Yes (populations)	Yes	No	Yes	Yes	Yes (as state variable as in Geber Method)
Variance partitioning		Yes (populations)	No	No	Yes	Yes	No (except biomass)
Eco-Evo assay		No	No (only a subset of a full reciprocal transplant)	No	Yes	Yes	Yes

absorbed by the ocean (Caldeira & Wickett, 2003), resulting in both increased concentration of dissolved inorganic carbon and reduced availability of calcium carbonate, crucial for the ability of organisms to form calcareous shells and skeletons (Doney et al., 2009). Calcifying phytoplankton such as coccolithophores are thus adversely affected by increased seawater CO_2 compared to other functional groups of phytoplankton such as diatoms that benefit from the surplus of CO_2 facilitating its acquisition (Bach et al., 2017; Collins & Bell, 2004; Dutkiewicz et al., 2015). Species can, however, potentially keep pace with fast environmental changes by rapid evolution (Jin et al., 2013; Lohbeck et al., 2012). Such evolution on an ecologically relevant timescale is potentially widespread in phytoplankton due to pronounced intraspecific diversity (Hattich et al., 2017; Zhang et al., 2018), fast generation times and enormous population sizes of up to millions cells per mL (Collins et al., 2014; Rengefors et al., 2017; Reusch & Boyd, 2013). In the face of climate change, the relevance of intraspecific diversity changes might be further enhanced as models predict a sharp decline of tropical phytoplankton diversity in the absence of adaptive evolution (Thomas et al., 2012).

Whether rapid evolution observed in highly controlled single species laboratory experiments has the potential to effectively propagate to altered phytoplankton community functioning in response to environmental changes, here CO_2 concentration, remains largely unknown. This knowledge gap partly results of the to date predominating separate assessment of plastic (physiological; e.g. Meyer & Riebesell, 2015), evolutionary (e.g. Lohbeck et al., 2012) or ecological (e.g. Sommer et al., 2015) changes (but see Collins & Gardner, 2009). The uncertainty about the evolutionary contribution further results from a mismatch between required and available data to apply the existing metrics to partition and quantify ecological and evolutionary diversity effects. The aforementioned methodological constraint to collect genotype/species abundances and their associated trait values arises in particular in microscopic communities as phytoplankton, where only a limited number of morphological traits can be assessed in situ (i.e. size, morphological defence). Other traits such as characteristics related to nutrient uptake and toxicity cannot be microscopically observed, and thus their measurement requires the isolation of single species or genotypes and subsequent trait measurements in clonal populations in laboratory settings. Such measurements are time and labour-intensive. Abundance measurements require the identification of species and genotypes. While species identification and quantification are feasible by microscopy (cell size >5 μm) and in parts by flow cytometry (cell size <5 μm), genotype identification relies on molecular tools such as microsatellite markers with a limited ability to provide quantitative data (Lohbeck et al., 2012; Wolf et al., 2019). To overcome the constrains on required observations on genotype frequency shifts to assess the relative eco-evolutionary contributions on carbon uptake in marine phytoplankton using the Price equation approach, Collins and Gardner (2009) proposed to assume additivity of evolutionary, ecological and physiological contributions. Precisely, they suggested calculating the evolutionary contribution indirectly by subtracting physiological and ecological contributions from the

total community change (Collins & Gardner, 2009). The underlying assumption might, however, not hold true if ecological and evolutionary processes interact (see Govaert et al., 2016).

In this study, we set out to effectively assess the relative ecological and evolutionary contributions to total phytoplankton abundance changes in response to changing environmental conditions, assuming that intraspecific changes are dominated by genotype frequency shifts. To this end, we developed and evaluated a novel experimental design that overcomes the above outlined constraints of current partitioning methodologies. This experimental design allows to separate and quantify the effects of the different components of community change on a target mean trait and/or community property by directly applying a set of interspecific and intraspecific diversity manipulations that are based on observed community changes. We validate the successful functioning of the new experimental design by comparing the outcome with partitioning of two established metrics and provide first data emphasizing interspecific over intraspecific diversity shifts as major driver for total phytoplankton abundance change in response to increased seawater CO_2.

2 | MATERIALS AND METHODS

2.1 | Experimental setup and design

During the experiment, communities are first allowed to respond to any novel (here high CO_2) environment (Sorting phase). This response comprises both interspecific and intraspecific changes, which we assume to be predominantly manifested in species and genotype frequency shifts, respectively (Figure 1; Supporting Information Section 1). In the second step, the relative ecological and evolutionary contributions to the total abundance change in response to high CO_2 concentration are tested in an Eco-Evo assay by manipulating interspecific and intraspecific diversity based on changes observed in the sorting phase (Figure 1).

