

P1.01 Eco- evolutionary coupling in competing marine phytoplankton communities

P2.01 Nutrient uptake-related trait variability and trade-offs – adaptive evolution and community functioning in competing phytoplankton species

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In the first phase (P1.01) of our project, we have successfully established a model system that consists of two stably coexisting marine phytoplankton species, the diatom *Chaetoceros affinis* and the coccolithophore *Emiliania huxleyi*. The two species largely varied in their responses to increasing seawater CO₂ concentration, i.e. *E. huxleyi* suffered and *C. affinis* gained in response to CO₂. To allow for intraspecific selection in the long-term experiments each of the two species was introduced with nine genotypes that also varied in their response strengths to CO₂ (shown in Hattich *et al.* (2017)).

Using this model system, we were able to achieve three major milestones in understanding the consequences and relative importance of inter- and intraspecific community changes and adaptation to shifting community properties, mean trait values and dynamics.

First, we were able to establish a new experimental method (the eco-evo assay) to disentangle and quantify the relative importance of inter- and intraspecific changes to community mean trait and property changes (Hattich *et al.* 2022). In this article, we moreover compared and validated the outcome of the new assay with existing, however, much more conceptual and data-demanding approaches. By this, we offer a valid and feasible method to the scientific community to quantify the different types of changes that take place on different levels of biological organisation for total community changes. By applying this decomposition method over a longer-term selection, we showed that community property and mean trait changes over 100 generations (160 d in Fig 1A and B) were largely driven by species compositional shifts, while genotype sorting gained in importance with time (Fig. 1C and D) (Hattich *et al.* 2023).

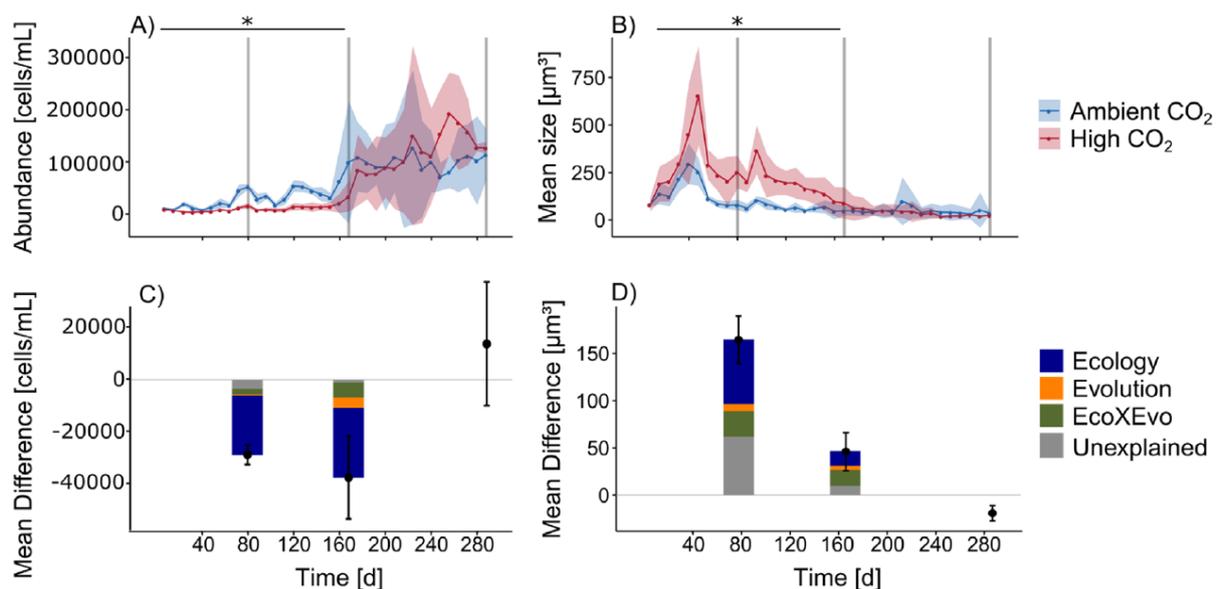


Figure 1:

Fig. 1. Upper panel: community total cell abundance (A) and mean size (B) changes in response to ambient (400 ppm) and high (1250 ppm) CO₂ conditions over 288 d (corresponding to approximately 180 generations) (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 CO₂ treatments (indicated by upper black line with asterisks) and vanished in the longer-term (>168 d). Lower panel: outcomes of the eco-evo assays that were applied to communities at different time points during

CO₂ selection (depicted by the gray lines in the upper figures) to quantify the inter- (Ecology) and intraspecific (Evolution) contributions to observed total changes in response to CO₂ condition. Bars show calculated effect sizes and standard errors of CO₂ treatments to total cell abundance (C) and mean size (D) responses. The colors of bar charts show the fraction of the total change that is explained by ecological (i.e. interspecific) and evolutionary (i.e. intraspecific) responses, respectively.

