Eco-evolutionary importance in competing marine phytoplankton communities

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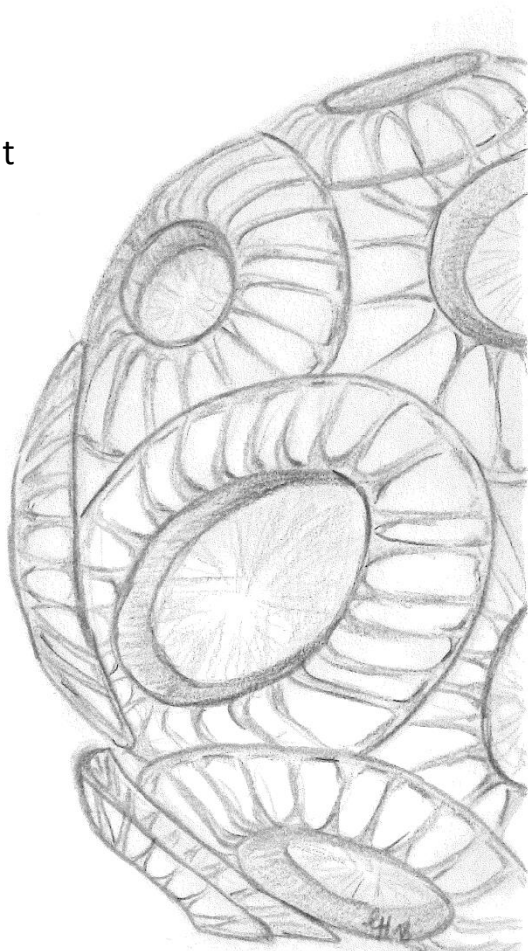

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Für meine Eltern

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Summary

Ecosystems have always been exposed to environmental changes. During the past centuries, however, human activities have accelerated these processes vastly. The awareness that ecological and evolutionary changes happen on a similar timescale, and that these processes interact, and consequently jointly determine community structure, only arose over the past decades. This might be especially important for organisms such as phytoplankton, which have short generation times and vast population sizes, characteristics that favour rapid evolutionary changes. Phytoplankton communities form massive blooms and provide the basis of aquatic food webs. Thus, understanding and predicting future phytoplankton community structures is essential in the light of rapid climate changes. Empirical studies have demonstrated species frequency shifts and adaptive evolution in response to novel environments. To date, however, most studies have treated ecological and evolutionary changes in isolation, and their relative contributions to the overall community change were not quantified. In this thesis, I aim to close this knowledge gap by simultaneously studying ecological and evolutionary processes of a marine phytoplankton community under a climate change driver, to ultimately uncover their relative importance for future community changes.

The model community I used for this eco-evolutionary study consisted of several genotypes of the coccolithophore *Emiliania huxleyi* and the diatom *Chaetoceros affinis*, belonging to different functional groups of phytoplankton. These two functional groups can together be responsible for up to 80 % of the primary production of all marine phytoplankton worldwide. Enhanced CO_2 concentration provided a valuable environmental driver, since it occurs worldwide, and both species used in the community experiment, are described to be adversely affected by this driver, thus likely pronouncing ecological changes in this particular community. Additionally, evolutionary changes were expected, as the potential of *E. huxleyi* to adapt to enhanced CO_2 conditions was previously demonstrated.

In order to estimate the model community's standing genetic diversity, exhibiting the starting point for genotype selection and the basis for rapid evolutionary changes, I assessed the plastic responses of populations of *E. huxleyi*, *C. affinis* and additionally of the coccolithophore *Gephyrocapsa oceanica* in response to an enhanced CO_2 environment (Chapter I). Surprisingly, all three species mostly buffered the effect of enhanced CO_2 concentration, if looked at the mean response over all genotypes of a species. However, the responses of single genotypes to elevated CO_2 ranged between neutral and negative impacts, especially among the two coccolithophores. In contrast to many previous investigations testing only some or single genotypes (laboratory strain) of a species, this study highlights that population responses can be assessed reliably by a

mix of different genotypes, ideally from fresh isolated retrieved from the field. This approach significantly reduces the effort to obtain reliable mean plastic responses of populations in future studies.

In the second part of this thesis, I introduce a new experimental assay (Eco-Evo assay), by which - for the first time - a total community change can be partitioned into its ecological and evolutionary contributions (Chapter II). This assay overcomes the limitations of existing eco-evolutionary partitioning metrics. The assay was first applied to the model communities that have been exposed to ambient and enhanced CO_2 concentrations for 50 generations, verifying its functionality. This Eco-Evo assay was then used to repeatedly assess the relative importance of ecological and evolutionary changes for a total community mean size and abundance change in response to CO_2 concentration after short-, mid- and longer-term (50, 105 and 180 generations, respectively; Chapter III). The study could highlight that the short-term total community responses to enhanced CO_2 concentrations were dominated by ecological changes, while evolutionary changes gained in importance in the mid-term. Longer-term responses were unexpectedly not different between the ambient and high CO_2 treatments, and no underlying ecological or evolutionary changes were observed. I argue that this longer-term response likely resulted from a feedback from genotype selection in response to nutrient limitation under both environments on ecological changes, irrespective of CO_2.

Overall, this thesis demonstrated that both ecological and evolutionary changes can contribute to total phytoplankton community mean trait and property changes. This, however, strongly depended on the time scale considered. A widespread use of the Eco-Evo assay could allow identifying universal eco-evolutionary mechanisms in phytoplankton communities, significantly enhancing our current understanding of phytoplankton community changes as well as future predictions retrieved from these.

Zusammenfassung

Schon immer waren Ökosysteme Umweltveränderungen ausgesetzt. In den vergangenen Jahrhunderten haben menschliche Aktivitäten diese Prozesse jedoch erheblich beschleunigt. Das Bewusstsein, dass ökologische und evolutive Veränderungen in einer ähnlichen Zeitspanne stattfinden, und dass diese Prozesse interagieren und somit gemeinsam die Struktur von Artengemeinschaften bestimmen, entstand jedoch erst in den letzten Jahrzehnten. Dies kann für Organismen wie Phytoplankton besonders wichtig sein, da kurze Generationszeiten und enorme Populationsgrößen evolutive Veränderungen begünstigen. Phytoplankton kann massive Blüten hervorrufen und bildet die Basis aquatischer Nahrungsnetze. Im Rahmen des Klimawandels, ist das Verständnis und die Fähigkeit Vorhersagen über zukünftige Zusammensetzungen von Phytoplankton Gemeinschaften treffen zu können, unerlässlich. Empirische Studien haben gezeigt, dass Klimawandel sowohl zu Veränderungen in der Artzusammensetzung von Gemeinschaften, als auch zu adaptiver Evolution von einzelnen Arten führen kann. Bislang jedoch haben die meisten Studien ökologische und evolutionäre Veränderungen isoliert betrachtet und ihre relative Bedeutung für Veränderungen der Gemeinschaft und derer Funktionen wurden nicht quantifiziert. In dieser Arbeit möchte ich diese Wissenslücke schließen, indem ich gleichzeitig die ökologischen und evolutionären Prozesse in einer marinen Phytoplankton Gemeinschaft unter dem Einfluss des Klimawandels untersuche, um schließlich deren relative Wichtigkeit für zukünftige Veränderungen zu bestimmen.

Die Artengemeinschaft, die ich für diese experimentellen Studien verwendet habe, bestand aus mehreren Genotypen der Coccolithophoride *Emiliania huxleyi* und der Diatomee *Chaetoceros affinis*, welche verschiedenen funktionellen Gruppen des Phytoplanktons angehören. Diese beiden funktionellen Gruppen können zusammen für bis zu 80 % der Primärproduktion des marinen Phytoplanktons weltweit verantwortlich sein. Eine erhöhte CO_2-Konzentration im Meerwasser wurde als Umweltfaktor gewählt, da dieser die Ozeane weltweit beeinflusst, und unterschiedliche Effekte auf die beiden Arten hat, wodurch letztendlich starke ökologische Verschiebungen in der Artzusammensetzung begünstigt werden könnten. Zusätzlich wurden evolutive Veränderungen erwartet, da schon in vorrangehenden Studien gezeigt wurde, dass sich *E. huxleyi* an erhöhte CO_2-Konzentration anpassen kann.

Die bestehende genetische Diversität bildet die Grundlage für mögliche Frequenzverschiebungen zwischen Genotypen und für eine schnelle evolutive Veränderung. Um diese in der hier benutzen Artengemeinschaft abschätzen zu können untersuchte ich die plastischen Antworten von *E. huxleyi*, *C. affinis* und zusätzlich *Gephyrocapsa oceanica* auf eine erhöhte CO_2-Konzentration (Kapitel I).

Überraschenderweise pufferten alle drei Arten den Einfluss der erhöhten CO_2-Konzentration, wenn man die durchschnittliche Reaktion aller Genotypen einer Art betrachtet. Die Reaktionen einzelner Genotypen auf erhöhtes CO_2 unterschieden sich jedoch stark zwischen neutralen und negativen Einflüssen, insbesondere bei den beiden Coccolithophoriden. Im Gegensatz zu vielen früheren Untersuchungen, bei denen ein Genotyp (meist ein einzelner Stamm der lange im Labor gehalten wurde) einer Art getestet wurde, wird in dieser Studie deutlich, dass die Reaktionen der Population genauer durch eine Mischung verschiedener Genotypen beurteilt werden können, idealerweise aus frisch isolierten Proben aus dem natürlichen Habitat. Dieser Ansatz verringert erheblich den Aufwand, um in zukünftigen Studien verlässliche mittlere plastische Antworten von Populationen zu erhalten.

Des Weitern stelle ich einen neuen experimentellen Ansatz (Eco-Evo-Assay) vor, mit welchem die relative Wichtigkeit von ökologischen und evolutiven Veränderungen für Eigenschafts- und Funktionsveränderungen von Artengemeinschaften unter Klimaveränderungen bestimmt werden können (Kapitel II). Dieser experimentelle Ansatz überwindet dadurch die Einschränkungen von bisher entwickelten Ansätzen welche ökologische und evolutive Wichtigkeiten für Veränderungen von Arten und Gemeinschaften berechnen. Der Ansatz wurde vorab in der zuvor beschriebenen Artgemeinschaft auf Funktionalität geprüft. Hierbei war die Gemeinschaft über 50 Generationen einer erhöhter CO_2-Konzentration ausgesetzt. Anschließend wurde das Eco-Evo-Assay verwendet, um wiederholt die relative Wichtigkeit von ökologischen und evolutiven Veränderungen für die durchschnittliche mittlere Größe der Artgemeinschaft und deren totale Abundanz in Reaktion auf die CO_2-Konzentration nach kurz-, mittel- und längerfristiger Dauer des Experiments (entsprechend 50, 105 und 180 Generationen) bestimmt (Kapitel III). Die Studie konnte somit zeigen, dass die kurzfristigen Reaktionen der gesamten Artgemeinschaft auf erhöhte CO_2-Konzentrationen von ökologischen Veränderungen dominiert werden, während evolutive Veränderungen mittelfristig an Bedeutung gewinnen. Unerwartet war, dass längerfristige Reaktionen nicht von den beiden CO_2-Konzentrationen beeinflusst waren, und zu dem Zeitpunkt keine ökologischen oder evolutiven Veränderungen beobachtet werden konnten. Ich argumentiere, dass diese längerfristige Reaktion wahrscheinlich darauf zurück zu führen ist, dass die Frequenzveränderungen zwischen Genotypen durch Nährstofflimitation bedingt waren, und letztendlich unabhängig von CO_2 zu ökologischen Veränderungen führten.

Insgesamt zeigt diese Dissertation, dass sowohl ökologische als auch evolutive Prozesse zu den Veränderungen von Eigenschaften und Funktionen einer Phytoplankton Artengemeinschaft beitragen können. Dieser Einfluss war jedoch stark von dem betrachteten Zeitraum abhängig. Eine weiträumige Anwendung des Eco-Evo-Assays könnte es erlauben universelle ökologische und evolutive Mechanismen in Phytoplankton Artengemeinschaften zu identifizieren, wodurch sich unser derzeitiges Verständnis der Veränderungen von Phytoplankton Artengemeinschaften und resultierenden Vorhersagen erheblich verbessern könnte.

General Introduction

Climate change and the ocean

The increased use of fossil fuels since the industrial revolution has and continues to result in an enhanced emission of CO_2, among other greenhouse gases. CO_2 is the most persistent and frequently emitted greenhouse gas, and its positive correlation to the earths' temperature is described for over a century (Arrhenius, 1896). Thus, initial concerns about the potential effects of increasing atmospheric CO_2 concentration on the earths' climate system were raised early on (Callendar, 1949), but the magnitude and rate of anthropogenic CO_2 emissions were only understood after monitoring of atmospheric CO_2 was initiated in the 1950s. This gave rise to an increasing effort to study potential scenarios of earth climate development under different emission scenarios by the Intergovernmental Panel on Climate Change (IPCC). Additionally, first common political regulations were initiated in 1997 in the Kyoto protocol to reduce the anthropogenic impact on earth' climate. However, well-intentioned, this initiative largely failed and between 2000 and 2010, the highest CO_2 emissions were recorded (Pörtner et al., 2014). In 2015, the Kyoto protocol was superseded by the Paris agreement, which set the ambitious goal of keeping global warming 'well below 2 °C' (IPCC, 2018). This ambitious goal is widely discussed to be an important step forward as it encourages innovative thinking and drastic policy changes (Falkner, 2016; Rosen, 2015). Nevertheless, it remains uncertain how the reduction to net-zero emissions should be met in order to keep temperatures below 2 °C.

Climate change impacts the ocean at a different rate compared to the atmosphere (Archer and Brovkin, 2008). This difference can be explained by long equilibration times and the high absorption efficiency for CO_2 and temperature by the ocean. Consequently, the ocean functions as a sink for 26 % of the so far anthropogenically emitted CO_2 and 90 % of the thereof resulting heat (Caldeira and Wickett, 2003; Hoegh-Guldberg and Bruno, 2010). Therefore, the ocean has changed in abiotic conditions compared to pre-industrialisation. Over this period, surface temperatures have increased by about 0.6 °C (Boyce et al., 2010; Pörtner et al., 2014), pH decreased by 0.1 unit, and therefore carbonate chemistry shifted (Caldeira and Wickett, 2003). More specifically, the amount of oceanic inorganic carbon increased while the amount of saturated calcium carbonate, which is crucial for the ability of organisms to form calcareous shells and skeletons, decreased (Doney et al., 2009). These abiotic changes are not uniform across the entire ocean, and coastal waters are predicted to be adversely altered compared to the open ocean. In open oceanic regions,

from the tropics to mid-latitudes, increasing temperatures enhance stratification, reduce mixing depth, and as such decrease nutrient availabilities (Behrenfeld et al., 2006; Doney, 2006; Lewandowska et al., 2014). Along coastal regions, this temperature effect does not occur as such due to upwelling and riverine runoff, which can additionally lead to eutrophication and enhance pH fluctuations (Cai et al., 2011; Feely et al., 2008). The described shifts in abiotic conditions will accelerate even if the Paris agreement of <2 °C is achieved, but to a much lower extend (IPCC, 2018). Nevertheless, some oceanic ecosystems are under high threat even before the year 2100, which highlights the need for immediate and significant reduction of CO_2 emissions (Gattuso et al., 2015; IPCC, 2018). Moreover, suggested CO_2 mitigation approaches by means of climate engineering in order to reach net-zero emissions should be implemented with great caution as they could put marine ecosystems under additional risk. For example, CO_2 injection into the deep sea and nutrient fertilisation to fuel the biological carbon pump have been discussed to artificially enhance the transport of atmospheric CO_2 into the ocean but are potentially associated with far reaching and unpredictable consequences for the marine ecosystem (Boyd et al., 2007; Caldeira and Wickett, 2005).

Phytoplankton communities in times of climate change

Phytoplankton is defined as microscopic photosynthetic protists inhabiting the photic layer of all waterbodies, from fresh over brackish to marine waters. High turnover rates of these primary producers from diverse phyla yield up to 50 % of the primary and oxygen production of the earth (Field et al., 1998). Phytoplankton communities form the basis of marine food webs and productivity changes propagate to higher trophic levels (Chavez et al., 2003). Such associated ecosystem functions and services can potentially be altered when phytoplankton composition, diversity and consequently productivity (i.e. biomass) are shifted by anthropogenically-induced changes of ocean's abiotic conditions. For example, shifts to smaller sized phytoplankton groups under warming increase the length of food webs and thus reduces the transfer efficiency to higher trophic levels (Barnes et al., 2010; Boyce et al., 2015; Boyce and Worm, 2015; Stibor et al., 2004). Cell size decline due to increased temperature has also been discussed to drive the observed phytoplankton productivity decline in open oceans and was hypothesised to ultimately affect future fishery yields (Boyce et al. 2010; Boyce and Worm, 2015). This example illustrates that advancing predictions of future phytoplankton community structure and functioning is important and necessitates a shift away from bulk measurements towards the assessment of species composition. Diversity change in phytoplankton communities can also impact elemental cycles since major functional groups determine those cycles by their biogeochemical signatures

(Litchman et al., 2015). Moreover, major phytoplankton groups show unique environmental sensitivities, which have the potential to promote diversity changes. An excellent example is the expected adverse effect of enhanced CO_2 on calcifying coccolithophores compared to other functional groups of phytoplankton (Bach et al., 2018). This can result in compositional shifts under the persisting increase of seawater CO_2 concentrations that again have the potential to affect carbon cycling and thus global climate processes (Bach et al., 2016; Riebesell et al., 2009). In the North Atlantic, in late bloom stages for example, silica is depleted before nitrate leads to a switch from diatom to coccolithophore dominance (Leblanc et al., 2009; Sieracki et al., 1993). The question is whether or not such succession pattern and the bloom formation of coccolithophores will be altered in the future when calcification becomes more costly under reduced pH, resulting from increased CO_2 concentration. So far no conclusive answer to this question was found by neither, laboratory plasticity, nor mesocosm experiments (Eggers et al., 2014; Eggers and Matthiessen, 2013; Meyer and Riebesell, 2015; Schulz et al., 2017). Also uncertain is if experimental evolution of coccolithophores to high CO_2 (Lohbeck et al., 2012) could affect phytoplankton community structure (Bach et al., 2018; Collins, 2011; Scheinin et al., 2015). Evolution on an ecologically relevant timescale is potentially wide spread in phytoplankton due to fast generation times and enormous population sizes (Collins et al., 2014; Rengefors et al., 2017). Consequently, several studies highlight the need to simultaneously assess ecological and evolutionary changes and quantify their relative contribution to phytoplankton community shifts in response to environmental changes (Collins and Gardner, 2009; Litchman et al., 2012, 2015; Riebesell and Gattuso, 2015). This will lead to a better understanding of future phytoplankton community changes.