2.1.1 | Sorting phase

The experimental model community comprised two phytoplankton species, *Chaetoceros affinis* and *Emiliania huxleyi*, with nine genotypes each (for detailed information on variations among the different genotypes, see Hattich et al., 2017). These communities were exposed to ambient and high (novel) seawater CO_2 environments in the sorting phase (Figure 1). The growth medium used throughout the experiment was prepared from artificial seawater (according to Kester et al., 1967) with a salinity of 35, and contained 19.6 ± 0.7 µmol/L nitrate, 1.0 ± 0.1 µmol/L phosphate, 3.8 ± 0.6 µmol/L silicate, f/8 vitamin and trace metal concentration (Guillard, 1975). Seawater pCO_2 was manipulated by aerating the growth medium with CO_2-enriched air containing 400 (ambient) and 1,250 (high) ppm for 24 hr. The applied high CO_2 concentration of 1,250 ppm and resulting shifts in carbonate chemistry

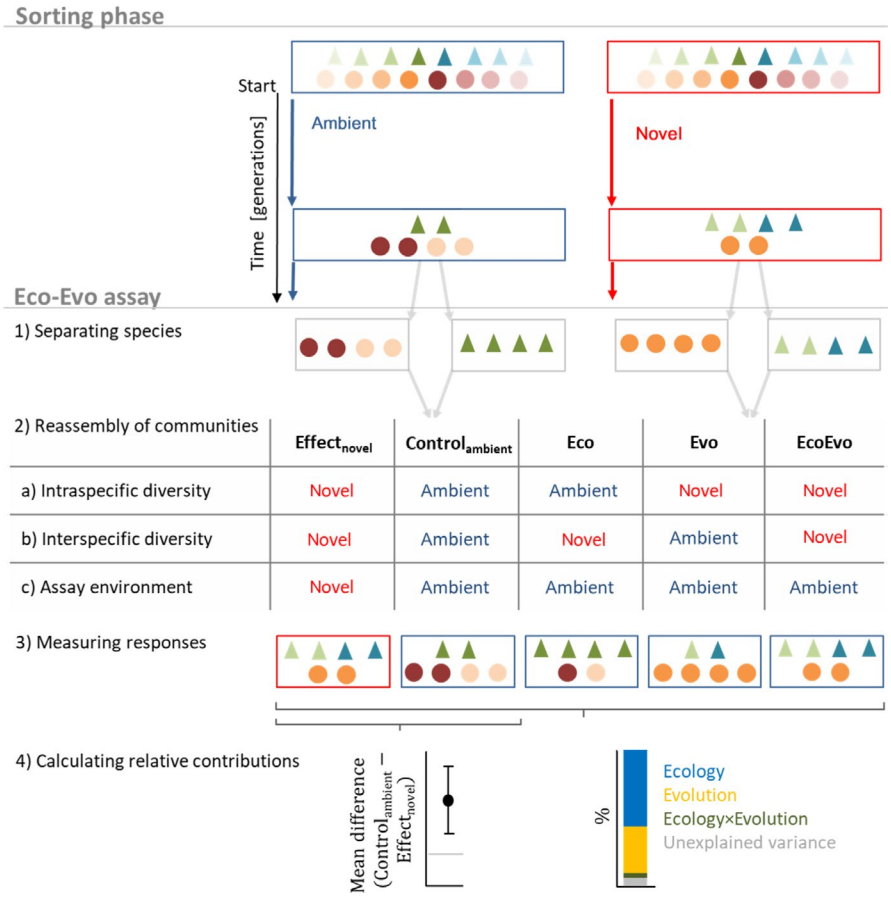

FIGURE 1 Stepwise description of the experimental design to partition and quantify the relative importance of ecological and evolutionary contributions to total change in a community trait or property in response to a novel environment. The sorting phase shows a model system including a minimum of two species (triangle and circle) with several genotypes (colour of triangles and circles). In response to ambient (blue box) and novel (red boxes) environments hypothetical species and genotype sorting (dominating interspecific and intraspecific changes) are depicted as shifts in absolute number and proportion of these symbols and their colouring, respectively. The Eco-Evo assay gives detailed information on the different steps that have to be taken to quantify the relative ecological and evolutionary contributions to total community changes observed under any novel environment at a given point in time of the sorting phase