Second, we demonstrated that altered genotype sorting over the long term (~180 generations = 288 d in Fig. 2) can significantly alter community composition, properties and mean trait values (Listmann *et al.* 2020; Hattich *et al.* 2023). In more detail we showed that up to approximately 100 generations CO₂ significantly affected species compositional shifts as expected, i.e. the diatom significantly gained and the coccolithophore suffered in response CO₂. We found that contrary to species sorting, genotype sorting turned out not being driven by CO₂ but likely by the experimental conditions, leading to genotype exclusion in the same manner in both CO₂ treatments and thus to the same genotype dominating all treatments after 100 generations of selection. Once one *E. huxleyi* genotype monodominated in all treatments the community dynamics abruptly changed. The composition flipped from the previously dominating diatom to dominance of *E. huxleyi* (Listmann *et al.* 2020) (Fig. 2). At the same time total abundance increased by 10fold and mean cell size declined to 20% compared to the onset of the experiment (Hattich *et al.* 2023) (Fig. 1A and B). The experimental environment was characterized by similar recurrently pulsed nutrient conditions which led us to investigate the variability of nutrient upake-related traits in our model system in the second phase of the priority programme (see below).

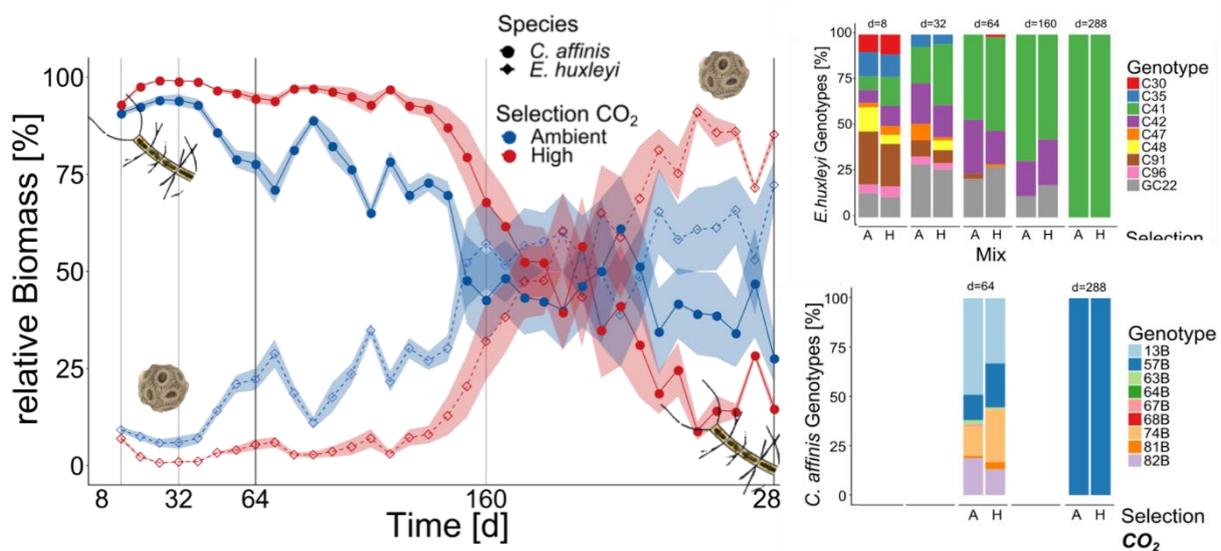


Figure 2: Species and genotype sorting in response to two different CO₂ treatments over 288 days of selection (after Listmann *et al.* 2020). Lower row: Total community change in abundance and mean size in response to CO₂ (after Hattich *et al.* 2023).

Third, in a community context we demonstrated not only the occurrence of rapid evolution in the form of genotype sorting but also evolutionary adaptation (Listmann *et al.* 2020), although less pronounced as initially hypothesized. Reciprocal assay experiments demonstrated weak and complex adaptation effects only in the diatom, never the coccolithophore species. In *C. affinis*, we found different responses to CO₂ depending on the presence and absence of the other species (i.e. with and without competition) that even resulted in a weak adaptive effect to increased CO₂ in the second assay after 288 days, evident as a significant interaction of selection and assay treatment. This response was mainly driven by low growth rates of *C.*

affinis selected in mix-cultures to both high CO₂, as well as low growth rates of *C. affinis* selected under ambient CO₂ in mono-cultures. This complex result demonstrates how effects of both abiotic and biotic factors interact and need to be jointly considered to avoid overestimation of adaptive responses to abiotic factors alone.