"Nothing in evolution or ecology makes sense except in the light of the other" (Pelletier et al., 2009)

Evolutionary and ecological processes together shape the diversity of populations and communities (Pelletier et al., 2009). Evolutionary processes involve allele or genotype frequency shifts within species, while ecological processes at the community level are reflected in species frequency shifts. The timescale at which ecological and evolutionary processes play out was traditionally believed to be diverging, since evolutionary processes were considered to be too slow to impact ecological processes on contemporary timescales (Pelletier et al., 2009; Schoener, 2011). Therefore, evolutionary processes were seldom considered in ecological studies. However, during the past decades, scientists have learned that rapid/contemporary evolution, especially due to genotype selection, is wide spread and happens on

timescales similar to ecological processes (Carroll et al., 2007; Schoener, 2011). As a result, selection on both the evolutionary and the ecological level is jointly influenced by the abiotic environment and by biotic interactions within and between both levels (Vellend, 2010). The consequential inclusion of evolutionary processes in ecological studies showed that evolutionary processes are indeed impinging over very short time scales upon ecological interactions (Fussmann et al., 2003). For example, communities possessing different standing genetic diversities upon which selection can act, show radically altered community dynamics. This has been shown for predator-prey dynamics with single or multiple algal genotypes subjected to grazing (Becks et al., 2010; Yoshida et al., 2003). Conversely, different ecological interactions resulting from altered species diversity may impact within-species adaptive evolution. For example, in bacteria, competition with other species enhanced the degree of evolutionary diversification (Lawrence et al., 2012), while other studies predict that increased species diversity can lower species' adaptive capacity by reducing the niche space that is left vacant (De Mazancourt et al., 2008). In summary, the increasing number of eco-evolutionary studies over the past years (Fig. 1) shows that interactions between ecological and evolutionary processes are wide spread (e.g. Yoshida et al., 2003; Fussmann et al., 2007; Thibodeau et al., 2015; Frickel et al., 2016), but their relative contribution to observed changes (Fig. 1 partition*), especially in the light of climate change remains largely unknown (Fig. 1 partition* AND environmental change).

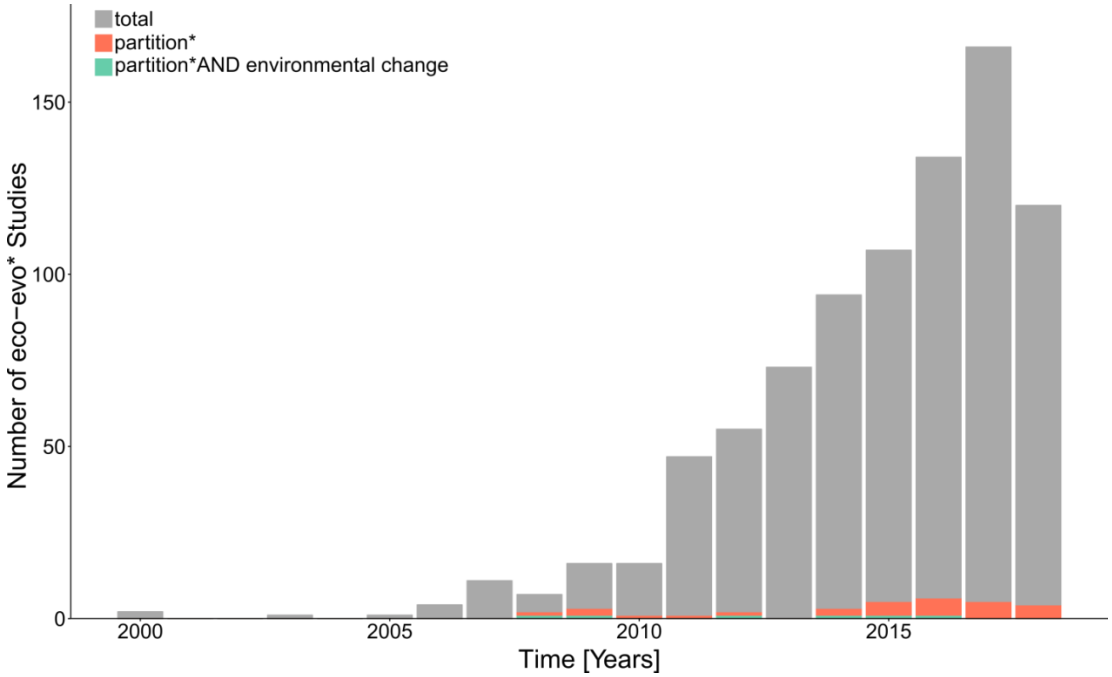

Figure 1: Number of studies in the field of ecology and evolution found for each year between 2000 and 2018 by a web of science search on the 4th of October 2018. Bar chart shows total number of studies found by a search for evo-evo*, colours indicate the number of studies which included partitioning (red; eco-evo* partition*) and partitioning and environmental change (turquoise; eco-evo* partition* AND environmental change).

Partitioning total change into ecological and evolutionary contributions

The relative contributions of ecology and evolution can be assessed with existing partitioning metrics, namely the Price equation (Price, 1970), reaction norm based approaches (Woltereck, 1909), variance partitioning after Lepš et al. (2011). However, these approaches are to date rarely applied (low amount of studies in the partitioning area in Fig. 1). Comparing the outcome among existing partitioning studies reveals that ecological and evolutionary contributions on mean traits are quite variable across systems (Hairston et al., 2005), and depend on the chosen trait (Govaert et al., 2016; Stoks et al., 2016) as well as on exposure time (Becks et al., 2012; Govaert et al., 2016). Consequently, no common pattern of eco-evolutionary contributions, which could aid the prediction of future ecosystem changes, could hitherto be extrapolated (Ellner, 2013; Rudman et al., 2017). Therefore, our efforts to assess the relative importance of ecological and evolutionary contributions to observed community changes should be increased. Further, the use of community properties such as biomass or abundance over mean traits is believed to deliver a more uniform and comprehensive partitioning as these traits constitute the consequence of all trait changes in a community (Govaert et al., 2016). Additionally, the choice of the particular metrics to partition eco-evolutionary importance can significantly influence the outcome: All approaches underlay different biological assumptions with specific data requirements, which ultimately assign the observed changes differently to ecological, evolutionary, plasticity and interaction components and thus are suited to answer specific questions (Govaert et al., 2016; van Benthem et al., 2016; Fig. 2). Thus, eco-evolutionary partitioning metrics will further be explained in the following paragraphs with a particular emphasize on the applicability of these metrics to partition the ecological and evolutionary contributions to community property changes.

The Price equation was originally developed to study evolution, by linking offspring to their parents (Price, 1970). However, this approach stands out with its generality and simplicity, resulting in the ability to include several levels of complexity and allowing the use across fields (Queller, 2017). To study the relative importance of ecological and evolutionary processes in an asexually reproducing community, a Price in Price equation can be applied (Collins and Gardner, 2009). This requires following changes of groups over time, which here are first species, and second, the genetic linages (genotypes) within species. Specifically, the trait values of these groups and their relative abundance need to be known to allow the calculation of abundance-weighted mean trait changes. As such, the Price equation is a suitable tool to quantify ecological and evolutionary components of trait changes, but not community properties. In addition, the Price equation approach requires data, which are often difficult to obtain

empirically (Fig. 2). This difficulty applies particularly to measurements of trait and frequency changes of genotypes.

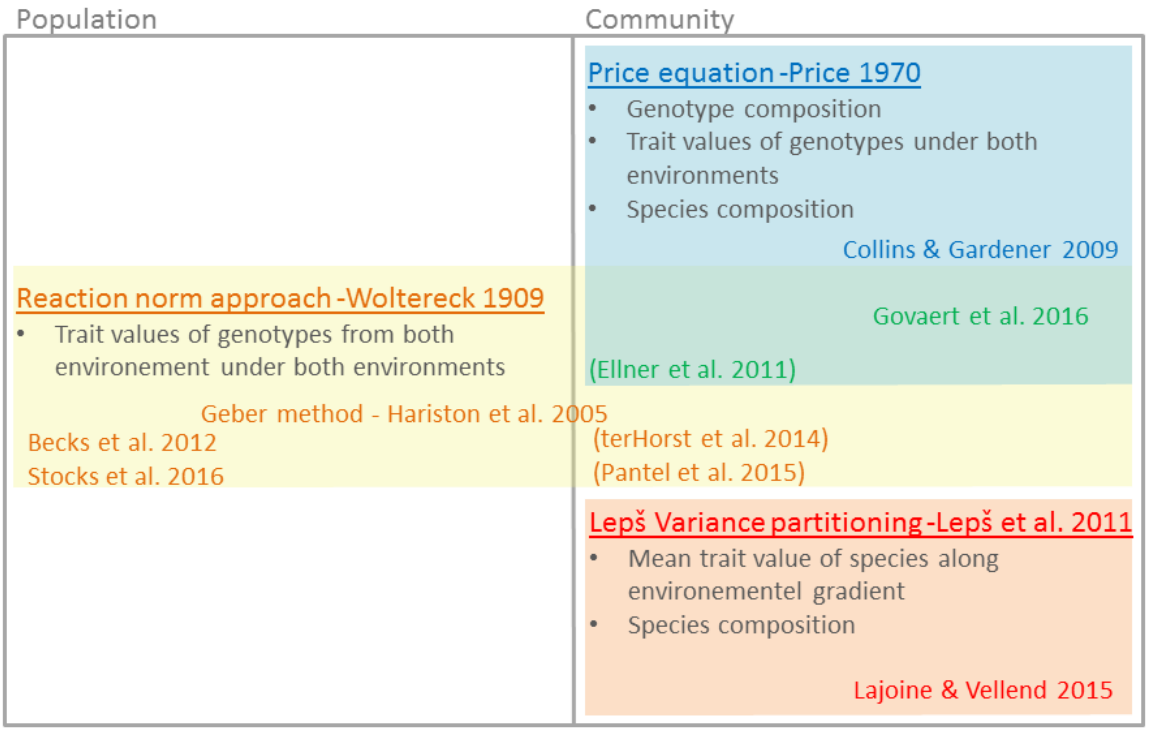

Figure 2: Applicability of existing partitioning metrics to the population and community level (coloured boxes) and their specific requirements and assumptions. If boxes overlap, both requirements need to be fulfilled. Reference examples are given for each approach. If the applicability to the community level is limited in my opinion, references are given in brackets. The figure was developed following personal communication with Lynn Govaert.

The reaction norm approach is the basis of the original (Hairston et al., 2005) and extended (Ellner et al., 2011) Geber method and the partitioning approach after Stoks et al. (2016). The Geber method was developed to demonstrate that ecological processes are fast enough to feedback onto ecological processes in populations and this approach defines the ecological component as the response to a contemporary environment (Hairston et al., 2005). For example, in Darwin's finches, population growth rate is linked to the evolution of beak size and an ecological response to precipitation (Hairston et al., 2005). In contrast, the partitioning approach after Stoks et al. (2016) does not include an ecological component, but disentangles constitutive evolution, from plasticity and evolution of plasticity. All reaction norm based approaches require the assessment of trait values of individual genotypes adapted to at least two different environmental states under both environments in a reciprocal transplant experiment. Thereafter, unweighted mean trait changes are calculated which do not require the knowledge of

relative genotype contributions. However, no reaction norm approach considers that ecological shifts constitute of species frequency changes on a community level. Consequently, partitioning matrices based on the reaction norm approach are applicable on the population level, while an upscaling to the community level is limited (Pantel et al., 2015; terHorst et al., 2014), or requires coupling the reaction norm approach with a Price equation approach (Govaert et al., 2016; Fig. 2).

Variance partitioning after Lepš et al. (2011) was developed to quantify the contribution of inter- and intraspecific trait variation to a total observed trait variation along an environmental gradient. Interspecific trait variation is mediated by species turnover and as such captures ecological changes. In contrast, intraspecific trait variation does not translate into evolutionary processes per definition, as it can be mediated by both, genetic changes via genotype turnover, and via non-genetic changes trough plastic responses. Nevertheless, the approach by Lepš et al. (2011) which specifically considers species turnover, is applicable to community mean trait changes and a valuable addition to the eco-evolutionary partitioning metrics if traits can be measured *in situ* (Fig. 2).

Thesis outline

The aim of this thesis is to advance the understanding of increased CO_2 effects on phytoplankton communities by assessing the plastic responses of single species and the relative contributions of ecological and evolutionary processes to a total observed community change.

In this thesis, the populations and communities comprised species from two major groups of phytoplankton, namely coccolithophores (*Emiliania huxleyi* and *Gephyrocapsa oceanica)* and diatoms (*Chaetoceros affinis)*. Both, coccolithophores and diatoms, are cosmopolitan bloomers together contributing up to 80 % of marine primary production (Poulton et al., 2013; Sarthou et al., 2005), but prevail under different conditions (Boyd et al., 2010). Different nutrient utilisation strategies (Edwards et al., 2012; Marañón, 2014) potentially enable coexistence of *E. huxleyi* and *C. affinis* in microcosms, which mimic natural nutrients fluctuations. Importantly, both groups of primary producers are adversely affected by enhanced CO_2 concentrations (Bach et al., 2018). This can consequently result in compositional changes in communities that are exposed to an enhanced CO_2 environment (e.g. Eggers et al., 2014). For the CO_2 sensitive *E. huxleyi*, a potential for adaptation to this environmental driver was demonstrated (Lohbeck et al., 2012). Since CO_2-driven changes in community and genotype composition have been demonstrated previously, I anticipated frequency shifts within and among species, when exposing this community of *C. affinis* and *E. huxleyi* to an increased CO_2 concentration. The aforementioned makes this community the ideal system, to study ecological and evolutionary changes in phytoplankton communities.

Chapter I

The first chapter comprises a series of experiments assessing the plastic responses of nine *C. affinis*, *E. huxleyi* and *G. oceanica* genotypes each, and the mean population response across all tested genotypes per species. Here, I addressed the research question, (i) how variable are phenotypic responses to increased CO_2 concentrations between and within species originating from one oceanographic region? Knowing the plastic responses of all single genotypes of *C. affinis* and *E. huxleyi* was the basis for the subsequent community experiments (see Chapter II and III for details). The observed standing genetic variation allows for genotype selection alongside ecological processes during long-term experiments and could help explaining the observed community shifts. Moreover, the use of plastic responses to model future distributions is far-spread but a potential source of uncertainty, as inconsistent responses are measured

for different strains and laboratory conditions (Meyer and Riebesell, 2015). Therefore, I used natural and fresh isolates of algae from the field and contrasted the responses of single genotypes of a population to the mean population response, based on the assumption that genotype frequency shifts are negligible in the short time frame of the plasticity experiments. I additionally asked the research question (ii) if the assessment of a plastic population response using mix of several genotypes could more accurately describe the plasticity of a species than the use of a random genotype?

Chapter II

The second chapter comprises a description of a novel assay approach (Eco-Evo assay), specifically developed to assess the relative contribution of ecological and evolutionary change to a total mean trait or property change of a phytoplankton community, as well as a community experiment to test the functionality of the assay. This assay fills a significant gap in current partitioning metrics. Specifically, the partitioning metrics can i) solely assess the relative contributions of ecology and evolution to mean traits and ii) require genotype and trait data which are in praxis difficult to obtain in phytoplankton (see Introduction, Partitioning total change into ecological and evolutionary contributions). Here, a community consisting of nine genotypes of *E. huxleyi* und *C. affinis* each was exposed to an ambient and high CO_2 environment and allowed to change in species and genotype frequency. After approximately 50 generations, their relative contributions to the observed abundance changes were assessed using the Eco-Evo assay. In this chapter, I address (i) whether the Eco-Evo assay functions and (ii) argue why it has the potential to improve our current understanding of ecological and evolutionary processes.

Chapter III

The third chapter comprised three Eco-Evo assays which repeatedly assessed the relative importance of ecological an evolutionary changes to total abundance and mean size changes of the *C. affinis* and *E. huxleyi* phytoplankton community exposed to high CO_2 conditions in a longer-term sorting phase. Here, the Eco-Evo assay (Chapter II) was applied after approximately 50, 105 and 180 generations (i.e. short-, mid- and longer-term, respectively). The assessment of the relative ecological and evolutionary importance is a sub-discipline of the field of eco-evolutionary dynamics (Hendry, 2016). This implies that ecological and evolutionary processes constantly change and often interact. This is supported by the fact that the relative contribution of ecology and evolution to rotifer population growth rate and age of reproduction in a *Daphnia*

community were, for example, shown to depend on the duration of the experiment (Becks et al., 2012; Govaert et al., 2016). Based on these examples and the assumption that ecological processes are faster than evolutionary processes, I ask (i) if ecological changes dominate phytoplankton community shifts at first and whether these become less important with time while the relevance of evolution increases?

Chapter I:

Inter- and intra-specific phenotypic plasticity of three phytoplankton species in response to ocean acidification

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****shared 1st authorship, + shared last-authorship***

Abstract

Phenotypic plasticity describes the phenotypic adjustment of the same genotype to different environmental conditions and is best described by a reaction norm. We focus on the effect of ocean acidification (OA) on inter – and intraspecific reaction norms of three globally important phytoplankton species (*Emiliania huxleyi, Gephyrocapsa oceanica*, *Chaetoceros affinis*). Despite significant differences in growth rates between the species, they all showed a high potential for phenotypic buffering (similar growth rates between ambient and high CO_2 condition). Only three coccolithophore genotypes showed a reduced growth in high CO_2. Largely diverging responses to high CO_2 of single coccolithophore genotypes compared to the respective mean species responses, however, raise the question if an extrapolation to the population level is possible from single genotype experiments. We therefore compared the mean response of all tested genotypes to a total species response comprising the same genotypes, which was not significantly different in the coccolithophores. Assessing species reaction norm to different environmental conditions on short time scale in a genotype-mix could thus reduce sampling effort while increasing predictive power.

Introduction

The expression of different phenotypes of a genotype in different environments is called phenotypic plasticity. It is described by the shape of the reaction norm of a trait value at different environments. No visible change in a focal trait despite a change in the environment (horizontal reaction norm) is defined as phenotypic buffering (Pigliucci, 2010). This does not preclude changes in other traits or on the molecular level. How phenotypic plasticity interacts with evolutionary adaptation is contentious (Lande, 2009); it is discussed to be both a non-mutual alternative to evolutionary adaptation and a strong driver for adaptation. In the plasticity-first scenario, a population/species survives environmental change due to pronounced plasticity until genetic mutations may occur and potentially fix the previously plastic trait such that the fitness under the new conditions increases (Levis and Pfennig, 2016). Provided that there is standing genetic/genotypic variation, mean population fitness can also increase at the level of populations, as a result from alteration of gene/genotypic frequencies over time caused by selection.

One prominent environmental change is ocean acidification (OA) (Caldeira and Wickett, 2003), describing that the anthropogenically introduced CO_2 dissolving in the oceans leads to subsequent changes in the carbonate system which potentially affects organisms, species and communities (Doney et al., 2009). In marine phytoplankton different effect sizes and signs in response to OA (i.e. varying reaction norms) have been observed between and within different taxa (Dutkiewicz et al., 2015). A reason for within species differences can be adaptation to different geographical regions (Kremp et al., 2012). Little is, however, known about inter- and intraspecific variation in reaction norms of populations and communities originating from one geographical region. Additionally, Valladares et al. (2014) summarize, that current mathematical models predicting alterations in communities due to climate change lack data on intraspecific genetic and phenotypic variation. Largely diverging responses to OA of different *E. huxleyi* genotypes among studies (Langer et al., 2009), raise the question if responses derived from one or a few genotypes can be directly extrapolated to the population and community level.