reflected the expected increase in anthropogenically introduced CO_2 by the end of the century (IPCC, 2014). After aeration, the prepared ambient and high CO_2-manipulated media were sterile filtered (0.2 μm pore size) into 0.5 L polycarbonate bottles serving as experimental units. At the onset of the sorting phase, five replicates for each environment (ambient and high CO_2) were inoculated with a defined total biovolume of 5.5×106 μm^3 of the phytoplankton start community. The species *C. affinis* and *E. huxleyi* that were used in this community belong to different functional groups of phytoplankton exhibiting different nutrient utilization strategies (Litchman et al., 2007) and are characterized by significantly diverging cell sizes (mean ± *SD* were 462 ± 192 μm^3 and 22 ± 8 μm^3, respectively). To account for the substantial difference in species' cell size, the experimental units were initiated with equal species' biovolumes (50%) in the start communities, which resulted in an unequal relative cell abundance at the onset of the sorting phase (98% for *E. huxleyi* and 2% for *C. affinis*). The nine genotypes per species were inoculated with equal cell numbers (11% each). The experimental cultivation was conducted in semi-continuous batch cycles, under constant rotation of 0.75 min^{-1} and a maximum light intensity of 350 μmol m^{-2} s^{-1} reached after 3 hr of dusk (17 L: 7 D cycle) in 20°C. *Chaetoceros affinis* reached saturated growth after 5 days and *E. huxleyi* not before 7 days. Communities including both species were thus in stationary phase after 8 days (approximately five generations) at which point an inoculum of each replicated community, including the underlying interspecific and intraspecific changes, was transferred to the next batch cycle. At the end of each batch cycle, 5 ml samples of each replicate were fixed in Lugol's iodine solution, from which abundance and biovolume (Hillebrand et al., 1999) of both species were assessed with an inverted light microscope (Zeiss, Axiovert 200 and Observer A1). Subsequently, an inoculum with a total biovolume of 5.5×10^6 μm^3 was transferred to the next batch.

To test whether community changes in response to CO_2 had occurred, we first assessed total abundance (cells/ml) changes between ambient and high CO_2 environment at the end of the sorting phase (after 80 days) using an ANOVA. The difference in species composition was calculated as the relative contribution (%) of *E. huxleyi* to the total abundance (cells/ml), and the effect of high CO_2 was tested at the end of the sorting phase (after 80 days) by means of ANOVA.

2.1.2 | Eco-Evo assay—(1) Assessment of species composition and separation of species

In the present study, species composition and total biovolume of the sorting phase communities were determined microscopically in all replicates at the end of the 10th semi-continuous batch cycle, which corresponded to 80 days and approximately 50 generations. The physical separation of the two species, necessary for the following diversity manipulations, was conducted using a 20 μm mesh (Hydro-Bios). Precisely, a defined volume of each replicate was pipetted onto the sieve, and the smaller *E. huxleyi* cells were collected in a sterile culture bottle underneath. The sieve was then turned around and the larger *C. affinis* cells were gently washed into another sterile culture bottle, using the same defined volume of artificial seawater. It is important to note that the physical separation of species is not restricted to the here chosen size fractionation, but other methods, such as cell sorting via flow cytometry or picking cells could be used if being more appropriate for other study systems.

2.1.3 | Eco-Evo assay—(2) Reassembly of assay communities

Subsequently, using the same experimental units as in the sorting phase, the separated species were reassembled into the following assay communities: $Control_{ambient}$, $Effect_{novel}$, Eco (ecology), Evo (evolution) and EcoEvo (eco-evolutionary interactions; Figure 1—Eco-Evo assay, step (2). The $Control_{\mathbf{ambient}}$ and $Effect_{novel}$ communities reflected all intraspecific and interspecific diversity changes of the communities sorted in response to the ambient and high CO_2 environment, respectively, and continued to grow in the original CO_2 conditions. The Eco, Evo and EcoEvo communities were reassembled to include, interspecific, intraspecific or both interspecific and intraspecific diversity changes as observed in the high CO_2 environment in the sorting phase. Apart from the specific diversity manipulations, these communities otherwise displayed ambient diversity changes and continued to grow in the ambient CO_2 environment. Comparison of these diversity manipulations to the $Control_{ambient}$ community allowed to capture the respective effect of ecological, evolutionary and eco-evolutionary changes for total community change in response to the high CO_2 environment. For a detailed description for the realization of each treatment combination, see Supporting Information Section 2. It is important to note that interspecific changes could be observed microscopically and were directly manipulated by altering species composition, while intraspecific diversity changes in genotype frequencies remained unknown and were indirectly manipulated by using inoculates from populations that underwent the required intraspecific changes.

Practically, all assay communities were inoculated with a total biovolume of 5.5×10^6 μm^3. To calculate the required transfer volumes of each species, the biovolume per ml of each separated species from a replicate and the aimed mean species compositions of the assay communities had to be considered (Table S1). Using mean species composition ensured that the size of the assay was not inflated, which would have resulted from fully crossing each observed relative species abundance with each intraspecific change. This way, small differences between replicates in terms of species sorting were not propagated to the assay (Supporting Information Section 3). The separated species originating from a replicate in the sorting phase were pipetted into one assay community according to the calculated volume and exposed to the required assay environment (Figure 1—Eco-Evo assay—step 2; Table S1). This was done to keep co-evolved populations of both species together. Assay communities

were then grown for one batch cycle under the same laboratory conditions as in the sorting phase.