In the second phase of our project (P2.01) we investigated, if nutrient uptake-related trait variability and trade-offs can explain community property and mean trait changes in competing phytoplankton species subjected to different nutrient regimes.

We demonstrated that the two species in our model system largely varied in cell size, and the maximum uptake rate for nitrate ($V_{\max N}$), with *C. affinis* being larger and showing higher $V_{\max N}$. While the nine *C. affinis* genotypes differed significantly in size and $V_{\max N}$, the *E. huxleyi* genotypes did not vary in a significant way regarding these traits (Hamer *et al.* 2022; Matthiessen *et al.* under revision). Measurements of affinity failed due to an experimental failure. With this information in mind we started a long-term community experiment in which nutrient regimes were manipulated with initially 10, 20, and 30 $\mu\text{mol/L}$ nitrate and one $\mu\text{mol/L}$ phosphate (10:1, 20:1, 30:1) and which ran over 130 phytoplankton generations.

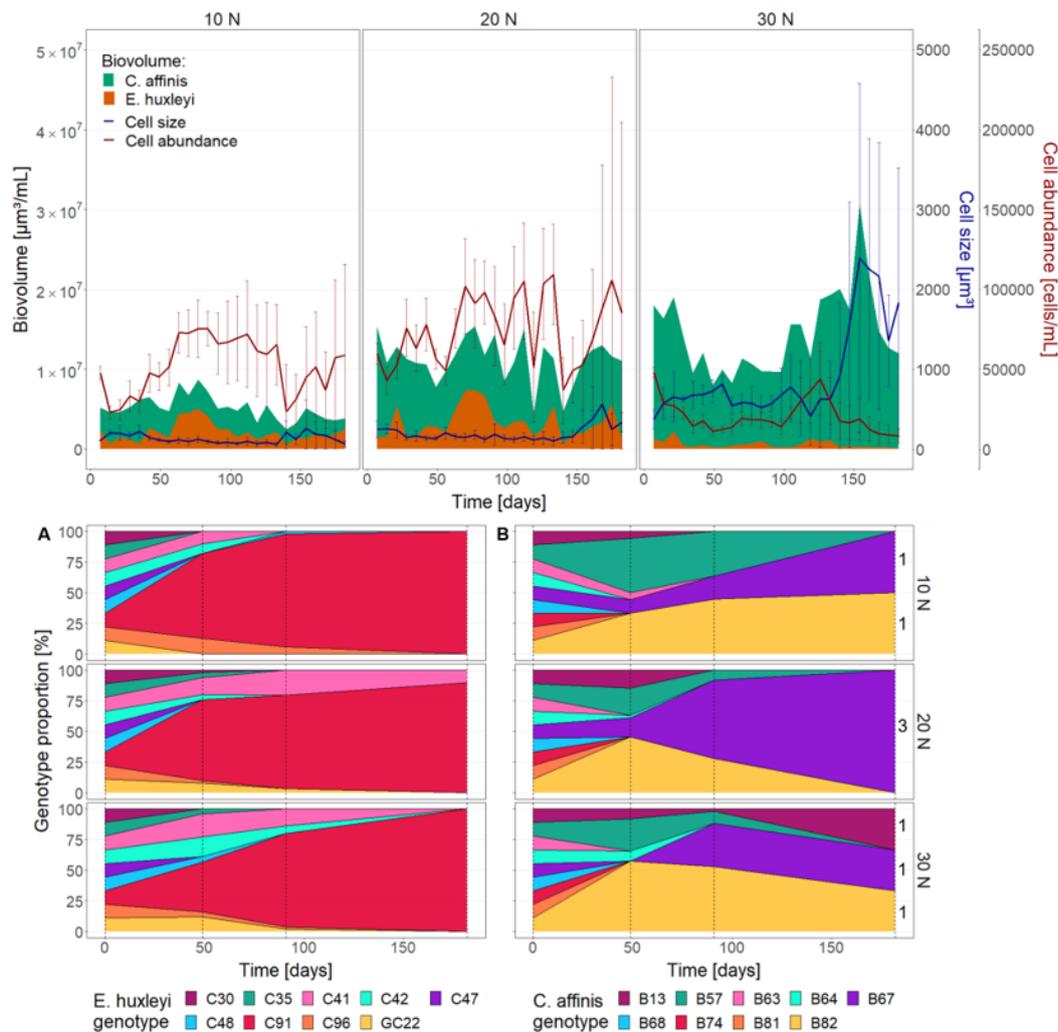


Figure 3: Upper graphs: Total biomass, mean abundance and cell size, and species composition in response to nutrient regimes over 180 d which approximately corresponds to 130 phytoplankton generations. Lower graphs: Genotype sorting of the two species in response to nutrient regime.