We compare (i), the intra- and interspecific reaction norms of three phytoplankton species in response to two different CO_2 conditions and (ii) the total multi-genotype species response to the mean intraspecific CO_2-response of the respective species. The species used include two common bloom forming coccolithophores, *Emiliania huxleyi* and *Gephyrocapsa oceanica,* and a diatom, *Chaetoceros affinis*, originating from one region. We expect that (i) the coccolithophores show a zero to negative reaction norm as a result of OA (Bach et al., 2015) compared to

a positive slope for the diatom as a result of profitable DIC use (Schaum et al., 2013). Furthermore (ii) genotypes of a species should differ in their growth response and (iii) the total species reaction norm is unequal to that of single genotypes but similar to the calculated mean reaction norm of all genotypes together.

Methods

From *C. affinis*, *E. huxleyi* and *G. oceanica* nine different genotypes each and one mix of all genotypes with equal initial abundances (Appendix I-1) were immediately exposed to ambient and high CO_2 concentration in order to obtain a two-point reaction norm within the acclimation phase. All cultures used were field isolates (2014- 2015) originating from one geographical region (Gran Canary, 27°59'N 15°22'W). This design allowed us to compare the within and among species plasticity of one community and the effect of intraspecific interaction on the short term CO_2-response by contrasting the multi-genotype total species (mixculture) to the mean intraspecific plastic (monoculture) response. All treatment combinations were three-fold replicated resulting in 180 experimental units (0.5 L polycarbonate bottle). Due to space limitation each species was tested separately (June to July 2016; Appendix I-2, Fig. I-2-1).

The ambient and high CO_2-treatment was manipulated by aerating the artificial-seawater (35 salinity; after Kester et al., 1967) for 24h with CO_2-enriched air (400 and 1250ppm, respectively) prior to the experiment. The dissolved inorganic carbon (Hansen et al., 2013) were 2164.68 ± 27.76 and 2307.94 ± 51.59 µmol*kg^{-1} with a total alkalinity (following (Matthiessen et al., 2012)) of 2442.04 ± 20.72 and 2456.30 ±2 0.63 µmol*kg^{-1} for ambient and high CO_2, respectively. Nutrients were added to the final concentrations of 19.98 ± 0.39 µmol*L^{-1} nitrate, 1.01 ± 0.07 µmol*L^{-1} phosphate and 4.40 ± 0.24 µmol*L^{-1} silicate for coccolithophores and 34.16 ± 0.30 silicate for the diatoms. The excess of silicate added to media used for diatoms ensured that all species were limited by nitrate in the experiment, a prerequisite to compare results among species. Vitamin and trace metals were added in f/8 concentration (Guillard, 1975). The prepared medium was sterile filtrated (0.2 µm) into the experimental units. Each experimental unit was inoculated with an initial total biovolume of 8280 μm_3*ml^{-1}of exponentially growing cells balancing the substantial differences in cell size of the species used.

The experiment was carried out under constant rotation (0.75 min^{-1}) at 20 °C and a 17:7 day:night cycle reaching a maximum light intensity of 350 µmol*m^{-2}*s^{-1} after 3h dusk and dawn. The development of each culture was followed by daily cell counts for the coccolithophores (Z2TM COULTER COUNTER®) and fluorescence measurements for the diatom (10AU FIELD AND LABORATORY FLOUREMETER by TURNER DESIGNS). The

total sampling volume was below 10 %. Cultures were terminated at the third day in stationary phase (experimental duration: 9-16 d).

For statistical analysis the software R was used (R core team 2016). Growth rates were determined for each replicate by fitting an exponential growth model inbuilt in the package "growthrates" (Maintainer and Petzoldt, 2016). The overall effect of CO_2, species and genotype on growth rate was tested using a nested ANOVA (growthrate~ CO_2*Species*(Genotype/Species)). Subsequent analysis of intraspecific plasticity and the effect of genotype was tested by separate ANOVAs for each species (growthrate~CO_2*Genotype) and genotype (growthrate~CO_2) and visualized as the difference in growth rates between ambient and high CO_2 (Borenstein et al., 2009). The difference between mean interspecific plastic effects and the multi-clonal total species response was tested for each species separately (growthrate~Mono/Mix-culture). Parametric assumptions were explored graphically.

Results

The growth rates (µ) between the species were significantly different ($F_{2, 132} = 355.586$, $p < 0.001$) with *E. huxleyi* and *G. oceanica* showing a 44 % and 28 % lower µ than *C. affinis* (Fig. I-1). Across all species µ was generally lower in high CO_2 and significantly depended on genotype ($F_{1, 132} = 8.433$, $p = 0.004$; $F_{24, 132} = 6.161$, $p < 0.001$; respectively). Analysis on the species level revealed that only the µ of *G. oceanica* was significantly lower in high CO_2 ($F_{1, 56} = 20.659$, $p < 0.001$). Further, the magnitude of the difference in µ between the CO_2-treatments was not uniform among all tested genotypes within each species (Fig. I-2). While the mean difference of *C. affinis* genotypes ranged from 0.109 to -0.273 with a substantial standard error, those of *E. huxleyi* had a narrow range from 0.029 to -0.097, with one genotype (C30) showing a significant lower µ under high than ambient CO_2 ($F_{1, 4} = 48.64$, $p = 0.002$). The general negative mean difference in *G. oceanica* genotypes ranged from -0.17 to -0.21. Two genotypes (GC59, GC58) were significantly negatively affected by CO_2 ($F_{1, 4} = 10.7$, $p = 0.031$; $F_{1, 4} = 42.12$, $p = 0.003$) which drove the overall significant negative effect of CO_2 on the µ of *G. oceanica*. Finally, the difference in µ between mono and mixcultures was significantly different only in *C. affinis* ($F_{1, 22} = 8.405$, $p = 0.008$) with a higher µ in the mix than in the monocultures.

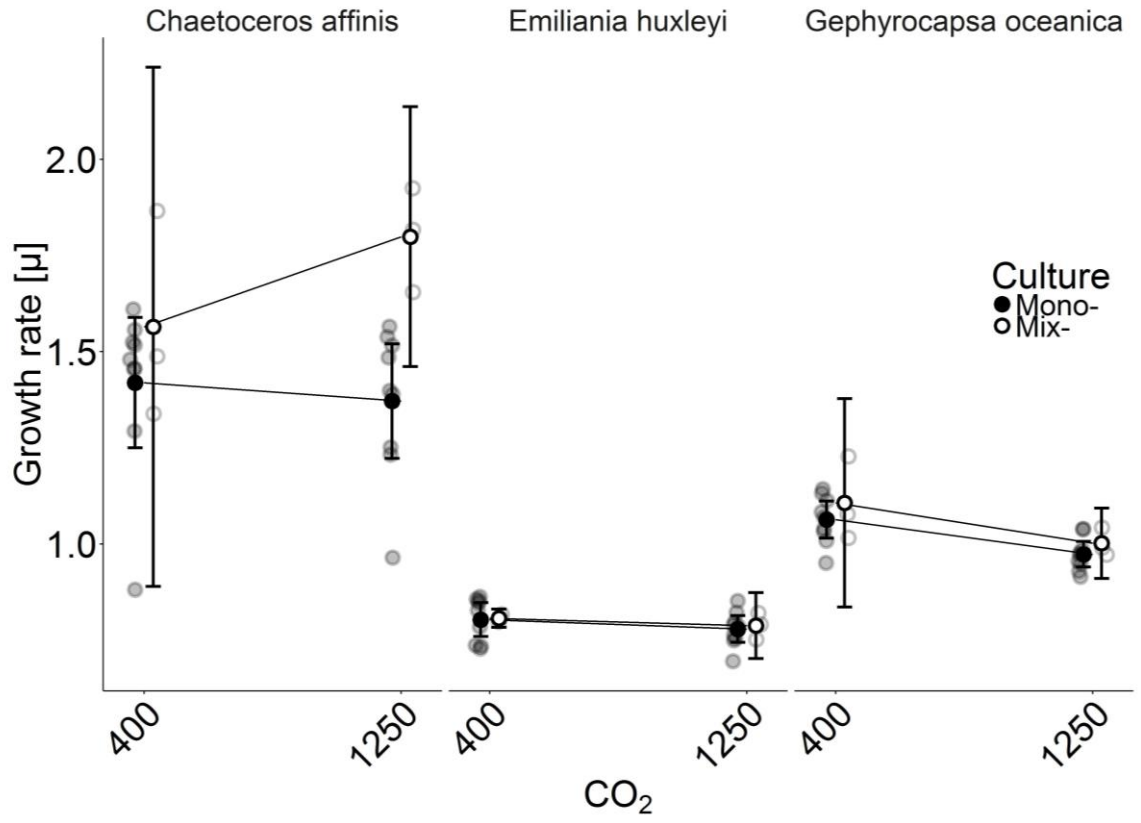

Figure I-1: Two-point reaction norm of growth rates in ambient and high CO_2 across mean of each genotype grown in monoculture (closed circle, N =9 (nine genotypes)) and a mixculture of all genotypes (open circle, N =3 (three replicates)) for each species. Mean and 95% CI are shown.

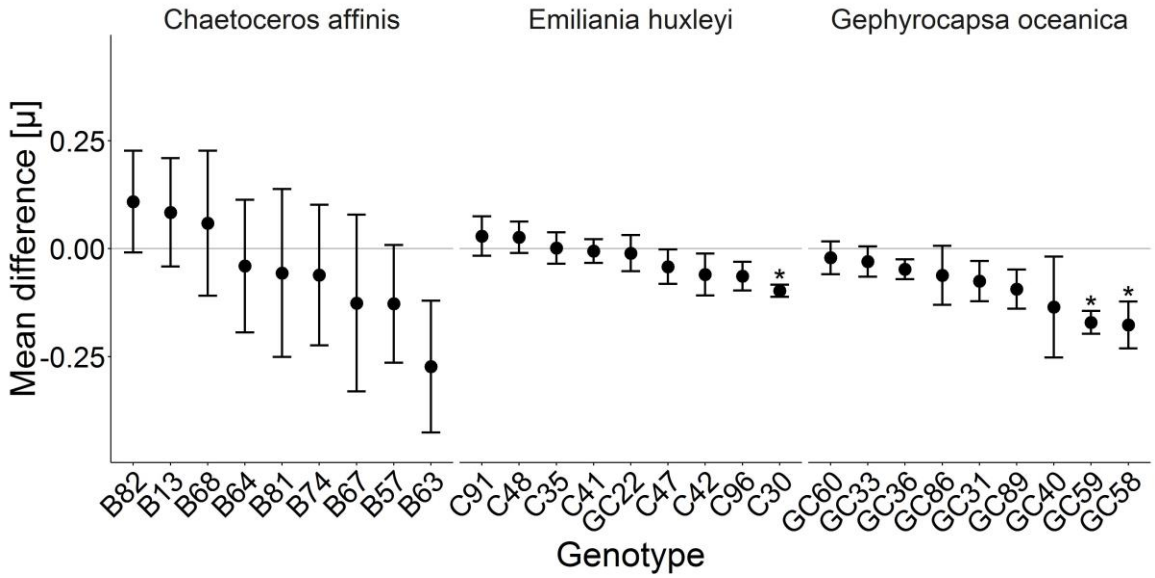

Figure I-2: Mean difference and its standard error of growth rates (m) between high and ambient CO_2 of each genotype and species. Grey line indicates no difference in growth between CO_2-treatments and asterisks highlight genotypes where growth rate was significantly affected by CO_2.

Discussion

We assessed the variation of phytoplankton acclimation reaction norms in two potential "loser" and one potential "winner" species under ocean acidification. Interestingly, all three species mostly buffered the effect of CO_2 and thus, showed a mean reaction norm slope similar or close to zero. However, within species, the response range varied. *C. affinis* showed the largest range in growth rates among genotypes tested. *E. huxleyi* and *G. oceanica* are ecologically more alike compared to *C. affinis* which could explain a more similar negative response among them. Due to the extensive literature (Meyer and Riebesell, 2015) showing negative effects of OA on coccolithophores we expected to see more genotypes showing a significant negative effect in growth under high CO_2, but note that most of those measurements were taken after acclimation, while our study was designed to address exactly the acclimation phase. Nevertheless, in line with literature we found that *G. oceanica* was most and significantly negatively affected by high CO_2 (Bach et al., 2015). Overall the weak effect of CO_2 could partly be due to the high variability among replicates masking a potential difference in growth rate between the two treatments. Additionally the experimental level of CO_2 in this study may be within the natural range (daily fluctuations, upwelling) species experience and can be phenotypically buffered (Riebesell, 2004).

The effect of CO_2 on single genotypes differed compared to the mean species response. *E. huxleyi* for example showed no overall effect of CO_2 on growth even though one genotype grew significantly slower under high CO_2. We observed the opposite in *G. oceanica* with an overall negative effect of high CO_2 on growth rate even though seven out of nine genotypes showed no difference. Our findings highlight the importance of testing many genotypes compared to the use of single genotypes, as has been done in most studies so far (Meyer and Riebesell, 2015), to avoid over- or underestimation of a species reaction norm to climate change.

The question remains how to minimize the sampling effort needed to study reaction norms of a representative set of genotypes of a species. We here show that the reaction norm of a culture containing the full set of genotypes compared to the mean of all genotypes cultured singly was similar in both coccolithophores but not in the diatom. The significant effect of culture condition on the slope in *C. affinis* could be driven by the high variability within the three replicates in the mixcultures. Nevertheless, our results suggest that the use of a mixculture of genotypes is sufficient to assess a species reaction norm on short time scales. This largely suggests that the total species reaction norm obtained from the mixcultures reflects the mean species plasticity if, as assumed here, genotype loss due to sorting is likely negligible (Appendix I-2).

Our experiments highlight the importance of investigating species reaction norms rather than reaction norms of single genotypes to better predict reactions to short-term environmental change. We show that analysing a mix of genotypes is an achievable and feasible way to identify realistic species reaction norms which are not overly influenced by randomly picked outlying genotypes from the standing genetic variability.

Data accessibility

PANGEA https://issues.pangaea.de/browse/PDI-13094

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Chapter II:

Experimentally decomposing total phytoplankton community change into ecological and evolutionary components

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Abstract

Community changes in response to environmental change comprise both, evolutionary and ecological processes, that occur on similar time scales albeit at different levels of biological organisation. While evolutionary changes due to environmental selection are reflected in altered genotype or allele frequencies within species, ecological changes are manifested in altered species composition. Predicting future phytoplankton community change thus requires understanding the respective relative contributions of ecological and evolutionary changes to a community change under a novel environment. In phytoplankton, the assessment of the relative importance of ecological and evolutionary change is methodologically limited, because existing numerical partitioning metrics are constrained by the impracticable requirement to measure frequency shifts and/or trait changes of genotypes in a community. As an alternative we here describe an experimental protocol, which neither requires determining genotype frequency nor of genotype trait changes. Experimentally a community is first allowed to respond to a novel environment with both species and genotype changes. Second, the resulting total community change under the novel environment is partitioned into ecological and evolutionary components using a newly developed assay (Eco-Evo assay). The relative contributions of ecological and evolutionary changes to total community changes are quantified by excluding either the species or genotype sorting, and measuring the resulting difference in community response. We demonstrate the functionality of the Eco-Evo assay in an artificial marine phytoplankton community exposed to ocean acidification for 50 generations and discuss how this assay can fill a gap in our current approaches towards the understanding of eco-evolutionary contributions to community change.

Introduction

Observed and further projected phytoplankton community reorganisation in response to climate change is recognized to be a combination of physiological responses of individuals, evolutionary adaptation of species, and ecological shifts among species (Collins and Gardner, 2009; Fussmann et al., 2007). Together these shifts at the basis of marine food-webs likely alter phytoplankton community properties or mean functional traits which in turn have the potential to influence major ecosystem functions in the sea (Boyce et al., 2010; Iglesias-Rodriguez et al., 2008; Stibor et al., 2004). Although often discussed to be essential for predictions on future phytoplankton change (Collins and Gardner, 2009; De Meester et al., 2011; Litchman et al., 2012; Riebesell and Gattuso, 2015) and its propagating effects, the relative contributions in particular of ecological changes (i.e. species frequency shifts) and evolutionary adaptation (here mainly through genotype frequency shifts of standing genetic variation) to total community change in response to climate change remain largely unknown. This knowledge gap is on the one hand caused by the dominating assessments of either plastic (physiological), evolutionary or ecological responses to climate change in isolation (but see Collins and Gardner, 2009). These studies allow for insights on each particular level of biological organisation, but largely ignore that ecological and evolutionary processes can happen on similar timescales and thus can influence one another (Carroll et al., 2007; Fussmann et al., 2007; Hairston et al., 2005; Schoener, 2011). On the other hand, the partitioning of total community changes in general and/or phytoplankton community change in particular is not possible with to date developed methods to partition ecological and evolutionary contribution.

With the existing partitioning metrics commonly applied to eco-evolutionary studies, namely the Price equation approach (Collins and Gardner, 2009; Govaert et al., 2016; Price, 1970), variance partitioning after Lepš (Lajoie and Vellend, 2015; Lepš et al., 2011) and reaction norm approach as applied in the Geber method (Ellner et al., 2011; Hairston et al., 2005; Pantel et al., 2015; terHorst et al., 2014), total community change cannot always be partitioned into its ecological and evolutionary components. The Geber method was mainly developed to show that evolutionary rates are sufficiently fast to feedback onto ecological processes in populations. The ecological component is thus defined as the response of the population to contemporary environment. In a community context, however, ecological changes also entail species abundance shifts, which are not considered in the ecological component of the Geber method. Therefore the extension of the Geber method to the entire community is restricted to an assessment of the effect of evolution of a single target species onto a community (Pantel et al., 2015; terHorst et al., 2014). As such this approach ignores that selection on both,

the evolutionary and the ecological level, are jointly influenced by the abiotic environment and by biotic interactions within and between both levels of biological organisation (Vellend, 2010). We conclude that the Geber method is of limited applicability to quantify the relative importance of ecological and evolutionary change for total community changes. In contrast, both, the Price equation and variance partitioning approach after Lepš include species abundance shifts as ecological component and can partition a mean trait change of a community into its ecological and evolutionary components. Here mean traits are measured by weighing the mean trait per species against their respective abundance. Thus community properties such as total abundance or biomass cannot be partitioned as it would weigh the response value against itself. The assessment of community property changes is, however, proposed to be more integrative and comprehensive than the assessment of the change of single traits in a community (Govaert et al., 2016). Community property changes result from the sum of all trait changes in a community and often relate to community functioning.

While the Price equation and variance partitioning after Lepš are applicable to partition community mean trait changes, their feasibility to phytoplankton is limited by their need to measure genotype abundance changes and/or their changes in trait values at minimum two time points or environments, respectively. The difficulty to assess phytoplankton genotype abundances and their traits is manly caused by its small size. Qualitative genotype identification could for example be addressed with microsatellite markers, which are relatively easy to obtain and use. Quantitative genotype assessment using microsatellite markers would, however, require a priori knowledge of present genotypes within a population and vast re-isolation work of single individuals of the community followed by growth to dense clonal populations (as done by Listmann, 2018). Trait measurements on single genotypes/species of phytoplankton under different environments can, with the exception of size, not be performed in-situ, but requires isolation of genotypes/species and subsequent assessment in clonal populations. Such measurements are not only time and labour intensive but also potentially inaccurate, since ecological interactions that might change responses are excluded (Govaert et al., 2016).