2.1.4 | Eco-Evo assay—(3) Measurement and statistical analyses of assay community responses

Total abundance of each assay community was determined via microscopy at the end of one assay batch cycle. Similar to the batch cycles in the sorting phase, in the assay both species of the community reached stationary phase at day 8, and a reliable outcome of the assay could be obtained. The total community change in response to the high CO_2 concentration and the effects of the interspecific and intraspecific diversity manipulations were statistically analysed using a one-factorial ANOVA with five levels comparing the $Effect_{novel}$, Eco, Evo and EcoEvo communities to the $Control_{ambient}$ communities (setting $Control_{ambient}$ as intercept). It is important to test for both the total (i.e. difference between $Control_{ambient}$ and $Effect_{novel}$) and the single effects (i.e. differences between $Control_{ambient}$ and Eco, Evo and EcoEvo, respectively), as effects of diversity shifts on the interspecific and intraspecific level can have opposite effect signs and potentially compensate one another. This may lead to cryptic eco-evolutionary dynamics (Kinnison et al., 2015). In such situations, no total community change would be observed between $Effect_{novel}$ and $Control_{ambient}$, but responses measured in Eco, Evo and/or EcoEvo communities would be significantly different to the $Control_{ambient}$ communities. This information is further needed for the following calculation of the relative contribution of ecological and evolutionary changes, as this calculation is only valid if (a) significant total community changes in response to the novel environment and/or (b) significant effects of the inclusion of interspecific and/or intraspecific shifts (in Eco, Evo and EcoEvo communities) were found.

2.1.5 | Eco-Evo assay—(4) Calculation of relative ecological and evolutionary contribution

The relative contribution of ecological and evolutionary changes for total abundance (cells/ml) of the community, their interaction and unexplained variance was calculated by dividing the respective absolute values of $Effect_{ecology}$, $Effect_{evolution}$, $Effect_{eco\times evo}$ and $Effect_{unexplained}$ (see respective Equations 5–8 below) by their sum (Equations 1–4):

$$\%\,\text{Ecology} = \frac{\left|\text{Effect}_{\text{ecology}}\right|}{\left|\text{Effect}_{\text{ecology}}\right| + \left|\text{Effect}_{\text{evolution}}\right| + \left|\text{Effect}_{\text{eco}\times\text{evo}}\right| + \left|\text{Effect}_{\text{unexplained}}\right|}, \quad (1)$$

$$\%\,\text{Evolution} = \frac{\left|\text{Effect}_{\text{evolution}}\right|}{\left|\text{Effect}_{\text{ecology}}\right| + \left|\text{Effect}_{\text{evolution}}\right| + \left|\text{Effect}_{\text{eco}\times\text{evo}}\right| + \left|\text{Effect}_{\text{unexplained}}\right|}, \quad (2)$$

$$\%\,\text{Eco}\times\text{Evo} = \frac{\left|\text{Effect}_{\text{eco}\times\text{evo}}\right|}{\left|\text{Effect}_{\text{ecology}}\right| + \left|\text{Effect}_{\text{evolution}}\right| + \left|\text{Effect}_{\text{eco}\times\text{evo}}\right| + \left|\text{Effect}_{\text{unexplained}}\right|}, \quad (3)$$

$$\%\,\text{U} = \frac{\left|\text{Effect}_{\text{unexplained}}\right|}{\left|\text{Effect}_{\text{ecology}}\right| + \left|\text{Effect}_{\text{evolution}}\right| + \left|\text{Effect}_{\text{eco}\times\text{evo}}\right| + \left|\text{Effect}_{\text{unexplained}}\right|}, \quad (4)$$

where $Effect_{ecology}$ and $Effect_{evolution}$ captured the absolute contributions of ecological and evolutionary changes to total community change and were calculated by subtracting the response of the $Control_{ambient}$ community from the response of Eco and Evo communities, respectively (Equations 5–6):

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

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

The $Effect_{eco\times evo}$ captured interactions between ecology and evolution and was calculated as the difference between the measured combined effect of both processes and the sum of the single ecological and evolutionary effects (Equation 7):

$$\begin{aligned}\text{Effect}_{\text{eco}\times\text{evo}} &= \left(\text{EcoEvo} - \text{Control}_{\text{ambient}}\right) \\ &- \left[\left(\text{Eco} - \text{Control}_{\text{ambient}}\right) + \left(\text{Evo} - \text{Control}_{\text{ambient}}\right)\right],\end{aligned} \quad (7)$$

and the *unexplained* variance (Equation 8), that encompassed divergence from additivity of ecological and evolutionary changes and their interaction from the total community change between $Effect_{novel}$ and $Control_{ambient}$ communities:

$$\begin{aligned}\text{Effect}_{\text{unexplained}} &= \left(\text{Effect}_{\text{novel}} - \text{Control}_{\text{ambient}}\right) \\ &- \left(\text{Effect}_{\text{ecology}} + \text{Effect}_{\text{evolution}} + \text{Effect}_{\text{eco}\times\text{evo}}\right).\end{aligned} \quad (8)$$

To display relative eco-evolutionary changes visually into perspective of the total effect sizes, we plotted the relative contributions underneath the mean differences between the community responses under the ambient ($Control_{ambient}$) and high ($Effect_{novel}$) CO_2 environments (Borenstein et al., 2009).