In this experiment, species composition was significantly affected by nutrient regime. At low nitrate concentration the two species occurred evenly, while *C. affinis* with their higher $V_{\max N}$ and larger cells gained in dominance with nutrients. As such the species compositional shifts were predictable and to a large part responsible for mean size and total biomass increase in response to increased nutrients (Fig. 3 upper graphs). On top after more than 80 generations (i.e. 130 d in Fig. 3), we found an increase in mean cell size and total biomass and their respective variability only in the replete conditions (Fig. 3 upper graphs). Genotype sorting, in particular for the highly variable genotypes of *C. affinis*, contrary to expectations could not be predicted based on genotype's cell size and other prior trait measurements in the different nutrient regimes. In more detail only one *E. huxleyi* genotype dominated in all nutrient regimes, and three different *C. affinis* genotypes remained after 130 generations (Fig. 3 lower graphs). The latter, however, did not co-occur but surprisingly dominated different replicates of the same nutrient treatment (Matthiessen *et al.* under revision).

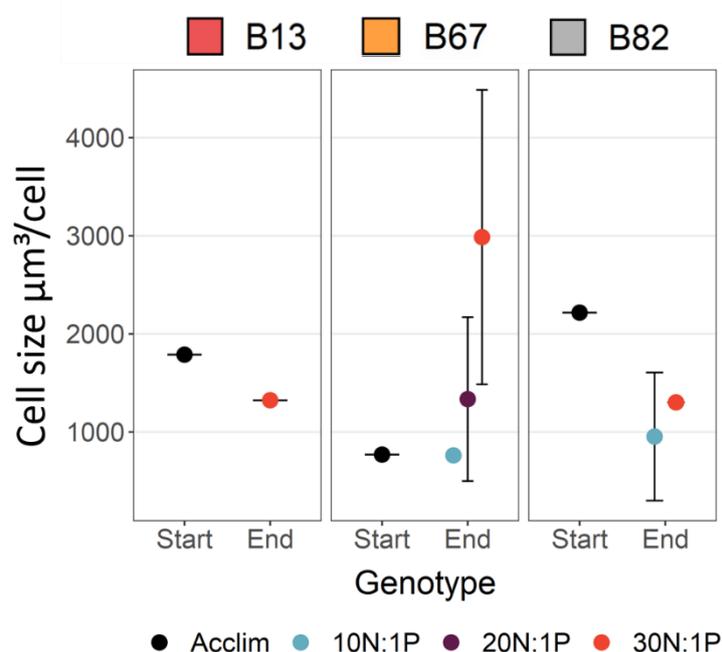


Figure 4: Size plasticity of remaining *C. affinis* genotypes over the nutrient treatments at the end of the long-term experiment compared to mean size at the onset.

We were able to demonstrate, however, that the increase in the mean and variability of cell size and total biomass after more than 80 generations in the treatments with the highest nitrate concentrations were driven by extraordinarily high size plasticity of the dominant remaining *C. affinis* genotype (genotype B67; Fig. 4). In fact, the plastic size range of *C. affinis* found at the end of the long-term experiment was on average twice as high than the size variability among genotypes prior to the onset (Matthiessen *et al.* under revision).

A short-term experimental test for size plasticity of the *C. affinis* genotypes in fact proofed no significant standing genotype variability in size plasticity in the first place and instead suggested

that evolution for plasticity took place in the long-term community experiment. This evolved size plasticity in turned translated to mean size and biomass increase and points to the importance of potential interactions between evolution and plastic changes to explaining community mean trait and property shifts in response to environmental changes (Matthiessen *et al.* under revision).

Collectively, our results of both project phases underpin that trait changes on all levels on which simultaneous shifts can occur potentially contribute to total community change and therefore should be considered when predicting community changes.