We propose to overcome these methodological limitations by experimentally quantifying the relative importance of ecological and evolutionary changes for phytoplankton community property or trait changes in response to environmental change. This experimental approach does neither require knowing abundance or trait value changes of single genotypes in the community, nor rely on an existing numerical partitioning metric. Rather, the experimental approach requires that species in the community can be physically separated to allow manipulation of species independent of

genotype composition in an assay (Eco-Evo assay). The Eco-Evo assay partitions the total community property or trait changes observed in a long term experiment in response to environmental change into its ecological and evolutionary components. Precisely, the effects of ecological or evolutionary changes in a community are assessed by respectively resetting the genotype or species abundance to the ambient environment and quantifying the resulting difference to the total community change under the novel environment. As such the Eco-Evo assay does not aim to understand ecological and evolutionary changes under both environmental conditions, but instead allows asking the question how much of the observed community shifts in response to a novel environmental is mediated by ecological and evolutionary changes.

Materials and Procedures

The here introduced Eco-Evo assay can be repeatedly applied over the course of a long-term experiment (sorting phase) to assess the relative contributions of ecological and evolutionary changes to total community change in response to a novel environment (Fig. II-1). Appropriate measures for a total community change can be a mean trait or community property change. Mean traits, such as body or cell size, can be meaningful on the community level if they are under selection by the chosen environmental driver. Community properties, as for example total biomass, abundance or nutrient content, can be of interest as they often relate to ecosystem functioning.

Sorting phase

The sorting phase allows the community to restructure in terms of species and genotype frequency shifts in response to an ambient and novel environment (Fig. II-1, Sorting phase). The novel environment reflects projected future ranges of any factor under climate change such as temperature, CO_2-concentration, salinity or nutrients and is compared against an ambient control. Generally the community of interest should be present with a minimum diversity of two stably coexisting species, each comprising multiple genotypes. Furthermore, the species in the community must be separable. Throughout the sorting phase, the community property or mean trait of interest that is expected to change under the novel environment should be monitored.

Our model community comprised of two phytoplankton species, *Chaetoceros affinis* and *Emiliania huxleyi,* with nine genotypes each (for details on genotypes see Hattich et al., 2017). During the sorting phase, here realized with semi-continuous batch-cycles, the community was exposed to ambient and high seawater CO_2 environment.

C. affinis and *E. huxleyi* belong to different functional groups (Litchman et al., 2007) and were characterized by diverging cell size (mean ± SD were 462 ± 192 µm³ and 22 ± 8 µm³, respectively). The differing cell size allowed for a physical separation of the two species by a sieve which is required for the subsequent assay (see Materials and Procedures, Eco-Evo Assay). The applied novel sweater CO_2 concentration and resulting shifts in carbonate chemistry reflected expected increase of anthropogenically introduced CO_2 of the next century (IPCC, 2014). CO_2 concentrations were manipulated by aerating the growth medium with CO_2-enriched air containing 400 (ambient) and 1250 (novel/ high) ppm for 24 h (details see Hattich et al., 2017). The growth medium was made out of artificial-seawater (according to Kester et al., 1967) with a salinity of 35 and contained 19.59 ± 0.65 µmol L^{-1} nitrate, 0.97 ± 0.09 µmol L^{-1} phosphate ,3.82 ± 0.55 µmol L^{-1} silicate, f/8 vitamin and trace concentration (Guillard, 1975). 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. At the start of the sorting phase, on January 10th 2017, five replicates for each CO_2 environment (i.e. ambient and high) were inoculated with a defined biovolume of 5.5 * 10^6 µm³ of the start community. In the start community the genotypes per species were present with equal cell numbers (11 %), while the species due to their substantial differences in cell size were present with equal biovolume (50 %). This resulted in 98 % *E. huxleyi* and 2 % *C. affinis* relative abundance at the onset of the experiment. In each semi-continuous batch cycles, the communities were grown to stationary phase. The growth took place under constant rotation of 0.75 min^{-1} on a plankton wheel with a maximum light intensity of 350 µmol m^{-2} s^{-1} reached after three h dusk and dawn of 17 L : 7 D cycle in 20 °C. The stationary phase was reached after eight days which corresponded to approximately five generations. Thereafter communities and underlying changes in species and genotype sorting in response to the applied environmental conditions were transferred to the next batch cycle. Practically, after each batch cycle, 5 mL samples from each replicate were fixed in Lugol's iodine solution. Abundance and biovolume (Hillebrand et al., 1999) were assessed with an inverted light microscope (Zeiss, Axiovert 200 and Observer A1) and subsequently a total biovolume of 5.5 *10^6 µm³ transferred to the next batch.

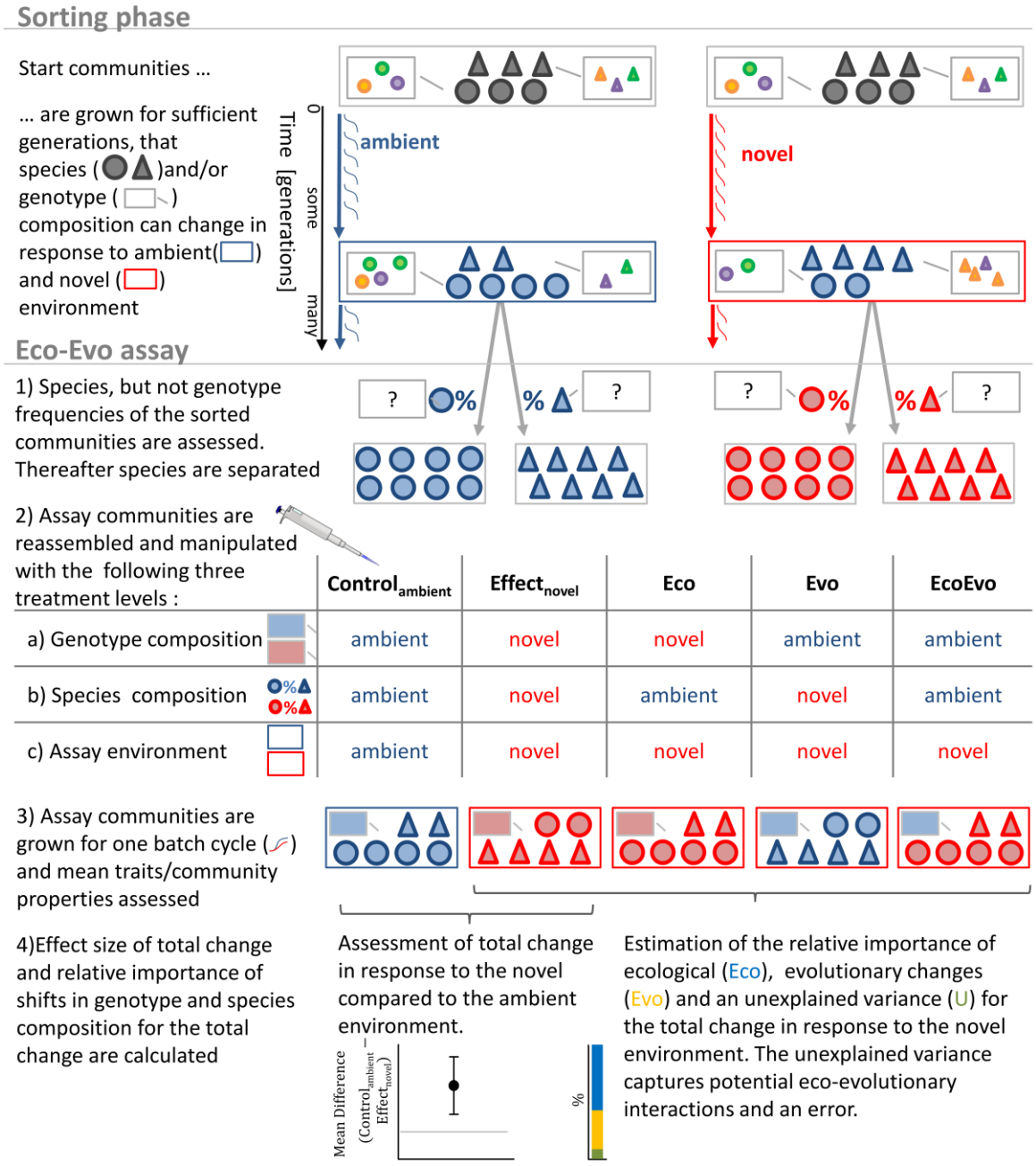

Figure II-1: Stepwise description of the experimental approach to quantify the relative importance of ecological and evolutionary changes for a total change in response to a novel environment. The sorting phase includes the general minimum requirements on the model system and depicts a hypothetical species and genotype sorting through time in response to ambient and novel environment. The Eco-Evo assay gives detailed information on different steps that have to be taken in order to quantify the relative contributions of species and genotype frequency shifts for community changes observed under novel environment at a given point in time in the sorting phase.

Eco-Evo Assay

The newly developed Eco-Evo assay measures the ecological and evolutionary contributions for community change by either excluding species or genotype frequency shifts in response to the novel environment, and by quantifying the resulting difference in a mean trait or community property. Thus the critical step of the Eco-Evo assay is the artificially reassembly of assay communities, which is necessary to exclude species or genotype frequency shifts. Precisely, carrying about the assay requires 1) the assessment of relative species abundances in the communities and physical separation of the species at a chosen point in the course of the sorting phase, 2) the artificial reassembly and compositional manipulation of assay communities, 3) the measurements of assay community responses, and 4) the calculation of relative ecological and evolutionary importance (Fig. II-1, Eco-Evo assay, step 1 to 4).

1) Assessment of relative species abundances in communities and physical separation of species

In order to allow manipulating species composition of assay communities independent of genotype composition (Fig. II-1, Eco-Evo assay, step 2), species composition of communities in the sorting phase at the chosen point in time have to be determined. Additionally species have to be separated from each other (Fig. 1, Eco-Evo assay, step 1).

In the present study, the species abundance and biovolume was determined on March 29th 2017 after ten semi-continuous batch cycles in the sorting phase that corresponded to approximately 50 generations. Separation of the two species was conducted over a 20 µM sieve. Precisely a defined volume of the community was pipetted into the sieve and the small *E. huxleyi* collected in a sterile culture bottle underneath. In the next step the sieve was turned around and the bigger *C. affinis* gently washed into another sterile culture bottle.

2) Artificial reassembly and compositional manipulation of assay communities

All assay communities are artificially reassembled using the species that were physically separated from communities at a chosen point in the course of the sorting phase. To assess the total change between ambient and novel environment the Eco-Evo assay comprises $Control_{ambient}$ and an $Effect_{novel}$ communities. The **$Control_{ambient}$** communities reflect the (a) genotype and (b) species compositional changes of the communities sorted in response to the ambient environment and continue to grow

under the ambient environmental conditions in the assay (Fig. II-1, Eco-Evo assay, step 2). The **Effect$_{novel}$** communities in contrast reflect the (a) genotype and (b) species compositional changes of the communities sorted in response to the novel environment and continue to grow under the novel environmental conditions in the assay (Fig. II-1 Eco-Evo assay, step 2).

To test for the importance of species, genotype and both species and genotype compositional changes together for total community change in response to the novel environment, the assay also comprises of Eco (ecology), Evo (evolution) and EcoEvo communities. The Eco, Evo and EcoEvo communities measure the response to novel environments either without genotype and/or species frequency shifts. Their contribution to the total change is thus the respective difference to the Effect$_{novel}$. The **Eco** communities exclude the observed shifts in species composition in response to the novel environment. Thus the Eco communities are reassembled using (a) species whose genotype composition was sorted in response to the novel environment, but (b) reflect relative species abundances found in communities sorted under the ambient environment and (c) are exposed to the novel environment in the assay (Fig. II-1, Eco-Evo assay, step 2). The **Evo** communities exclude evolutionary adaptation in response to the novel environment by reassembling the communities using (a) species whose genotypes composition was sorted in response to the ambient environment, but (b) start with the relative species abundances found in communities sorted in response to the novel environment and (c) are exposed to the novel environment in the assay (Fig. II-1, Eco-Evo assay, step 2). Here it needs to be stressed that genotype composition cannot be reassembled artificially to mirror genotype composition observed in the ambient environment but is manipulated indirectly by using species whose genotypes have been sorted in response to ambient CO_2. As a result, the Evo community response does potentially not only reflect the effect of differential genotype sorting between ambient and novel environment, but could additionally include plasticity and with time de novo mutations, as has been shown in a single species approach with the same coccolithophore in Lohbeck et al. (2012). The **EcoEvo** communities combine the manipulations of Eco and Evo communities and thus exclude both shifts in species and genotype abundances in response to the novel environment. That is the communities are reassembled using (a) species whose genotype composition was sorted in response to the ambient environment and (b) start with the relative species abundances found in the communities sorted under the same ambient environment, but (c) are exposed to novel environment in the assay (Fig. II-1, Eco-Evo assay, step 2). Hence, this response reflects the combined effects due to altered species and genotype composition.

Potential divergences compared to the additive effect of the single Eco and Evo treatments can result from eco-evolutionary interactions.

The artificially reassembly of assay communities is a critical step of the Eco-Evo assay. Thus handling bias should be avoided and not only assay communities that do require the manipulation of species composition independent of genotype composition (Eco and Evo community) artificially reassembled but also those who do not ($Control_{ambient}$, $Effect_{novel}$, EcoEvo). To keep species that potentially co-evolved together in the same assay community, the species originating from a common source community should further be pipetted into the same assay community. Moreover there are three practical options to realise species composition in the reassembled assay communities according to observations from the sorting phase. First every replicate of the ambient environment can be randomly assigned to a replicate of the novel environment and their respective relative species abundances used to reassemble assay communities. This, however, potentially results in high variability, if single replicates are diverging in their responses. Second, species from every replicate can be reassembled to multiple assay communities reflecting all relative species abundances found at the end of the sorting phase under ambient or novel environmental conditions. This would fully cross variance within and between species and allow the assessment of variability, but would result in an unfeasible high number of experimental units, which are not all independent of one another. Third, the mean relative species abundance of communities sorted in response to the ambient and novel environment can be calculated and assay communities reassembled accordingly. This has the advantage that variability between replicates resulting from differential species shifts in the sorting phase is not continued in the assay, while sample size is not inflated artificially.

In our example, assay communities were reassembled from species separated from communities after 50 generations in the sorting phase. It thus tested for the relative importance of ecological and evolutionary changes for the total community change observed after 50 generations. The species composition was manipulated according to the third option given above using the mean relative *E. huxleyi* and *C. affinis* abundance of the communities sorted in response to ambient and high CO_2 condition. Practically the required transfer volumes of each species to inoculate the assay communities with a total biovolume of $5.5 * 10^6$ µm³ were calculated. For this purpose the species' abundances found in the source community and the aimed species compositions of the assay communities had to be considered (Appendix II-1). Then species originating from a common source community were pipetted into the same assay community according to the calculated volumina and exposed to the required assay environment (Fig. 1, Eco-Evo assay, step 2; Appendix II-1).

3) Measurement of assay community responses

The assay communities are grown until stationary phase under the same laboratory conditions as in the sorting phase and the mean trait or community property of interest is measured. Thereafter total community change and the effects of shifts on ecological and evolutionary level should be statistically analysed. It is important to test for both, since changes on the ecological and evolutionary levels can have opposite effect signs and potentially compensate one another. In such situations, no total change is observed between $Effect_{novel}$ and $Control_{ambient}$, but responses measured in Eco, Evo and/or EcoEvo community are significantly different to the $Effect_{novel}$.

In this study assay communities were grown for one batch cycle and total abundance was determined via microscopy. Statistical analyses of assay results were conducted with ANOVA. First we tested for a total change in response to high CO_2 environment by comparing $Control_{ambient}$ with $Effect_{novel}$. Than effects of genotype and/or species compositional shifts were tested by comparing the responses of the Eco, Evo and EcoEvo communities, respectively, to the $Effect_{novel}$ (setting Effect novel as intercept).

4) Calculation of relative ecological and evolutionary importance

The calculation of the relative importance of ecological and evolutionary changes for the total change is only valid if in the assay (i) significant total changes in response to the novel environment and/or (ii) significant effects of genotype and/or species compositional shifts are found (see statistical analysis above).

The relative importance of ecological and evolutionary changes and unexplained variance (Fig. II-1 Eco-Evo assay, step 4) is calculated by dividing the respective changes captured in $Effect_{ecology}$, $Effect_{evolution}$ and U by their sum (equation 1-3):

$$\%\, Ecology = \frac{Effect_{ecology}}{Effect_{ecology}+Effect_{evolution}+U} \quad (1),$$

$$\%\, Evolution = \frac{Effect_{evolution}}{Effect_{ecology}+Effect_{evolution}+U} \quad (2),$$

$$\%\, U = \frac{U}{Effect_{ecology}+Effect_{evolution}+U} \quad (3),$$

where U is the unexplained variance and encompasses divergence from additivity of ecological and evolutionary changes measured in isolation from the measured total eco-evolutionary change (equation 4) and is given as:

$$U = |\, Effect_{ecology+evolution} - \left(Effect_{ecology} + Effect_{evolution}\right)| \quad (4),$$

where the $Effect_{ecology}$, $Effect_{evolution}$ and $Effect_{ecology+evolution}$ capture the single and interactive contribution of ecological and evolutionary changes to total change in response to the novel environment, respectively, and are given as:

$$Effect_{ecology} = |Eco - Effect_{novel}| \quad (5),$$

$$Effect_{evolution} = |Evo - Effect_{novel}| \quad (6),$$

$$Effect_{ecology+evolution} = |EcoEvo - Effect_{novel}| \quad (7).$$

These contributions are calculated as absolute effects since ecological and evolutionary changes can result in different effect signs, and thus in overall masked effects when measured in combination (equation 5-7).

Here, the relative contributions of ecological and evolutionary change to the total change were calculated as mean effects following the given descriptions. In order to set changes into perspective we further calculated the effect size as the mean difference between the community responses under ambient ($Control_{ambient}$) and high ($Effect_{novel}$) CO_2 environment and its standard error (Borenstein et al., 2009) and plotted the relative contributions of ecological and evolutionary change underneath this total change.

Additional measurements to assess the functionality and critical steps of the Eco-Evo assay

As a quality check for the here presented method, though methodologically not required by the Eco-Evo assay, genotype compositional shifts were measured in the sorting phase. Genotype composition, however, was assessed for *E. huxleyi* only. Practically, 20 *E. huxleyi* cells per replicate were re-isolated after 5, 20, 40 and 50 generations (end of batch cycle 1, 4, 8, 10 respectively). The isolated cells were grown for a minimum of two weeks prior to genotype identification with microsatellites (details see Hattich et al., 2017). The observed differences in species and genotype compositions resulting from the exposure to ambient and high CO_2 for 50 generations were analysed with ANOVA and ANOSIM, respectively. In the ANOVA, relative species composition was addressed as % contribution of *E. huxleyi*.