2.2 | Applying established partitioning metrics allowing the comparison to the Eco-Evo assay

We compared the contributions of the ecological and evolutionary changes found in the Eco-Evo assay to those estimated by two existing eco-evolutionary partitioning metrics based on reaction norms. Specifically, we applied (a) an extended version of the Geber method (Ellner et al., 2011) and (b) an extended version of the reaction norm approach (Govaert et al., 2016). These are the only metrics available that can be applied to community property shifts and that do not require genotype frequencies over time (Table 1). In both, the Geber method and the reaction norm approach, the ecological and evolutionary contributions to total community change are obtained from a regression model with two state variables that represent ecological and evolutionary states (Ellner et al., 2011; Govaert, 2018). However, the ecological state in the original description of both metrics reflects

the abiotic environment (and thus may include plastic responses). To allow a comparison to the Eco-Evo assay, we extended both metrics by adding a third indicator variable representing the species composition. Regarding data requirements, both metrics need input from a full-reciprocal manipulation of interspecific and intraspecific changes and the CO_2 environment. Hence, to apply the Geber method and reaction norm approach, we used abundance data of the Eco-Evo assay communities and three additional communities to meet the required fully reciprocal design. These additional communities were run in parallel to the main experiment under the same conditions. Here, species compositional changes and/or intraspecific changes in the novel community were manipulated to reflect how the respective components changed in response to the ambient environment representing the reciprocal manipulation to the Eco-Evo assay (Table S2). Overall, it is important to note that (a) not all existing metrics (Table 1) could be applied and (b) that the application of these metrics required more extensive measurements than the introduced assay.

All data analyses were done in R (R Development Core Team, 2016). All plots were made using the package GGPLOT2 (Wickham, 2009). Statistical models included a priori visual inspection of normality and homogeneity of variances.

3 | RESULTS

3.1 | Sorting phase

After 80 days, high-CO_2 seawater conditions (the novel environment) reduced total phytoplankton abundance by fourfold when compared to ambient CO_2 conditions (Figure 2; $F_{1,8} = 167.07$, $p < 0.001$). The contribution of *E. huxleyi* to total phytoplankton abundance after 80 days was with 62% significantly lower under high compared to 89% under ambient CO_2 concentrations (Figure 2; $F_{1,8} = 26.04$, $p < 0.001$).

3.2 | Eco-Evo assay

The Eco-Evo assay partitioned the total change in community abundance into its underlying ecological and evolutionary contributions. The manipulation of interspecific composition (Eco and EcoEvo communities) resulted in a threefold to fourfold total abundance decrease compared to the $Control_{ambient}$ communities (Figure 3a; $Control_{ambient}$ vs. Eco and EcoEvo, Eco: $F_{4,20} = 25.38$, $p < 0.001$, EcoEvo: $F_{4,20} = 25.38$, $p < 0.001$, respectively). In contrast, the manipulation of the intraspecific changes (Evo communities) did not affect total abundance compared to the $Control_{ambient}$ (Figure 3a; $Control_{ambient}$ vs. Evo; Evo: $F_{4,20} = 25.38$, $p = 0.92$). Consequently, the ecological change contributed 79.6% to the observed total phytoplankton abundance decline in response to high CO_2, while evolutionary change resulted in a negligible relative contribution of 1.3% (Figure 3b). The interaction between ecology and evolution explained 7.4%, while 11.7% of the total change in community abundance remained unexplained.

3.3 | Eco-evolutionary contributions calculated by existing partitioning metrics

The change in community abundance in response to high CO_2 was predominantly explained by ecological shifts when applying the reaction norm approach and the Geber method on data collected in our system (Figure 4). Specifically, ecology contributed 59% and 44% to the change in community abundance, while evolution had a negligible contribution of 2% and 0.7%, in the reaction norm approach and the Geber method, respectively.

4 | DISCUSSION

Here we show that an observed phytoplankton abundance decline in response to elevated CO_2 concentration could be predominantly explained by ecological changes and only to a low extent by contemporary intraspecific shifts due to evolution. Decomposition of the total response into the underlying ecological and evolutionary contributions was possible by means of a newly developed experimental design, the Eco-Evo assay. We validate this new experimental design by showing that the resulting relative ecological and evolutionary contributions to the total abundance decline were comparable to those calculated by using existing partitioning metrics such as the reaction norm approach and the Geber method.