Based on the described long-term community experiment, we were further able to demonstrate that the maintenance of intraspecific (i.e. genotype) diversity is context-dependent, i.e. that higher intraspecific diversity can be maintained in the presence of a competitor, provided it has a substantial proportion to total biovolume. Both nutrient fluctuation and regime showed

species-specific effects and especially structured genotype sorting of *C. affinis* (Hamer *et al.* 2022) (Fig. 5).

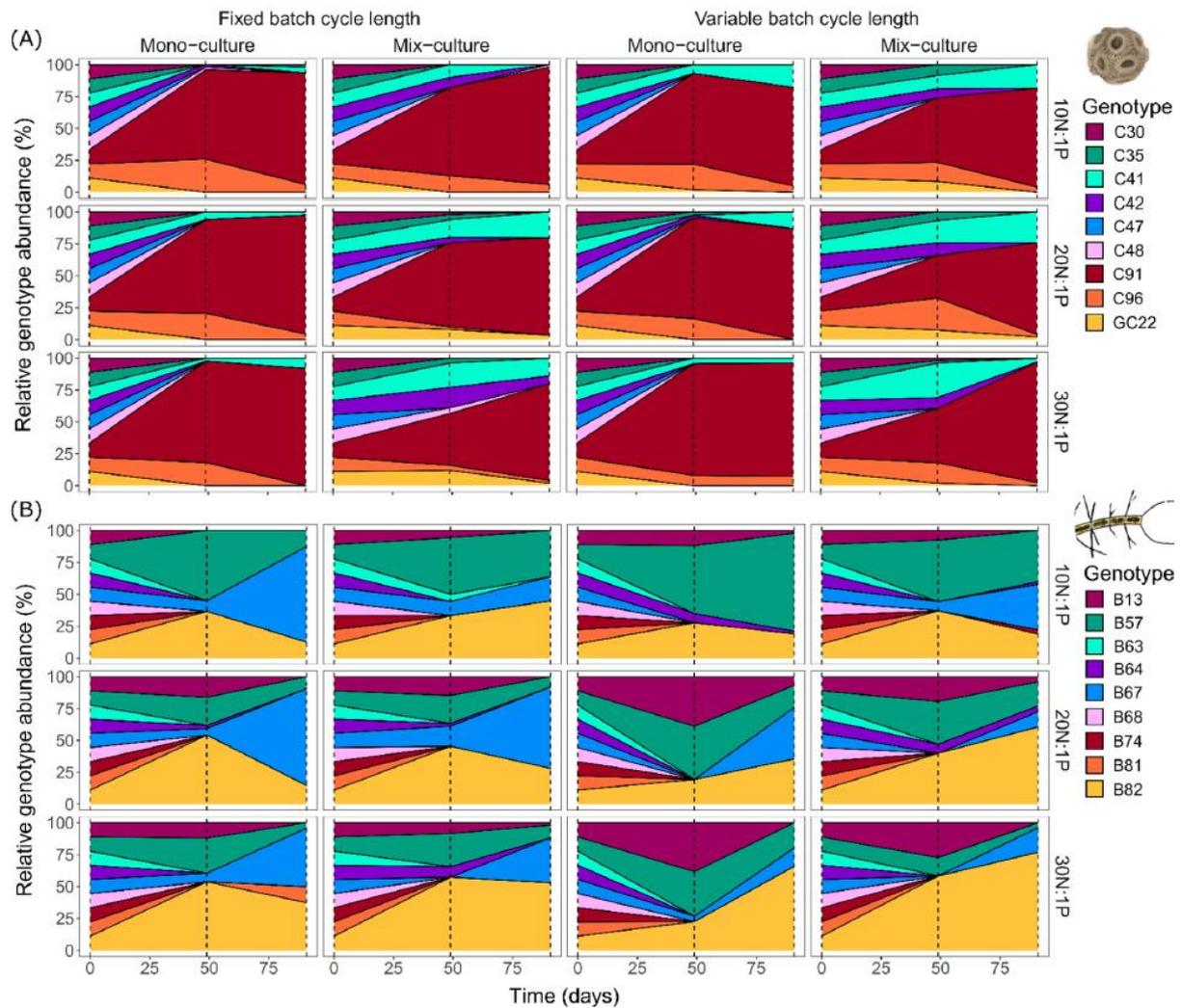


Figure 5: Mean relative genotype composition of *E. huxleyi* (A) and *C. affinis* (B) genotypes over time in mono-culture and mix-culture, at three nutrient regimes (10N:1P, 20N:1P, and 30N:1P) with fixed and variable batch cycle length.

References

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