Additionally, we analysed the potential error made by the reassembly to assay communities, a critical step of the Eco-Evo assay. To confirm that the artificially reassembled communities do mirror the total community response and species composition of communities in the sorting phase the communities of batch cycle eleven of the sorting phase were compared to the response in $Control_{ambient}$ and $Effect_{novel}$ communities. Therefore the total abundance and the ratio between *E. huxleyi* and

C*. affinis* of sorting and assay communities was analysed with an ANOVA. All data analyses, including a priory inspection of normality and heterogeneity of variances, and plotting using the packages "ggplot2" (Wickham, 2009) were undertaken in R (R Development Core Team, 2016).

Assessment

Sorting phase

The total abundance in communities sorted for 50 generations under high compared to ambient CO_2 conditions were by four fold reduced (Fig. II-2 A). This decline of phytoplankton abundance under high CO_2 coincided with a one-third lower *E. huxleyi* contribution under high compared to ambient CO_2 environment. Over time the relative abundance of *E. huxleyi* and *C. affinis* changed from a highly *E. huxleyi* dominated system at the start of the sorting phase (98% *E. huxleyi* cells/mL corresponding to equal contributions in biovolume [μm^3/mL]) towards a lower contribution of *E. huxleyi* cells/mL after 50 generations when the Eco-Evo assay took place (Fig. II-2 A). The reduction of *E. huxleyi* abundance was significantly greater under high compared to ambient CO_2 conditions (Fig. II-2 A; $F_{1,8} = 26.04, p < 0.001$) and resulted in 62% and 89% *E. huxleyi* cells after 50 generations in the sorting phase, respectively. In contrast to the species composition, the changes in *E. huxleyi* genotype composition found in the communities after 50 generations of sorting were not significantly altered by high CO_2 (Fig. II-2 B; $R = -0.03, p = 0.929$). All communities were composed of the same three out of nine initially present *E. huxleyi* genotypes after 50 generations (Fig. II-2 B). Nevertheless changes in genotype frequency shifts, even though not driven by CO_2, happen on a timescale equivalent to species frequencies changes. This was especially apparent in the observed doubling of one genotype and reduction to near detection limit of another genotype (C91 and C47 respectively; Fig. II-2B) within five generations (one batch cycle).

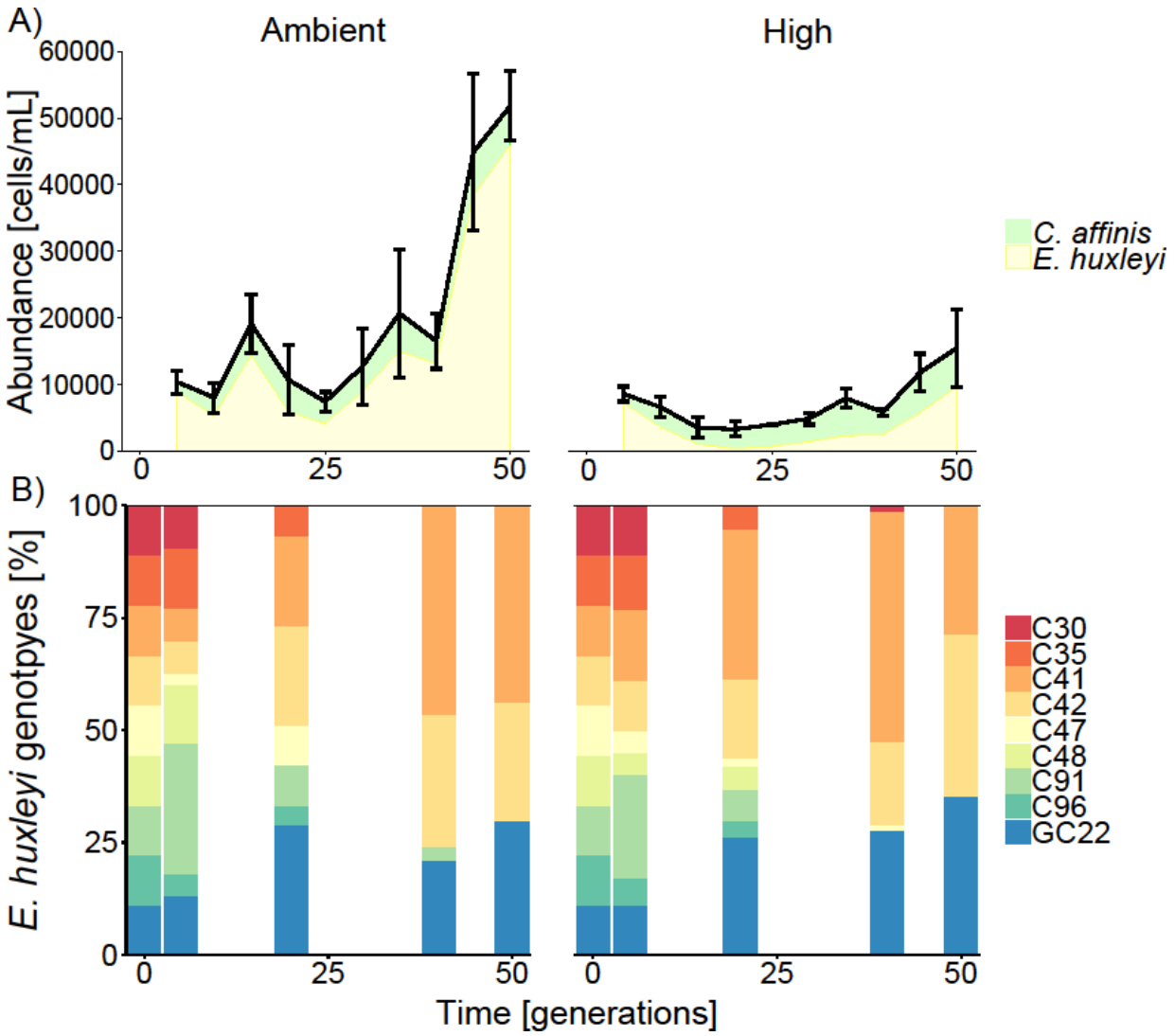

Figure II-2: Figure shows A) total abundance and underlying species composition change), and B) Genotype sorting of *E. huxleyi,* under ambient and high CO_2 concentrations trough out the sorting phase that lasted for 50 generations. Mean and 95%CI of n=5 for total abundance and mean of N=83, 45, 71, 68 and N=82, 57, 76, 38 for relative *E. huxleyi* genotype composition in ambient and high CO_2 concentrations respectively (N of generation 5, 20, 40, 50).

The analysis of differential species and genotype sorting that underlay the total abundance decline under high compared to ambient CO_2 environment after 50 generations confirmed the functionality of the experimental design in the sorting phase. Further it allowed the anticipation of expected ecological and evolutionary contributions to the total change against which the partitioning of the Eco-Evo assay can be validated (see Materials and Procedures, Eco-Evo Assay). The changes on the species and genotype level observed throughout the sorting phase are generally in good agreement with the literature. The significant lower contribution of *E. huxleyi* to communities sorted for 50 generations under high compared to ambient CO_2 environment is in

agreement with the consistent observation across studies that calcifying haptophytes decline in communities under ocean acidification (Eggers et al., 2014; Riebesell et al., 2017; Schulz et al., 2017). The observed similar *E. huxleyi* genotype sorting under ambient and high CO_2 conditions is in contrast to the differential sorting observed in response to ocean acidification in Lohbeck et al. (2012). The absence of an effect of CO_2 on genotype sorting observed could, however, result from overriding effects of the general experimental conditions characterized by nutrient pulses and competitive interactions. Similar overriding/ modulating effects of initial community composition (Eggers et al., 2014), temperature (Paul et al., 2016), or nutrient availability (Alvarez-Fernandez et al., 2018) on CO_2-effects are described on the community level. Since selection processes within and between species are argued to be comparable (Vellend, 2010), modulating effects of laboratory conditions on the genotype composition under high CO_2 are likely. The observed diverging and similar changes in species and genotypes respectively, which underlie observed decline in total abundance in response to ambient and high CO_2 during the sorting phase, suggest the detection of significant ecological but weak evolutionary contributions to the observed change in response to high CO_2 in the Eco-Evo assay.

Eco-Evo assay

The Eco-Evo assay captured the effect of high CO_2 concentration on communities after 50 generations and partitioned the total change into ecological and evolutionary contributions. First, the total abundance in high compared to ambient CO_2 conditions was by four fold reduced (Fig. II-3, $Control_{ambient}$ and $Effect_{novel}$ respectively) which mirrored the pattern observed after 50 generations in the sorting phase (Fig. II-2 A, 50 generations). Not only the abundance decline was of comparable magnitude in both, Eco-Evo assay and sorting phase (Fig. II-4) but also the ratio between *E. huxleyi* and *C. affinis* was preserved in reassembled assay communities (Fig. II-4; $F_{1, 17} = 2.16$, $p = 0.16$). Second, the Eco-Evo assay revealed a strong effect of species and weak effect of genotype sorting on the abundance decline in response to high CO_2. Precisely, excluding species sorting to the high CO_2 conditions in the Eco and EcoEvo communities resulted in a two to three fold abundance increase compared to total community change observed in response to the high CO_2 environment (Fig. II-3 $Effect_{novel}$ vs. Eco and EcoEvo; Eco: $F_{3,16} = 22.25$, $p < 0.001$; *EcoEvo* $F_{3,16} = 22.25$, $p < 0.001$ respectively). Evo communities, however, which only exclude genotype sorting showed a similar response to the total change under high CO_2 (Fig. II-3 $Effect_{novel}$ vs. Evo; Evo: $F_{3,16} = 22.25$, $p < 0.97$). Consequently ecological change was calculated to contribute 90 % to the observed total phytoplankton abundance change in response to high CO_2 concentration, while

genotype sorting resulted in a negligible relative contribution of 0.5 % (Fig. II-5, calculations see methods 2.2 step 4). Finally, 9.5 % of the total change remained unexplained.

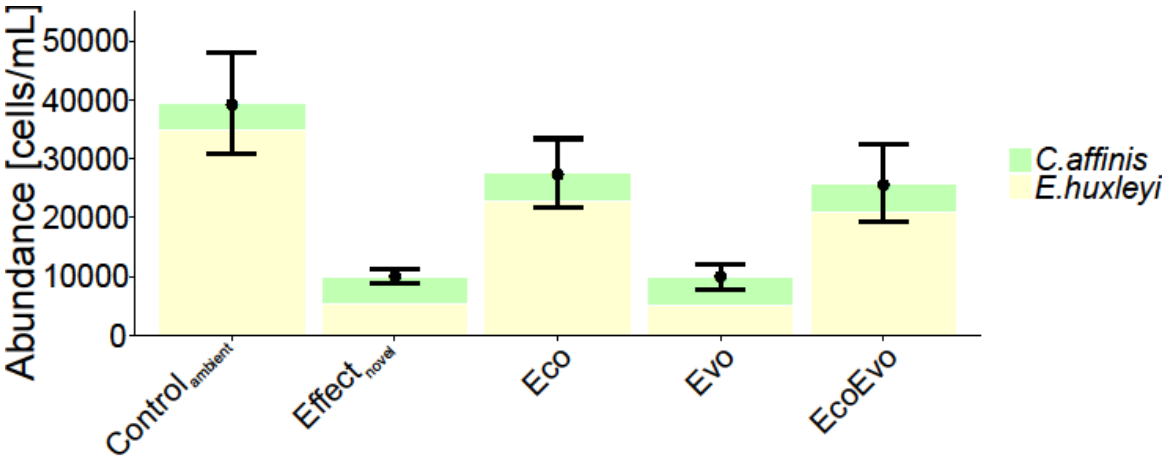

Figure II-3: Results of the Eco-Evo assay conducted with communities sorted for 50 generations. Mean of total abundance and standard deviation are shown for each treatment (n=5). Both $Control_{ambient}$ and $Effect_{novel}$ continue the compositional changes observed in the communities after 50 generations of sorting, while Eco, Evo and EcoEvo treatment, exclude species, genotype and both species and genotype composition changes in response to the novel environment, respectively (Fig. II-1, Eco-Evo assay, step 2). The mean contribution of the two species to total cell abundance is shown in the underlying bar charts.

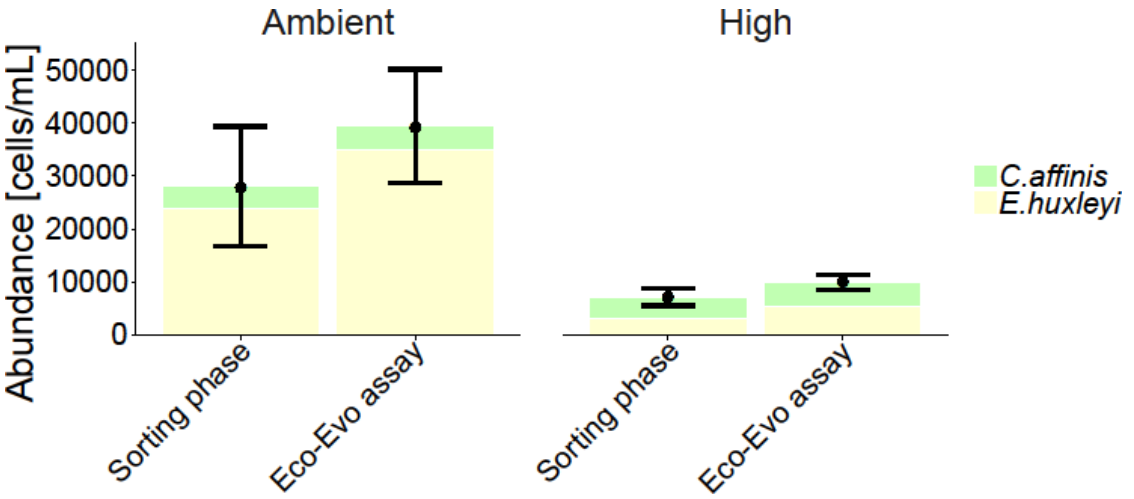

Figure II-4: Effect of artificial reassembling, without manipulating species or genotype frequencies, in the Eco-Evo assay under ambient and high CO_2 concentration compare to the respective community response found after 50 generations in the sorting phase. Mean and standard deviations, n=5.

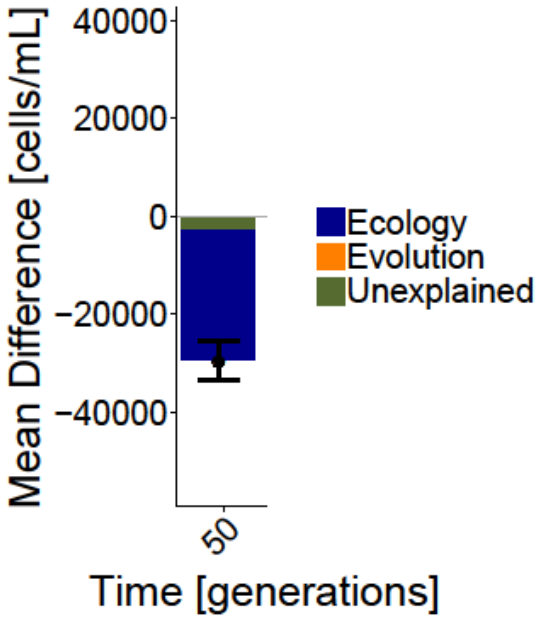

Figure II-5: Mean difference and its standard error of the abundance in communities sorted for 50 generations under ambient and high CO_2 concentration are shown (n=5). Underlying bar chart shows the relative importance of ecology and evolution to the total community changes in response to novel CO_2 environment and the unexplained variance.

Our example showed that the Eco-Evo assay technically works. Potential bottlenecks of i) unprecise artificial reassembly, or ii) potential shifts in species frequencies within the batch cycle of the assay, which could prevent detecting ecological and evolutionary relative abundance in the assay are not a concern. Those assay communities that were artificially reassembled, but not compositionally manipulated (i.e. $Control_{ambient}$ and $Effect_{novel}$) showed that in the assay species composition and the magnitude of the total change of the sorting phase was well reflected. The error made in artificially reassembling the communities is thus negligible. Further the initial composition left a strong signature to the end of the batch cycle, although small changes in species sorting occurred in response to the assay environment. Precisely, the $Control_{ambient}$, Eco and EcoEvo assay community starting with high *E. huxleyi* abundances showed this enhanced share at the end of the assay (Fig. II-3), approving that the assay allows detection of altered species and genotype composition. This is in line with other biodiversity ecosystem functioning experiments using phytoplankton, in which the species level has been initially manipulated and equal or significantly stronger effects of initial species composition compared to an environmental driver was shown (Eggers et al., 2014).

Comparing the compositional shifts in the sorting phase to the Eco-Evo assay outcome showed that the partitioning into ecological and evolutionary components was sound. In detail, the major ecological and negligible evolutionary contribution (Fig. II-5) into which the Eco-Evo assay partitioned the total cell abundance change under high CO_2 was largely confirmed by the observed strong species (Fig. II-2 A) and weak genotype compositional shifts (Fig. II-2 B) between the CO_2 environments after 50 generations in the sorting phase. Here, species frequency shifts propagated to a total abundance change, since the decline in *E. huxleyi* cells could not be balanced by *C. affinis*. Possible reasons are that, i) *C. affinis* was co-limited by silicate (Appendix II-2), and/or , ii) *C. affinis* low affinity for nutrients (Litchman et al., 2007) prevented the uptake of surplus nutrients emerging from the reduced *E. huxleyi* abundance, and/or iii) *C. affinis'* big size compared to *E. huxleyi* resulted in relatively lower cell abundance on the same amount of resources (Appendix II-3). Genotype composition shifted over time (Fig. II-2 B), and indeed happened on the same time scale as species frequency changes. The genotype frequency shifts were, however, not driven by the environmental driver applied, but more likely by other factors such as the competition for nutrients in the system (Listmann, 2018), and should therefore not be detected in the assay. The negligible evolutionary importance in this case is thus not an artefact of experimental duration, but reinforces the assay's functionality and applicability.

An additional comparison of the Eco-Evo assay results to those of existing numerical partitioning metrics to demonstrate the assay's functionality was not possible. Firstly, no numerical partitioning metric thus far allows for an assessment of a community property such as "abundance". Existing partitioning metrics generally require the calculation of abundance weighted mean traits. The calculation of abundance weighted mean traits is however not meaningful with community properties, as for example abundance would be weighed against itself. Second, existing numerical partitioning metrics with their unique definitions of ecological and evolutionary processes assign proportions of total change differently into ecological and evolutionary components (Govaert et al., 2016). For example, the same fraction of total change captured in the ecological component of the extended Geber method as used in Ellner et al. (2011), is in the reaction norm approach after Stocks (Stoks et al., 2016) assigned to plasticity and its evolution instead. This example highlights that a comparison of the partitioning by the Eco-Evo assay and a numerical partitioning metric will have different outcomes depending on which metric is used as a baseline and as such could not verify the assays functionality.