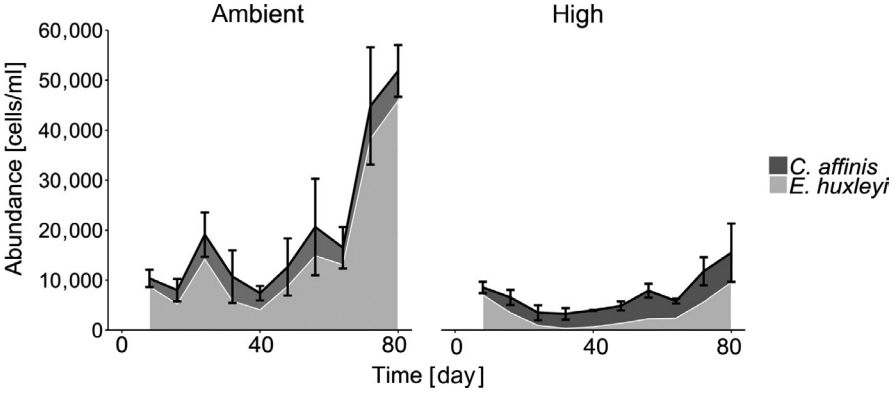

FIGURE 2 Total abundance (cells/ml) of the community and underlying species composition of *Chaetoceros affinis* and *Emiliania huxleyi* under ambient and under high (novel) CO_2 concentrations during the sorting phase of 80 days, corresponding to about 50 generations. Mean values and 95% CI of $n = 5$ replicates are shown

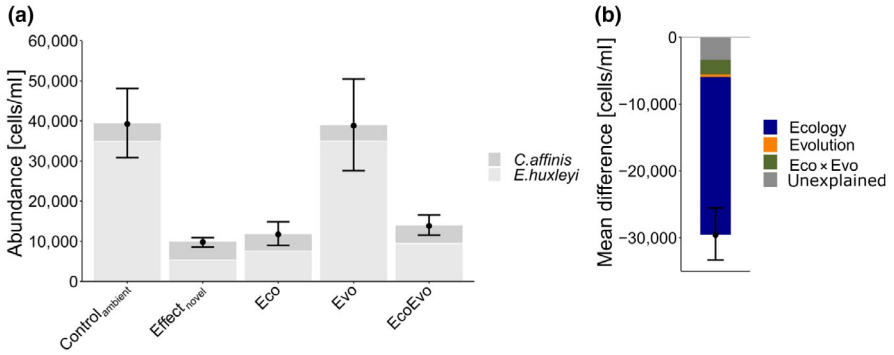

FIGURE 3 (a) Total cell abundance and underlying relative contribution of *Chaetoceros affinis* and *Emiliania huxleyi* in the different Eco-Evo assay communities reassembled from communities sorted for 80 days (approximately 50 generations) under ambient and under high (novel) CO_2. $Control_{ambient}$ and $Effect_{novel}$ reflected compositional changes of communities sorted in ambient and high CO_2 for 80 days and remained in their original environmental conditions, respectively. The Eco, Evo and EcoEvo communities encompassed species compositional changes, intraspecific changes and both species compositional and intraspecific changes in response to high CO_2, respectively, while grown under ambient CO_2. Mean values and standard deviations of $n = 5$ replicates are shown. (b) Mean difference of total cell abundance and its standard error in communities sorted for 80 days between ambient and high CO_2. Underlying bar chart colours show the relative importance of ecology and evolution and their interaction (Eco × Evo) to the total community changes in response to high CO_2, and the unexplained variance

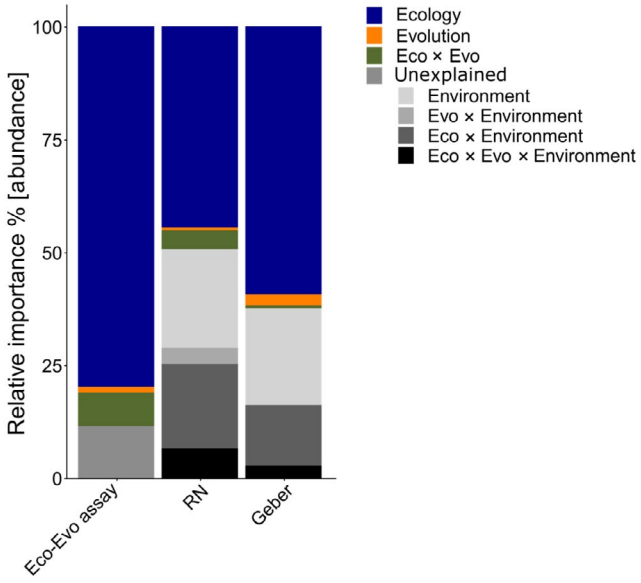

FIGURE 4 Comparison of relative ecological and evolutionary contributions to total change in abundance calculated by the here introduced Eco-Evo assay, the Geber method (Geber; Ellner et al., 2011) and the reaction norm approach (RN; Govaert, 2018; Govaert et al., 2016)

To date, a spectrum of altered phytoplankton functional community responses is described in response to increasing seawater CO_2 concentrations and can be attributed to underlying species diversity shifts to increasing seawater CO_2 concentrations (Eggers et al., 2014; Paul et al., 2016; Schulz et al., 2017; Sommer et al., 2015). In agreement with the observation that calcifying haptophytes often decline under increased seawater CO_2 (Eggers et al., 2014; Riebesell et al., 2017; Schulz et al., 2017), we here observed a one-third lower contribution of *E. huxleyi* in the high CO_2 environment after 80 days. At the same time, it has been reported that diatoms and small picoplankton can profit from a higher supply of inorganic carbon (Bach et al., 2017; Kroeker et al., 2013). Such enhanced growth of large diatoms under elevated CO_2 concentration has the potential to result in an overall increase of total phytoplankton biomass (Eggers et al., 2014). We could, however, not observe an enhanced growth by the diatom *C. affinis* in response to increasing CO_2 concentration (Hattich et al., 2017) that could balance the reduction of *E. huxleyi* cells. Consequently, in our model community, the total abundance declined in response to elevated CO_2. Possible reasons for *C. affinis* not profiting from the *E. huxleyi* decline are that the diatom *C. affinis* with a low nutrient affinity (Litchman et al., 2007) could not take up the emerging surplus resources from the reduced *E. huxleyi* abundance and translate them into enhanced growth, and/or that *C affinis* might have run into co-limitation of phosphate by silicate (Figure S4). Furthermore, *C. affinis*' large size compared to *E. huxleyi* likely resulted in a relatively lower cell abundance on the same amount of resources (Figure S5). The lower initial population size of *C. affinis* could have potentially constrained intraspecific responses due to the early exclusion of rare genotype. This, however, was not the case, reflected in the presence of more *C. affinis* genotypes compared to *E. huxleyi* after eight batch cycles (Listmann et al., 2020). The strong total abundance decline in our model community could additionally be favoured by the low number of species included which did not allow for response diversity and hence functional redundancy (Elmqvist et al., 2003). Functional redundancy is discussed, for instance, to buffer the negative effect of increased CO_2 concentration on arctic phytoplankton communities (Hoppe et al., 2017). Besides the direct negative CO_2 effect on total abundance, the reduction of *E. huxleyi* could potentially lead to indirect effects on higher trophic levels. An increase of mean size in communities under high CO_2 driven by the reduction of the smaller *E. huxleyi* cells could, for example, increase the size of associated grazers (Boyce et al., 2015) and thus the size of

secondary consumers. Changes in phytoplankton size were shown to alter food web length in mesocosm experiments (Stibor et al., 2004) and affect transfer efficiency (Barnes et al., 2010).

Considering the above discussed strong interspecific diversity changes, it is not surprising that ecological changes were identified as the major contribution to the observed total abundance decline under high CO_2 concentration. Low contributions of evolutionary changes in this experiment, however, were not an artefact caused by a too short sorting phase not allowing for evolutionary changes. Genotype compositional shifts indeed occurred on a comparable time scale as species sorting (compare shifts over time Figure 2; Figure S3); however, the selection on genotypes over time was contrary to selection on species not predominantly driven by CO_2 (Supporting Information Section 4, Figure S3). Genotype sorting in this model system was suggested to result from the general experimental conditions characterized by recurring nutrient pulses which strongly affected intraspecific competitive interactions (Listmann et al., 2020). In fact modulating or overriding impacts on CO_2 effects at the community level by, for example, initial community composition (Eggers et al., 2014), temperature (Paul et al., 2016) or nutrient availability (Alvarez-Fernandez et al., 2018) were described elsewhere. As the assay quantifies the contributions of ecology and evolution to the total change exclusively driven by the manipulated CO_2 concentration, changes over time driven by other factors were not captured. This explains the observed low evolutionary contribution to total phytoplankton abundance decline, despite substantial genotype sorting over time. The herein observed low evolutionary contribution does not mean that intraspecific changes are generally of low ecological significance. Pronounced evolutionary contributions are, for example, described in soil bacteria (terHorst et al., 2014), in *Daphnia* communities over a salinity gradient (Govaert et al., 2016) and in semi-natural meadows subjected to mowing and fertilization (Lepš et al., 2011). The latter study showed that the contribution of intraspecific diversity to trait changes differs markedly depending on the considered trait and the environmental factor (Lepš et al., 2011).