Discussion

The functionality of the proposed Eco-Evo assay to disentangle the importance of species and genotype frequency shifts for total observed community change was successfully tested using a simple model system. A wide spread application to plankton communities comprising other functional groups such as diazotrophs, mixotrophs, heterotrophs, and other calcyfiers and silicifiers and manipulating other environmental factors will likely improve our ability to predict future phytoplankton change (Litchman et al., 2012) and its propagating functional effects. The Eco-Evo assay thus allows to study relative contributions of ecological changes and evolutionary adaptation to phytoplankton change in response to climate change, which are often discussed to be important but in practice remained largely unknown. This knowledge gap could not be filled by inferring the relative contribution of ecological and evolutionary change to total phytoplankton community change, from existing studies in terrestrial and fresh water systems. These studies show idiosyncratic results depending, for example, on the system (Hairston et al., 2005), trait (response variable) of choice (Govaert et al., 2016; Stoks et al., 2016) and duration of exposure (Becks et al., 2012; Govaert et al., 2016) and cannot be condensed to a common pattern that allows extrapolations to other systems (Ellner, 2013; Rudman et al., 2017). Further, Collins and Gardner (2009) is, to the best of our knowledge, the only study that aimed to assess the relative importance of ecological and evolutionary processes for carbon uptake of a marine phytoplankton community. When applying the Price equation approach they, however, faced the problem that the required observations on genotype frequency shifts where not provided by their dataset. Therefore Collins and Gardner (2009) proposed that future studies could assume additivity of effects, which might not hold true if ecological and evolutionary processes interact and to calculate the evolutionary contribution indirectly, by subtracting physiological and ecological contribution from the total observed change in mean growth rate (Collins and Gardner, 2009). That this study is the only published approach to apply an existing numerical partitioning metric to marine phytoplankton highlights the difficulty and methodological constraints to gain insight in the relative contributions of ecological and evolutionary change to total community change.

The Eco-Eco assay does overcome this methodological constraint and is applicable to diverse marine phytoplankton communities. The concepts used to partition a total community change into ecological and evolutionary contribution in the Eco-Evo assay is comparable to the variance partitioning after Lepš et al. (2011) and reaction norm approach after Stoks (Stoks et al., 2016) for example. All above approaches quantify the difference of the total community change under a specific environment to the response of the same community while species compositional shifts or evolutionary

adaptation to the environment are excluded. The novelty of the Eco-Evo assay presented here is that the expected response of the community without changes on species or genotype level is not calculated but measured experimentally. This is an advantage in communities, where trait values cannot be obtained in-situ. Minimal prerequisites for the here presented Eco-Evo assay are a two-species community, with two genotypes each, which are exposed to an environmental driver with two levels. The empirical partitioning requires that the chosen species can be physically separated and reassembled into the assay communities. Using a model system whose organisms can be genotyped is not required. Phytoplankton populations naturally displays high levels of genetic diversity (Godhe et al., 2016; Lebret et al., 2012; Ruggiero et al., 2017; Rynearson and Armbrust, 2005). This results in a low risk to capture the same genotype twice even in a small volume of one L (Rynearson et al., 2006; Rynearson and Armbrust, 2005), even if genotypes are not assessed prior the start using genetic markers. Further the assessment of genotype frequency shifts is not required for the Eco-Evo assay. In other words, monitoring species changes is sufficient and makes the approach applicable to a wide range of phytoplankton communities.

In contrast to existing numerical partitioning metrics the presented experimental approach is not only possible for mean traits but also for community properties. Using community properties has recently been proposed to overcome the problem of attaining different relative importance of ecological and evolutionary contributions to mean trait changes, depending on the targeted trait (Govaert et al., 2016). Note that the choice of the community property can likewise influence the outcome. In our example, total cell abundance showed clear significant responses to elevated CO_2, while total biovolume (as proxy for biomass), showed less clear effects (Listmann, 2018). The change in cell abundance was largely driven by the smaller *E. huxleyi*, while the bigger *C. affinis* dominated changes in total biovolume (Appendix II-4). Effects were only found in cell abundances, since *E. huxleyi* abundance was reduced under elevated CO_2, while *C. affinis* abundance was unaffected. Thus, if a community change or the responses of its single species to a novel environment are unknown, a set of different community properties should be investigated. Correlations of the chosen community properties of interest with species abundances or biomass can elucidate which species mainly drives which changes (Appendix II-4). If different species dominate the regulation of particular community properties, their responses to a novel environment and underlying relative importance of ecological and evolutionary changes may diverge. Alternatively, the inclusion of a diverse set of community properties, while weighting these with regard to their importance for the specific research question, as done in functional diversity studies (Petchey and Gaston, 2006), will broaden the applicability of the proposed assay.

In conclusion, we successfully demonstrated partitioning and quantification of phytoplankton community change in response to high CO_2 into its ecological and evolutionary components using the novel Eco-Evo assay. Its potentially wide applicability, as well as the possibility to assess the relative importance of ecological vs. evolutionary processes repeatedly during progressing phytoplankton community change in response to environmental change, will advance studies on the relative importance and thus functional relevance of rapid evolution in ecological communities. Important next steps are thus the use of this empirical approach in diverse phytoplankton communities to better understand eco-evolutionary processes and to quantify their relative importance across marine ecosystems and time.

Comments and recommendations

The question is whether relative importance of ecological and evolutionary changes observed in a rather simplified community in the laboratory, is more than a proof of principle and is found in natural plankton communities in the field. This interesting question could be approached if the Eco-Evo assay is extended not only to test communities that were sorted in response to an environmental change in the laboratory, but in communities which are naturally exposed to an environmental gradient. This however requires finding cosmopolitan species, which can be separated from one another and are major players in communities along an environmental gradient. Alternatively to study the community change along a naturally accruing environmental gradient, it could also be of interest to study changes within one habitat by using dormant stages. The major challenge is, however, the physical separation of the different species in natural communities. Flow cytometric cell sorting could be applied, but would require downscaling of the assay to very small inocula or to culture the sorted species prior to the assay to obtain high enough abundances. Additionally the use of sorting fluid potentially impedes subsequent culturing of cells and should be considered.

Another potentially interesting extension of the herein proposed assay could be the inclusion of a component addressing phenotypic plasticity. Extending the Eco-Evo assay to further allow the separation of plasticity from the evolutionary component would, however, require that i) relative genotype abundances can be analysed directly prior the assay start and that ii) genotypes can be subsequently separated and artificially reassembled (Appendix II-5). Such extended applications of the assay will allow a better comparison with existing partitioning metrics, which include plasticity (Govaert et al., 2016; van Benthem et al., 2016).

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Chapter III:

Both ecology and evolution contribute to future phytoplankton community change

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Abstract:

Predictions of total phytoplankton community change in response to ongoing climate change require quantifying the relative importance of ecological and evolutionary contributions, which are currently unknown for most phytoplankton communities. Here, we exposed a phytoplankton community over 185 asexual generations to ambient and high CO_2 concentrations and assessed the relative importance of ecological vs. evolutionary processes for total community changes after short-, mid- and longer-term (until 50, 105 and >105 generations, respectively). We expected the relative contributions of ecology and evolution to change over time, with ecology as major contributor at first. Here we show that mid- and short-term total changes significantly depended on the CO_2 concentration and could be largely attributed to ecological changes. However, at mid- compared to short-term, the relative contribution of evolution increased. Over longer-terms community abundance increased while mean cell size decreased, irrespective of the CO_2 environment. In conclusion, future phytoplankton communities and the therein arising functional changes depend on both, ecology and evolution, yet the particular contributions vary temporally.

Introduction

Phytoplankton are a diverse group of globally distributed phototrophic marine microorganisms that are at the base of most marine food webs (Chavez et al., 2003; Sommer et al., 2002; Stibor et al., 2004). Climate change has the potential to change phytoplankton communities and the functioning they provide (Eggers et al., 2014; Lewandowska et al., 2014). Whereas seawater warming favours smaller phytoplankton groups, (Polovina and Woodworth, 2012; Sommer et al., 2016, 2015), e.g. picoplankton over diatoms, and can lead to a decline in total biomass/productivity (Boyce et al., 2010; Lewandowska et al., 2014), effects of increasing seawater CO_2 concentrations are more diverse. The varying effect sizes and signs of increased seawater CO_2 concentration found for different communities (Eggers et al., 2014; Paul et al., 2016; Schulz et al., 2017; Sommer et al., 2015) likely depends on the species present. In the concept of 'winners and losers', calcifying coccolithophores were shown to be negatively impacted by increased CO_2 concentration, while both larger diatoms and smaller picoplankton could profit from the higher supply of inorganic carbon (Bach et al., 2017; Kroeker et al., 2013). However, recent studies showed pronounced phenotypic differences in response to increased CO_2 concentration among genotypes within (Hattich et al., 2017) and between populations (Schaum et al., 2013). This genetic diversity possibly facilitates genotype abundance shifts and alter species sensitivity, which potentially result in community property changes (Collins and Gardner, 2009).

Evolutionary adaptation is a potentially important mechanism for phytoplankton to keep pace with climate change (Collins et al., 2014; Lohbeck et al., 2012; Rengefors et al., 2017). Both, adaptation via genotype sorting of standing genetic diversity and new mutations of a single genotype have been documented in response to increased seawater CO_2 concentration (Lohbeck et al., 2012, 2014) and temperature (O'Donnell et al., 2017; Schlüter et al., 2014). However, these studies observed adaptation in the absence of interactions with other species. This raises the question of the relative importance of evolutionary adaptation compared to ecological species frequency shifts in phytoplankton community change in response to climate change (Koch et al., 2014; Litchman et al., 2012). Especially under the consideration that ecological interactions such as competition between species or predator prey dynamics can alter the direction and strength of evolutionary selection (Collins, 2011; Kleynhans et al., 2016; Lawrence et al., 2012), and vice versa that evolution affects ecological dynamics (Becks et al., 2012; Fussmann et al., 2007; Hairston et al., 2005), this knowledge is fundamental.

A major gap in research addressed by this study is how total community changes in phytoplankton mean traits or community properties in response to changing environment are mediated by species and genotype abundance shifts, i.e. by ecological

and evolutionary changes. Using a community consisting of two coexisting species (*Emiliania huxleyi* and *Chaetoceros affinis*) with an initial genetic diversity of nine genotypes each exposed to two CO_2 concentrations (ambient and high) in a longer-term sorting experiment, we repeatedly assessed the relative contributions of ecological and evolutionary changes to community shifts with an experimental approach (Eco-Evo assay; Chapter II). Following the premise that community structure and functioning are jointly determined by ecological and evolutionary processes, we expect total abundance and mean size changes in response to CO_2 concentration to be driven by both ecological and evolutionary changes. However, based on the assumption that evolutionary changes require more time than shifts in species frequencies, we hypothesise 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.

Results

Over the experimental sorting phase of 36 batch cycles, 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 ten times higher compared to the onset (Fig. III-1 A; Time: $F_{1, 342} = 208.88, p < 0.001$). Over the same time mean cell size declined to a fifth of the initial cell size (Fig. III-1 B; Time: $F_{1, 342} = 353.04, p < 0.001$). Further, the time course revealed two distinct phases with CO_2 being a significant driver for total cell abundance and mean cell size in the short to mid-term (i.e. until 50 and 105 generations, respectively), but not in the longer-term (i.e. > 105 generations; Fig. III-1 A, B; Time*CO_2 for abundance: $F_{1, 342} = 326.29, p < 0.001$; Time*$CO_2$ for mean size: $F_{1, 342} = 327.91, p < 0.001$).

In the short to mid-term the total cell abundance in high CO_2 conditions was reduced to a third compared to ambient CO_2 conditions (Fig. III-1 A), which was driven by a decline in *E. huxleyi* abundance (Appendix III-1; Appendix III-2 B). This was an expected result given that growth rates (Rokitta and Rost, 2012) and relative abundances (Eggers et al., 2014) of calcifying phytoplankton have been shown to decrease in response to elevated CO_2 concentrations in other short-term studies. Mean cell size doubled in response to high CO_2 concentration (Fig. III-1 B, CO2: $F_{1, 342} = 142.55, p < 0.001$), driven by a shift towards a proportionally higher abundance of the larger *C. affinis* (Appendix III-3 B; Appendix III-4; Appendix III-5 B). A comparable increase in community cell size in response to CO_2 concentration has been previously attributed to a shift towards a higher proportion of larger diatoms (Sommer et al., 2015).

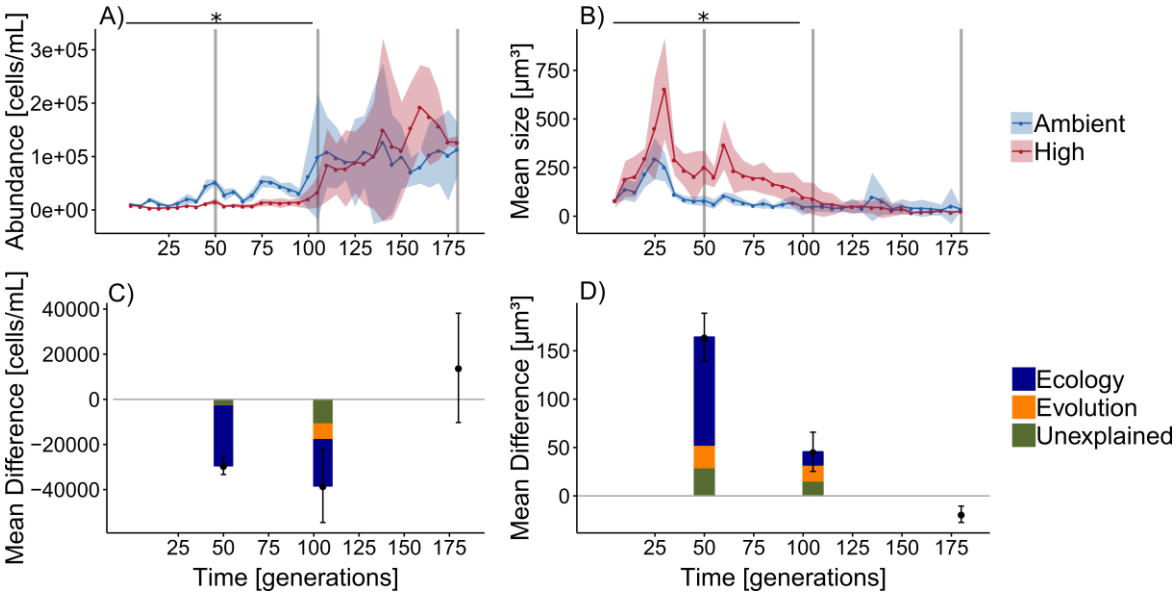

Figure III-1: Top graphs show community total cell abundance (A) and mean size (B) changes in response to ambient (400 ppm) and high (1250 ppm) CO_2 condition in the sorting phase (Mean and 95 % CI). Here, short to mid-term responses (until 50 and 105 generations, respectively) were significant different between CO_2 treatments (indicated by upper black line with asterisks) and in the longer-term CO_2 effect vanished (>105 generations). Lower graphs show outcome of the Eco-Evo assays undertaken using communities at a given time point in the sorting phase (depicted by the grey line in the upper graph) to quantify the ecological and evolutionary contributions to observed total changes in response to CO_2 condition. Calculated mean difference and its standard error of total cell abundance (C) and mean size (D) between the $Control_{high}$ and $Control_{ambient}$ community in the Eco-Evo assay are shown. The underlying bar charts show how much of this total change is explained by ecological and evolutionary changes. The relative importance was calculated from assay results (Appendix III-7 A, B; Appendix III-8 A, B), which was not valid at 180 generations as no significant total change or significant effects of species or genotype changes were found (Appendix III-7 C; Appendix III-8 C).

The relative importance of the, in the short- to mid-term, observed strong species shifts compared to genotype sorting for total community changes in response to high CO_2 concentration was quantified experimentally using an Eco-Evo assay. The Eco-Evo assay was based on the experimental exclusion of either species or genotype shifts or both (but see methods; Chapter II). In line with our expectations, we found that in the short-term ecological change largely explained the CO_2 driven total abundance and mean size changes while in the mid-term the contribution of evolutionary changes to total change slightly increased (Fig. III-1 C, D). Specifically, in the short-term 90 % of the total abundance decline in response to CO_2 concentration could be attributed to ecological changes (Fig. III-1 C). In the mid-term, only 53 % of the total abundance decline could be explained by ecological but 18 % by evolutionary changes, respectively (Fig. III-1 C). Similarly, in the short-term, 68 % of the increased mean cell size in the high CO_2 treatment could be explained by ecological changes and only 14 % by evolution.

In the mid-term, the relative contributions of ecological and evolutionary changes to mean cell size shifts equalled with 30 % and 36 %, respectively (Fig. III-1 D).

The absence of a total abundance and mean size change in response to CO_2 concentration in the longer-term could not be attributed to significant compensatory effects due to either ecological or evolutionary restructuring as shown by the Eco-Evo assay at generation 180 (Fig. III-1 C, D; Appendix III-7 C; Appendix III-8 C). As such neither species nor genotype abundances were significantly different between the CO_2 concentrations in the longer-term.

Discussion

In line with our predictions, this experiment showed that i) phytoplankton community properties and mean trait changes in response to climate change have both an ecological and evolutionary component, and ii) these changes are in the short- to mid-term largely driven by ecology, but with temporally decreasing importance. Unexpectedly, the effects of elevated CO_2 concentration vanished in the longer-term and no underlying ecological or evolutionary responses to CO_2 concentration could be found.

In contrast to our expectations, evolutionary changes were not too slow to become apparent in the short- to mid-term. Instead they occurred from the onset of the experiment but were independent of CO_2 concentration as a selection factor resulting in equal genotype sorting of *E. huxleyi* in both CO_2 environments (Appendix III-5 B, C; Listmann, 2018). In an analysis of evolutionary adaptation via reciprocal exposure experiments Listmann (2018) identified local experimental conditions characterized by nutrient fluctuations and strong nutrient limitation in the stationary phases as predominant selection factors on genotypes in this model system. However, this genotype selection could not be captured in the Eco-Evo assay since the changes happened irrespective of the CO_2 environment. This could explain the low contribution of evolutionary changes to total abundance and mean cell size changes in the short-term (Chapter II). The higher contribution of evolution in the mid-term was possibly caused by slight compositional shifts of the remaining genotypes of *E. huxleyi* between CO_2 concentrations (Appendix III-5 C). Not only richness, but compositional shifts can affect ecosystem functioning at the species level (Hillebrand et al., 2008; Hillebrand and Matthiessen, 2009). Generally, the weak selection force of high CO_2 concentration on genotypes of *E. huxleyi* was against the expectations considering that the genotypes used in this community displayed a diversity of phenotypic responses to CO_2 concentration (Hattich et al., 2017). This diversity was even greater than the diversity

among strains (Zhang et al., 2018) used in Lohbeck et al. (2012) who in fact observed strong genotype selection in *E. huxleyi* in response to enhanced CO_2 concentration. One possibility for this deviation could be the presence of the second species which could have altered the selection environment through competitive interactions. De Mazancourt et al. (2008) theoretically predicted lower evolutionary rates and rather species compositional changes to take place when pre-adapted species are present in a community. Among the two species, the diatom *C. affinis* was potentially favored by enhanced CO_2 concentration, since the mean plastic response expressed by a mix of genotypes was positive compared to *E. huxleyi* (Hattich et al., 2017). Against this reasoning, genotype selection of the here used populations occurred not only irrespectively of the CO_2 environment in communities but also in populations in the absence of the second species (Listmann, 2018). The alternative explanation for the low evolutionary contribution to the total community changes could be that the selective force of CO_2 concentration was overridden by nutrient conditions, producing always only one "winning" genotype of a species (Appendix III-5 A, C; Listmann, 2018). On the species level, CO_2 effects can be modulated or weakened by other drivers such as nutrients (Alvarez-Fernandez et al., 2018; Eggers et al., 2014; Paul et al., 2016). Here we demonstrated modulation of CO_2 effects by other factors (possibly nutrients) for the first time at the genotype level. The observed different selection pressure dominating sorting at different levels of biological organization was likely due to stronger intra- than interspecific competition for nutrients in this experiment. This finding raises the question whether species and genotype sorting are regularly driven by different selection factors, or if this finding is specific to CO_2 concentration and nutrients. Overriding effects of nutrient limitation also likely explained the observed shift towards smaller cells in general (i.e. for *E. huxleyi* as the smaller species and for a small *C. affinis*; Appendix III-5 B; Appendix III-4). Smaller cells are characterized by higher surface:volume ratios, and hence by higher nutrient affinity constituting a competitive advantage under nutrient limitation (Finkel et al., 2010; Marañón, 2014).