Applied to our experimental system, the established partitioning metrics uniformly showed that ecological contributions dominated the observed total abundance changes, which largely validated the here for the first time implemented experimental design of the Eco-Evo assay. However, the ecological contribution to the total change calculated by the Eco-Evo assay was up to 35% higher than estimated by the established metrics. Diverging eco-evolutionary partitioning results by different metrics using the same data are not unusual (van Benthem et al., 2016; Govaert et al., 2016) and can partly result of distinct underlying definitions of the included components (van Benthem et al., 2016). One example is that the Geber method does in contrast to other approaches not account for the directionality of changes from an ancestral to an affected community, but instead defines and calculates relative ecological and evolutionary contributions to a mean change using both ambient and novel as ancestral environment. Another potential source of variation is the inclusion of different numbers of components and interaction terms. The Geber method and RN approach include three additional interaction terms compared to the Eco-Evo assay which likely explains the observed divergence of eco-evolutionary contributions. The number of interaction terms does in contrast not affect the calculated absolute effect of ecological and evolutionary changes and their interaction on the abundance decline, which is reflected in identical absolute effects for the reaction norm approach and the Eco-Evo assay (Figure S6). Altogether, these comparisons underpin that the ecological contribution predominantly drove the observed abundance decline in response to enhanced CO_2 across all applied partitioning metrics. Extrapolating the findings of this single assessment should, however, be done with care, since relative ecological and evolutionary contributions to total community change were in other studies shown to differ over time (Becks et al., 2012; Govaert et al., 2016). In the presented study, evolutionary contributions might increase with time, considering that *E. huxleyi*, which predominantly drove total community responses, has the potential to adapt to increasing CO_2 concentrations after hundreds of generations (Lohbeck et al., 2012). In taking advantage of the effect of timing, we strongly recommend that future studies should assess ecological and evolutionary contributions at different points in time, elucidating when these processes are most important.

We demonstrated that the Eco-Evo assay eases the quantitative assessment of eco-evolutionary changes on the community level, providing a more integrative and comprehensive understanding of natural systems compared to single-species assessments (De Meester et al., 2019; Govaert et al., 2016). Major advantages are that this direct assessment on the community level (a) allows to quantify ecological and evolutionary contributions to community property changes (Table 1) and (b) is possible without prior identification of genotype traits and compositional shifts. Community properties, such as total abundance, particulate nutrient concentrations or resource use efficiency, relate to community functioning and are the results of aggregate trait changes within a community. Skipping genotyping clearly represents an advantage in communities where trait values and genotype frequency shifts cannot be obtained in situ and widens the potential pool of plankton communities subject to future investigation. These can comprise more (Figure S7) and other functional groups, for example, diazotrophs, mixotrophs, heterotrophs, other calcifiers and silicifiers, but also higher trophic-level consumers as long as the assemblage members can be physically separated and reassembled into assay communities. An application of the Eco-Evo assay to other communities than plankton also displaying evolution on a contemporary timescale is possible, for example, to bacteria and annual plants. While applying the Eco-Evo assay to bacterial communities, in which strains can be easily separated by plating them on agar, could be beneficial, the application to annual plant communities is likely more labour-intensive than a VP approach. The number of potentially applicable communities reduces when extending the Eco-Evo assay to separate plasticity from the evolutionary component (Table S3). This extension requires (a) direct assessment and (b) separation and artificial reassembly of genotypes. However, such an assessment would more holistically account for the potential interaction of plasticity and diversity changes than, for example, metrics

based on the Price equation (e.g. Govaert et al., 2016), relying on the assumption that plastic responses of organisms in isolation are the same as they would occur in a community context. Depending on the study system of interest, any relevant environmental driver can be applied (Supporting Information Section 1 and Figure S1), including multiple environmental drivers. However, the assessment of main responses to each of the included driver is highly labour-intensive as it increases the number of communities under selection and inflates the number of assay units. Alternatively, one could use communities naturally exposed to environmental gradients or changing environments within one habitat (e.g. applying dormant stages; Härnström et al., 2011). Such application would also help to understand whether the relative importance of ecological and evolutionary changes observed in simplified laboratory communities can be extrapolated to natural communities.

Our study contributes to advance the understanding of the relative importance and thus functional relevance of rapid evolution in ecological communities. While we provide a first estimate of eco-evolutionary contributions to total phytoplankton community shifts, further quantifications of a suit of community mean trait and property changes in response to a variety of environmental drivers at different time points are required to improve our general understanding of observed phytoplankton changes. The here shown community property changes driven by diversity changes among and within species will help to better predict future phytoplankton change and in consequence its propagating effects on key ecosystem functions. Using interspecific and intraspecific diversity manipulations to assess contemporary occurring eco-evolutionary contributions to total community changes moreover brings a central idea of the field of biodiversity ecosystem functioning into the field of eco-evolution. So far, the integration of eco-evolutionary aspects to understand community changes rather than shifts in populations remains scarce. Thus, a more integrative view of ecological and evolutionary concepts to explain contemporary occurring intraspecific and interspecific diversity effects would likely be beneficial to understand the functional consequences of future community change.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest.

AUTHORS' CONTRIBUTIONS

Eco-Evo assay was designed by B.M. and G.S.I.H., the following Study testing the assay was designed by G.S.I.H., L.L., B.M.; Laboratory work carried out by G.S.I.H., L.L., C.P. and data analysis by G.S.I.H.; L.G. extended and applied the Reaction norm approach and Geber method; G.S.I.H. drafted the manuscript and all other authors revising the manuscript and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Information of the 10 semi-continuous Sorting Phase batch cycles can be found in https://doi.org/10.1594/PANGAEA.887780. Raw data from the subsequent Eco-Evo assay and the calculated relative importance of interspecific and intraspecific diversity changes are archived in https://doi.org/10.1594/PANGAEA.896220.

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