Evolutionary changes within species have the potential to alter ecosystem function. Over time, the increase in abundance and decline in mean cell size coincided with a dominance shift from *C. affinis* to *E. huxleyi* (Appendix III-5 B). The proportional increase of *E. huxle*yi was observed in all communities from mid-term to longer-term and raises the question, how the initially inferior species that was on top negatively affected by high CO_2 concentration could overcome these disadvantages and become superior under both environmental conditions. One possibility is that the selection force of CO_2 concentration on the genotype level was modulated - or overridden - by nutrient limitation, which resulted in the selection of the same genotype under ambient and high

CO_2 conditions in the longer-term (discussed in the previous paragraph and in detail in Listmann, 2018; Appendix III-5 A, C). Taken together, the simultaneous predominance of one *E. huxleyi* genotype and the dominance shift of species from the diatom to the coccolithophore (Appendix III-5) suggest that evolutionary changes in response to the nutrient regime led to the observed community composition independent of CO_2 concentration in the longer-term. As such, evolutionary changes in turn affected ecological changes and ultimately propagated to the community level. The consistently observed decline of calcifying coccolithophores in communities under increased CO_2 concentration (Eggers et al., 2014; Riebesell et al., 2017; Schulz et al., 2017) was consequently not found to hold true in the longer-term when both ecological and evolutionary changes were allowed. Similarly, the short to mid-term increase in mean cell size in response to high CO_2 concentration, which is also in line with literature (Sommer et al., 2016), was reversed in the longer-term. This size reduction was caused by the increased proportion of *E. huxleyi* (Appendix III-3 B; Appendix III-5 B) and as such likely resulted from the suggested effect of evolutionary changes on species sorting. Reliable predictions on phytoplankton mean trait and property changes are essential as for example such diverging effects on size structure observed in short- compared to longer-term can have strong implications on predicted future pelagic ecosystem function. Studies have shown that phytoplankton food webs are mainly size structured (Boyce et al., 2015) and that decreasing mean size can increase food chain length (Stibor et al., 2004), in turn decreasing transfer efficiency to higher trophic levels (Barnes et al., 2010).

In conclusion, the simultaneous investigations on ecological and evolutionary changes to shifts in community mean traits or properties in response to climate change does set evolutionary changes into an ecological context and further enhances our understanding of their relative importance and potential eco-evolutionary feedbacks. We could show that the relative ecological and evolutionary importance exhibits temporal variations and that shifts on the species and genotype level have indeed the potential to alter community mean traits and properties. Particularly, feedbacks between ecology and evolution have possible far reaching consequences on our capability to predict future phytoplankton community changes and its consequences for ecosystem functioning.

Methods

The experimental study first comprised a longer-term sorting phase, allowing the phytoplankton community to shift in species and genotype composition in response to predicted future increased CO_2 concentration and second, an Eco-Evo assay testing for the relative importance of species and genotype frequency shifts to the total observed community change.

Phytoplankton community and experimental set-up of sorting phase. The experimental community studied consisted of two coexisting species, the diatom, *Chaetoceros affinis* and the coccolithophore, *E. huxleyi.* To allow for genotype selection, each species was represented by nine genotypes. All genotypes used in this study were isolated from near shore waters of Gran Canaria in 2014 and 2015 (for detailed information for genotypes and dates of isolation, see Hattich et al., 2017). In the experimental sorting phase that started January 10th, 2017, the community was exposed to ambient and high CO_2 conditions, each five times replicated. Artificial seawater with a salinity of 35 psu was aerated for 24 h with CO_2-enriched air set to 400 and 1250 ppm to obtain ambient and high CO_2 concentrations, respectively. Nutrients were added to obtain final concentrations of 0.99 ± 0.09 µmol/L phosphate, 19.60 ± 0.54 µmol/L nitrate and 4.35±0.80 µmol/L silicate. This medium was sterile filtered (0.2 mm pore size) into 0.5 L polycarbonate bottles serving as experimental units. The sorting phase was carried out in a semi-continuous batch cycle design, at 20.99 ± 1.24 °C and a 17 L: 7 D 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 dusk and dawn, respectively. To allow for more natural conditions, the cells were held in suspension by constant rotation (0.75 min^{-1}) on a plankton wheel. At the onset, communities were inoculated with the same biovolume (2.75mio µm³) of *E. huxleyi* and *C. affinis* to overcome substantial differences in size. Within each species, genotypes were present in equal contributions (11 %). Thereafter, changes in species and genotype frequencies in response to the CO_2 environment were transferred to the next batch cycle. Precisely, every eight days, abundance, species composition and mean cell size (details see “community property “abundance” and mean trait “size”.”) were analysed from a Lugol’s iodine-fixed sample using an inverted light microscope (Zeiss, Axiovert 200 and Observer A1; 20 x and 40 x magnification) and consequently, a new batch cycle initiated by transferring an inoculum of 5.5mio µm³ to new media. Each batch cycle corresponded to approximately five generations and ran into the stationary phase, where strong competition for nutrients should have taken place.

Eco-Evo assay. The Eco-Evo assay is an experimental protocol developed to quantify ecological and evolutionary components to total community changes. The assay consists of five communities in total, manipulated on the treatment levels of genotype

composition, species composition and assay environment (here CO_2 concentration), and were five times replicated each. The $Control_{ambient}$ and $Effect_{novel}$ communities reflected and quantified the total community change in response to high CO_2 environment. The Eco, Evo and EvoEvo communities, respectively, excluded ecological changes, or evolutionary changes, or both at once to test for their contribution to the total observed changes in response to high CO_2 (Chapter II). Precisely, the Eco treatment excluded shifts in species abundances to high CO_2 concentration by directly manipulating species composition as observed in the ambient treatment while genotypes have been sorted according to high CO_2 concentration. In the Evo communities genotype frequencies were indirectly manipulated by exposing the mean genotype frequency resulting from selection under ambient CO_2 concentration to the high CO_2 concentration in the assay, while species composition was held as observed in the high CO_2 environment. The Evo communities consequently excluded genotype frequency shifts but also potential new mutations and plastic effects. The EcoEco communities combined manipulations of single Eco and Evo communities and simultaneously excluded species and genotype frequency shifts in response to high CO_2 concentration. The Eco-Evo assay was carried out after 50, 105 and 180 generations (i.e. short-, mid- and longer-term, respectively). This required the assessment of species frequencies and separation of species of the community over a 20 µm sieve before the assay communities could be reassembled. In order to calculate the relative importance of ecological and evolutionary change the difference of the Eco and Evo communities to the $Effect_{novel}$ communities was divided by the sum of the single Eco and Evo communities effects and the unexplained variance, U. This unexplained variance could result of interaction effects between ecology and evolution and potential error that could not be separated in this assay and was calculated as the difference between the total change measured in the EcoEvo communities and the sum of the changes measured in the Eco and Evo communities (Chapter II).

Community property "abundance" and mean trait "size". The effect of ambient and high CO_2 concentration on the phytoplankton community was assessed throughout the sorting phase as well as in the Eco-Evo assay in terms of changes in total abundance and mean cell size. The master trait cell size affects many processes as for example, nutrient uptake, edibility and sinking rates and thus, is functionally important (Marañón, 2014). The mean cell size of a community is the sum of the cell abundances per species multiplied with their respective size, divided by the total cell abundance. The combination of size and cell abundance affects the productivity, i.e. biomass that can be build up by a community, but was not assessed directly considering that opposing effects of size and abundance would be hidden. Both, abundance and size were analysed

with an inverted light microscope (Zeiss, Axiovert 200 and Observer A1). Size was calculated after Hillebrand et al. (1999) from the diameter/ width and length measurements of each five *E. huxleyi*/ *C. affinis* cells per replicate.

Statistical analysis and visualization. Abundance and mean size changes that happened over time in response to the CO_2 environment were analysed with a generalized least square model (GLS). The GLS was parameterized to account for an autocorrelation with time (correlation = corAR1 (form = ~1 | Time)). We account for heterogeneity between CO_2 conditions and time points ((weights = varIdent (form = ~1 | CO_2 * Time)).

To test whether the observed community change in response to CO_2 conditions was significant in the assay, the $Control_{ambient}$ and $Effect_{novel}$ communities were compared with an ANOVA. Further, the differences of Eco, Evo or EcoEvo communities compared to the $Effect_{novel}$ communities were tested using an ANOVA, with $Effect_{novel}$ as the intercept. This statistical analysis validated the calculation of the relative ecological and evolutionary importance from mean assay effects, if significant effects of changes on species or genotype level or total changes were found (Chapter II). To better understand their effect size, the relative ecological and evolutionary importance was shown in relation to the mean difference observed in the communities between ambient and high CO_2 conditions (Borenstein et al., 2009).

All data analyses, 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).

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General Discussion

Overall, this thesis provides an understanding of the plastic responses of single genotypes of species from two major groups of phytoplankton to environmental change, as well as important mechanistic insights into the relative contributions of ecological and evolutionary changes which underlie future total phytoplankton community change. It is well understood that phytoplankton community changes in response to environmental change result from unique environmental sensitivities of the major functional groups and that they can consequently alter marine ecosystems and the goods and services they provide for mankind (Litchman et al., 2015). However, the importance of adaptation for future community changes remained previously uncertain (Collins and Gardner, 2009; Litchman et al., 2012). Therefore, as a potential starting point for adaptation, in a first approach, I studied the phenotypic response diversity of three species of phytoplankton, originating from one community in the North Atlantic, to increased CO_2 concentration (Chapter I). In a second step, I introduced an experimental assay (Eco-Evo assay), which allowed quantification of ecological and evolutionary contributions to phytoplankton community property changes (Chapter II). In a third step, I applied the Eco-Evo assay to a phytoplankton community and demonstrated that both, ecological and evolutionary changes can translate to community functioning and that the relative contributions of ecology and evolution shift with time (Chapter III). In the following paragraphs, I will discuss the novelty and achievements of the single chapters of my thesis and their potential to improve predictions of future phytoplankton community shifts. Thereafter, I integrate knowledge gained from the single chapters into a larger framework.

Assessing plastic responses to predict adaptive potential and future shifts

My thesis sheds light on the controversy of intraspecific diversity among phytoplankton populations (Chapter I). Plastic responses of individuals or genotypes to climate change have been widely assessed in the past because they can uncover the adaptive potential of species and can ultimately be used to predict future ecosystem shifts by means of ecosystem models, for example. However, reported inconsistent responses and regional differences highlight the need to increase the study effort towards testing more genotypes of different populations (and species) to increase the predictability. For example, plastic responses of *Emiliania huxleyi* under enhanced CO_2 concentrations range from positive to negative, and further depend on other drivers such as nutrients, light availability and strain identity (Meyer and Riebesell, 2015). Consequently, individual plastic responses do not necessary reflect a mean species

response. Thus, future community shifts to enhanced CO_2 concentration predicted by a model that is parameterised with a single plastic response can strongly deviate from the conclusions reached when considering a species' mean response. Therefore Dutkiewicz et al. (2015) applied a meta-analysis to obtain the relevant response diversity around a mean CO_2 response of six major functional groups of phytoplankton to better parameterise their model. Nevertheless, although more reproducible and reliable in its outcome, this approach ignored the fact that plastic responses might additionally depend on the origin, and thus the experienced environment of a population. As an example, the response to enhanced CO_2 concentration of *E. huxleyi* and *Ostreococcus tauri* were shown to depend on their geographic origin (Schaum et al., 2013; Zhang et al., 2018). Notably, for *O. tauri*, site-specific differences in response to enhanced CO_2 concentration were even as great as differences between phytoplankton functional groups reported in the literature. This led to the suggestion that sampling location could better predict future responses to enhanced CO_2 concentration than relatedness (Schaum et al., 2013). In contrast, our study showed that the responses to enhanced CO_2 in phytoplankton species originating from one location in the North Atlantic were strongly dependent on species identity, i.e. the response to enhanced CO_2 concentration was more similar among the two tested coccolithophores species compared to the diatom species (Chapter I). Further the plastic responses of coccolithophores from different oceanographic regions (Dutkiewicz et al., 2015) are wider than the diversity of plastic responses of *E. huxleyi* and *Gephyrocapsa oceanica* populations from one single geographic region (Chapter I; Fig. 3A). Altogether considering regional differences of species/functional groups responses to environmental change has the potential to result in more realistic predictions of regional community shifts.

The herein tested assessment of mix-genotype culture plastic responses eases studying mean population responses and could reduce the existing data limitation (Chapter I). The precision of model predictions with increasing complexity through the inclusion of regional effects or multiple drivers for example, is to date, mainly constrained by the availability of data (Valladares et al., 2014). Because testing regional (population-specific) differences in the species responses to an environmental driver often requires unrealistic high numbers of experimental units, they are rarely tested. This, often unrealistic, number of experimental units mainly results from the need to test plastic responses of several genotypes in each of the investigated population to be able to calculate a reliable population plastic response to an environmental driver. Certainly, assessing the plastic response of one particular single genotype of a population does not necessary reflect the mean population response (Chapter I). My data show that the response measured in a mix-genotype culture (i.e. all genotypes of a

population in equal contributions) reflects the mean population response (Chapter I). Consequently, the effort needed to obtain a reliable population response could be reduced by the assessment of mix-genotype cultures. To illustrate the advantage of assessing population responses in mix-genotype cultures, I suggest this line of thoughts: Imagining one would like to test the population response to ambient and high CO_2 concentrations, across at least six geographic regions (ranging from arctic to tropics), in six species (one per functional group as used in Dutkiewicz et al. , 2015), by the separate assessment of ten genotypes each and a minimum replication of three, the experiment would contain 2160 experimental units. In contrast to this almost unfeasible approach, assessing the population specific responses in mix-genotype cultures (i.e. mixing genotypes of one region and functional group each) would require handling 216 experimental units only for the given example. Similarly, the assessment of species responses to multiple drivers, which is considered an important step in climate change research (Philip W. Boyd et al., 2018; Riebesell and Gattuso, 2015), quickly results in a high number of experimental units. Mean species responses to several environmental drivers could likewise be assessed by mix-genotype cultures. In conclusion, if only mean population responses/mean species responses to a set of environmental drivers are required, the herein proposed assessment of mix-genotype communities could fill the gap as it allows the assessment of mean population/ species responses in shorter time.

The trade-off of the assessment of mean population responses by mix-genotype cultures is, however, that the intraspecific diversity is not resolved. Following the recently suggested significant ecological importance of intraspecific compared to interspecific diversity (Des Roches et al., 2017), the interest in intraspecific diversity measurements likely increases. How to make such assessment of intraspecific diversity, which requires the assessment of plastic responses of several genotypes, feasible is, however, uncertain. High-throughput phenotyping is possible by automating *in vivo* fluorescence measurements in well plates and could aid the assessment of plastic (growth) responses of several genotypes (Gross et al., 2018). However, I think that the assessment of phenotypes in culture bottles is preferable since i) the larger volume of culture bottles allows the assessment of other important traits (e.g. nutrient uptake or stoichiometric changes) and ii) the possibility to rotate bottles allows for more natural growth conditions by holding cells in suspension. This is important since laboratory conditions can alter plastic responses of individuals (Meyer and Riebesell, 2015) and moreover the range of plastic responses displayed in a population. Indeed, growth rates displayed by the same subset of *E. huxleyi* genotypes in response to a comparable increase in CO_2 concentration was more diverse in the experiment presented in Chapter I than in a study by Zhang et al. (2018) with diverging growth conditions (Fig. 3 B).

Therefore, I want to highlight the importance of mimicking conditions in the laboratory, which are as close to natural abiotic conditions as possible to obtain meaningful plastic responses and would argue that automation is a good idea, but should rather be applied to bottle cultures than well-plates.

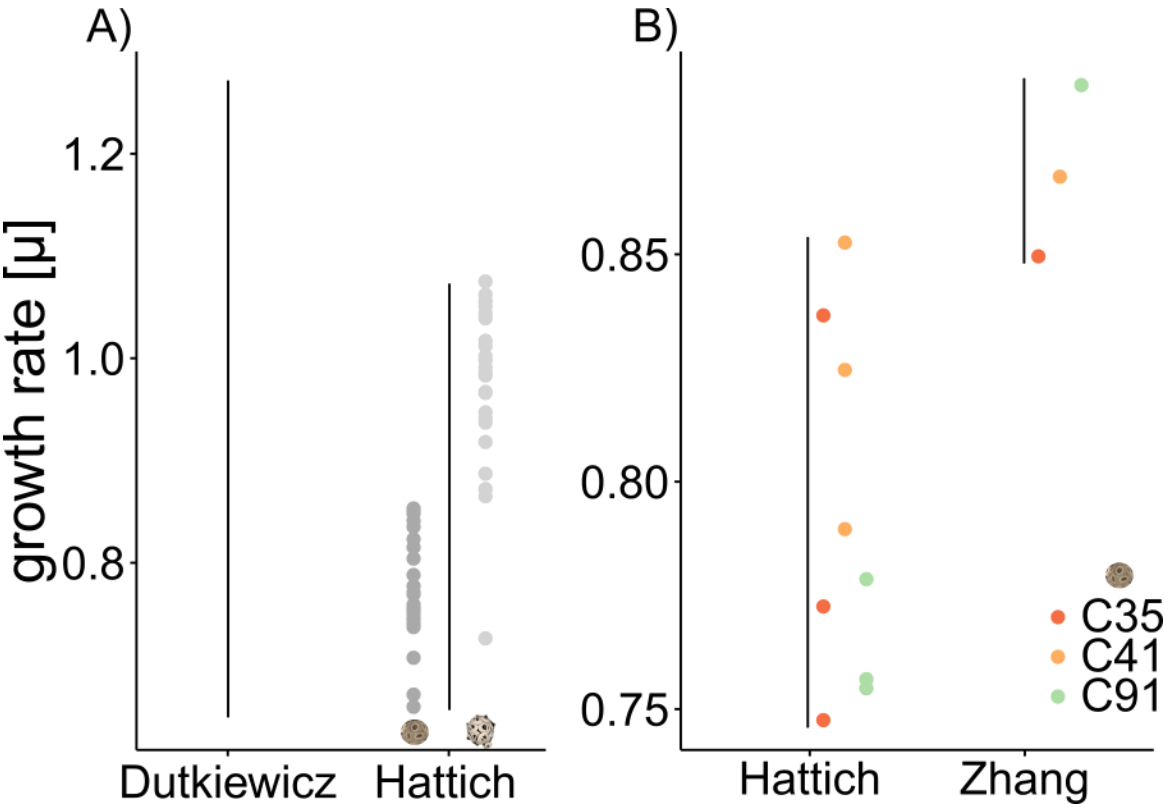

Figure 3: Comparison of the ranges of growth rates (black line) displayed by coccolithophores in response to enhanced CO_2 concentrations (700 - 1250 ppm) in different studies (Dutkiewicz et al. ,2015; Hattich et al., 2017 (Chapter I); Zhang et al., 2018). Growth rates are shown, since log response ratios would not capture the actual response diversity to enhanced CO_2. (A) In order to demonstrate that the overall range in responses, irrespective of origin of a species, does not reflect regional effects, the response range of coccolithophores reported in Dutkiewicz et al. (2015) is contrasted to the responses of the two coccolithophores, *E. huxleyi* (dark grey dots) and *G. oceanica* (light grey dots) reported in Chapter I of the present thesis (Hattich et al., 2017). The range of coccolithophores responses reported in Dutkiewicz et al. (2015) might be wider than in Hattich et al. (2017), since the responses of four coccolithophores species studied in the laboratory and as well as in mesocosms, with a variable range of CO_2 enhancement were included. (B) Further, the same *E. huxleyi* genotypes (indicated by different colours) display different response to enhanced CO_2 concentration depending on the laboratory conditions (i.e. nutrients and light availability, temperature, exponential versus stationary phase, constant versus manual rotation twice a day), which results in diverging phenotypic diversities (comparison Hattich et al., 2017 and Zhang et al., 2018).

Closing the gap – experimental quantification of ecological and evolutionary contributions to phytoplankton community changes

Even though the importance of understanding the relative contributions of ecological and evolutionary change to total phytoplankton community changes has been frequently highlighted (Collins and Gardner, 2009; Fussmann et al., 2007; Litchman et al., 2012), to date, no study succeeded in such quantification. Applications of existing partitioning metrics to total phytoplankton community changes were restricted by their data requirements, highlighting the need for novel partitioning approaches overcoming this limitation (see introduction; Chapter II). For example, Collins and Gardner (2009) attempted the quantification of ecological and evolutionary changes underlying a total phytoplankton community change using a Price equation metric. However, their approach lacked data on genotype frequency and trait value changes, thus not allowing to partition changes in carbon uptake of a phytoplankton community into ecological and evolutionary components. In another study, frequency of genotypes of one single freshwater phytoplankton species were tracked in response to enhanced CO_2 concentration (Collins 2011). By defining ecology as the competition among strains of *Chlamydomonas reinhardtii*, this study allowed partitioning of ecological and evolutionary contributions in freshwater phytoplankton in response to enhanced CO_2 concentration. As such, Collins (2011) assessed changes at the population, rather than at the community level. Moreover, I argue that selection of standing genetic diversity depends on competition among strains and as such competition among strains should be captured in the evolutionary and not ecological components.

The herein introduced Eco-Evo assay (Chapter II) was specifically developed to assess ecological and evolutionary contributions to community level changes and the ecological component therefore captures changes in species abundances. The main difference to existing metrics (see e.g. Govaert et al., 2016; Lepš et al., 2011) is that total community shifts resulting from ecological and evolutionary changes were assessed experimentally. Therefore, total community changes are not calculated as abundance-weighted mean trait changes from genotype trait- and genotype and species frequency-shifts. This is advantageous since firstly, the difficulty to measure genotype trait- and frequency-shifts get dispensable. Second, community properties can be partitioned, which is not meaningful if abundance-weighted mean changes are calculated. Consequently, the Eco-Evo assay is of broader applicability in phytoplankton communities, where measurements of genotype traits and frequencies are practically constrained and for the first time allows the partitioning of community properties.

My approach does not require measuring genotype frequency shifts and therefore increases the applicability to decompose total phytoplankton community

changes into ecological and evolutionary contributions. The trade-off is, however, that it does not allow for understanding the role of plasticity for adaptation (Lande, 2009; Levis and Pfennig, 2016). In the Eco-Evo assay, genotypes are manipulated indirectly, since frequencies are unknown and genotypes cannot be separated and reassembled. As a result, the evolutionary component not only captures genotype frequency shifts, but rather changes in intraspecific diversity, which could also be caused by plastic responses of genotypes or *de-novo* mutations. This limits the comparability to existing methods, which normally separate genotype frequency shifts from plastic responses (e.g. Price, 1970). However, in eco-evolutionary studies there are strong arguments against such separation calling to assess phenotypes rather than genotypes (Hendry, 2016, 2013). First, selection does not directly act on the genotype, but on the phenotype expressed, which depends on the genotype as well as the plastic response of the genotype in a specific environment (Hendry, 2016). As such, genotypes are only under indirect selection via the expressed phenotype. Second, a genotype does not have direct ecological effects on other organisms, but the expressed phenotype determines the ecological effect (Hendry, 2016). Additionally, I would argue that third, plasticity could be included in the evolutionary component as it can evolve (Schaum et al., 2016) and thus contribute to evolutionary changes. Fourth, the classification of genotypes with microsatellites is in natural communities, in my opinion, contagious since the number of genotypes differentiated depends on the number of microsatellite markers used (Debora Iglesias-Rodriguez et al., 2006). Further quantitative analysis and thus measuring frequency changes is still difficult. Specifically, both quantitative allele-specific quantitative polymerase chain reaction (Lee et al., 2016) and the here used (semi-) quantitative method via re-isolation require prior knowledge of genotypes present in a community (to develop genotype specific primers and to ensure that all genotypes present can be unambiguously identified with a set of microsatellite markers, respectively). All in all, this line of arguments for the study of phenotypes rather than genotypes, further argues for defining evolutionary changes as shifts in intraspecific trait variation. Nevertheless, differentiating between evolutionary and plastic responses could be possible if the Eco-Evo assay was extended by a plasticity component (only possible if genotypes can be analysed and separated; Chapter II). I, however, think that mechanistic insight into the role of plasticity for evolution cannot be gained from community responses and consequently the benefit of including a plasticity component is low. Specifically, community responses integrate plastic and evolutionary responses across species. Therefore, the (potentially diverging) responses of single species are unknown and inseparable and the role of plasticity for evolution of species cannot be understood by these measures.

Both ecological and evolutionary changes can contribute to functional shifts

This thesis bridges between eco-evolutionary and biodiversity ecosystem functioning studies. Eco-evolutionary studies, observing ecological and evolutionary contributions to changes in populations cannot be up-scaled to predicting shifts at the community level since ecological and evolutionary contributions to mean traits differ strongly between targeted traits, study duration and systems (Becks et al., 2012; Govaert et al., 2016; Hairston et al., 2005). The assessment of community properties over trait changes of target species in a community was therefore discussed to be more comprehensive as it results from the sum of all trait changes per species (Govaert et al., 2016), but community properties cannot be partitioned with existing metrics (Introduction, Partitioning total change into ecological and evolutionary contributions). Biodiversity ecosystem functioning studies have shown that ecosystem functioning depends on species, but also genotype diversity (Crutsinger et al., 2006; Prieto et al., 2015; Reusch et al., 2005). For example, species diversity was described to increase productivity via selection and complementary effects (Loreau and Hector, 2001). Likewise, increased intraspecific diversity in terrestrial plants (Crutsinger et al., 2006) and seagrass (Reusch et al., 2005) was found to increase their productivity. Moreover, the potential of intraspecific diversity to increase complementarity between species and ultimately alter selection effects among species has been demonstrated (Schöb et al., 2015). Species dominance was discussed to increase the importance of intraspecific diversity (Hillebrand et al., 2008). This potential of inter- and intraspecific diversity to impact one another highlights the importance of their simultaneous assessment and the need to uncover their relative contributions to ecosystem functioning.

Using the Eco-Evo assay (Chapter II), I could - for the first time - demonstrate that the mean trait and community property changes which were over time shifting in response to an environmental driver were significantly driven by both ecological and evolutionary changes and that their importance also differed with time (Chapter III). The strong total abundance decline in response to high seawater CO_2 concentration at the short- to mid-term (until 50 and 105 generations, respectively), and increases under both, ambient and enhanced CO_2 conditions in the long-term (>105 generations), were partitioned into considerable ecological and evolutionary contributions over time. Precisely, abundance changes were dominated by ecological changes in the short-term and evolutionary changes significantly contributed only in the mid-term. In the longer-term no underlying changes on species and genotype abundances in response to high CO_2 could be detected. Likewise, ecological and evolutionary contributions for mean size were found over time. Mean size was, however, adversely affected by CO_2 and increased

at the short to mid-term in response to the high CO_2 concentration while it thereafter decreased in both ambient and high CO_2 conditions.

Such ecological and evolutionary driven shifts in mean size and total abundance (Chapter III) are potentially important, since both changes in size and abundance are discussed to have far reaching impacts on ecosystem functioning (Barnes et al., 2010; Boyce et al., 2010). Precisely, Boyce et al. (2010) showed that increasing temperatures in the past century correlated with a 1% annual decline of mean primary production worldwide and discussed the potential of such reduction to alter fisheries yields and biogeochemical cycling. From the here presented data I would thus expect that the short- to mid-term abundance reduction could reduce fisheries yields. At longer-term this predicted effect on fisheries yields would, however, not hold true since total abundance under enhanced CO_2 concentration was no longer reduced in comparison to ambient CO_2 concentration. Phytoplankton size is important since plankton food-webs are mainly size structured and size changes can consequently result in functional shifts (Stibor et al., 2004). Precisely, a decline in phytoplankton size by 50 % (from 100 μm^3 to 50 μm^3), which is comparable to the here observed longer-term changes, reduces the size of associated grazers to 10 % of their initial size (1000 μm^3 to 100 μm^3; Boyce et al. 2015). A comparable decrease in phytoplankton size was shown to increase food-web length in mesocosm experiments from a three-level to a four-level food chain (Stibor et al., 2004), and thus reduced transfer efficiency (Barnes et al., 2010). While in the longer-term the size trend irrespective of CO_2 concentration could be expected to decrease transfer efficiency to higher trophic levels, the short- to mid-term responses in under enhanced CO_2 concentration could have adverse effects on the plankton food web. In summary, the opposing effects at short- and mid-term compared to the longer-term underline the importance of long-term studies, which allow for both ecological and evolutionary changes, to reach conclusive predictions of future ecosystem functioning.

Assessing a community from different perspectives helps understanding the underlying evolutionary changes and potential feedbacks on ecology

With this thesis I further showed that studying different levels of complexity in one particular system allows gaining novel insights that would possibly not have been available with a single view on the system. The applied microcosm experiments are still relatively artificial, but their great level of control enables mechanistic insights, making this a valuable tool in ecology (Srivastava et al., 2004). In this thesis I used microcosms to study plasticity of individuals (Chapter I), genotype sorting in populations (Listmann, 2018) and ecological and evolutionary changes in communities (Chapters II and III).

In Figure 4 I integrated the results of these experiments at different levels of complexity to answer a series of question (Questions 1-4), which can help explaining the overarching question: "What drives the observed comparably low importance of evolutionary changes for the total community change at short- and mid-term". Taken together, these experiments revealed a relatively low evolutionary contribution to total community change, probably resulting from a weak selection force of the applied driver (i.e. enhanced CO_2 concentrations) on the different genotypes. This might be caused by the overriding effects of nutrient limitation (see discussion in Chapter III). The observed low evolutionary contribution after short-term could have also been falsely attributed to low standing genetic diversity of the used population (Fig. 4, Question 1). The contrary, however, was demonstrated in the plasticity experiment, where a high level of variance between genotypes indicated a high standing genetic diversity (Chapter I). In fact, one would have expected a strong potential for adaptation (i.e. CO_2-driven selection upon genotypes), considering that genotype frequency shifts of a less diverse population of the same species *E. huxleyi* from Bergen, Norway were shown to be significantly affected by high CO_2 concentrations in laboratory experiments (Lohbeck et al., 2012; Zhang et al., 2018). There are two striking differences between the present study (Chapter III) and the adaptation experiment using the Bergen *E. huxleyi* population (Lohbeck et al., 2012). First, the presence of a second (competing) species and second the growth of the cultures into the stationary phase (Chapters II and III). The presence of a second species could have allowed the system to respond via species sorting, which may slow down evolutionary rates (De Mazancourt et al., 2008). However, genotype frequencies measured in this study showed strong genotype sorting over time, which interestingly occurred irrespective of the driver CO_2 (Fig. 4, Question 2; Chapter III). The possibility that competition with a second species has altered selection pressure could also be disproven: Genotype sorting of simultaneously grown mono-species cultures mirrored changes observed in communities (Fig. 4, Question 3; Listmann, 2018). However, all cultures of the present investigations (Chapter III; Listmann, 2018) were grown to the stationary phase, which consequently resulted in more natural conditions such as bloom formation under replete nutrient conditions ending in a stationary phase characterised by nutrient limitation (Fig. 4, Question 4; Chapter II and III; Listmann, 2018). Thus, nutrient limitation likely drove genotype selection and explains the low evolutionary contribution in the Evo-Evo assay, since this evolutionary response to nutrients was not captured in the evolutionary component explicitly testing changes in response to high CO_2 concentrations (Chapter II and III).

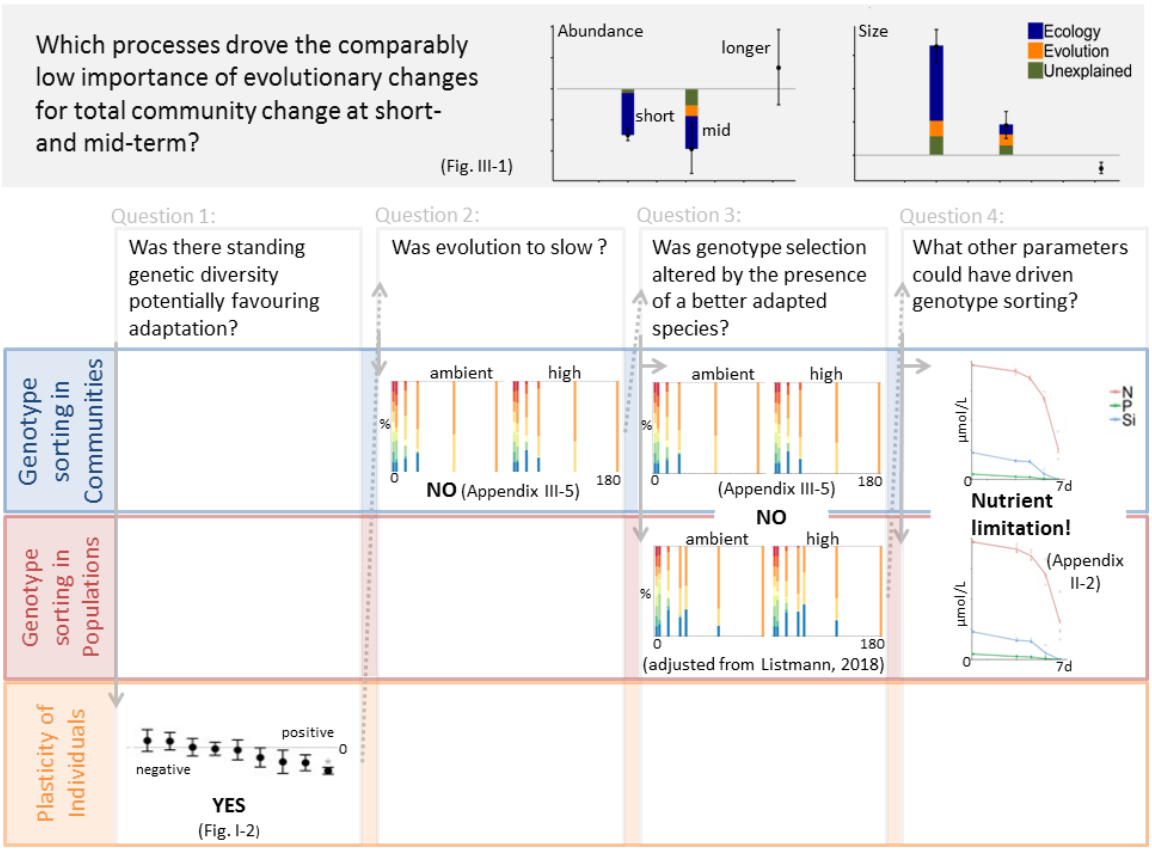

Figure 4: This figure integrates the results from the different experiments using the model community of *E. huxleyi* and *C. affinis*. The overarching question resulting from Chapter III is: "What drives the observed comparably low importance of evolutionary changes for the total community change at short- and mid-term?" (shown in top; Fig. III-1 from Chapter III). Tis overarching question could be answered when considering the plasticity of used individuals (Question 1; Fig. I-2 from Chapter I), genotype sorting in populations (Question 3, 4; graph adjusted from Listmann, 2018), genotype sorting in communities (Question 2, 3, 4; Appendix III-5 of Chapter III) and the nutrient conditions (Question 4; Appendix II-2 of Chapter II). These graphs show plasticity and sorting and of *E. huxleyi* only, since total abundance and mean size changes mostly correlated with this species (Appendix III-2; Appendix III-3).

The observed strong genotype selection, possibly in response to nutrient limitation in turn likely affected species composition. From mid- to longer-term the dominance of one *E. huxleyi* genotype coincided with strong shifts at the species level, i.e. a dominance shift from *C. affinis* to *E. huxleyi* (Chapter III). As a result, *E. huxleyi* was no longer affected by the applied CO_2 environment. This observed feedback from evolutionary to ecological changes is of high importance, since it ultimately led to longer-term compositional and functional changes. It is, however, important to note that coexistence of species was most likely enabled through the niche differentiation between velocity (the diatom) and affinity-adaptation (the coccolithophores; Sommer, 1984), but coexistence on the genotype level was not given. Instead, the results suggest that competitive exclusion due to nutrient limitation took place (Chapter III; Listmann,

2018). This exclusion could be caused i) by the microcosms, which did not provide more niches, or ii) by the present trait variation on the genotype level, which seemed insufficient to avert competitive exclusion by niche differentiation. Thus, the strength of competitive exclusion among genotypes might have been unnaturally high and the overriding effects of nutrients over CO_2 concentration did not exclusively reflect natural processes. Since coexistence theory, however, states that intraspecific competition must exceed interspecific competition to allow stable species coexistence (Chesson, 2000), the observed stronger intraspecific competition for nutrients and consequent overriding effects on CO_2 concentration might yet capture realistic responses. Either way, this thesis demonstrates the significance to mechanistically understand eco-evolutionary processes in phytoplankton communities, also with regard to potential feedbacks between ecological and evolutionary processes.

Outlook and Future Perspectives

This thesis provides an important step forward to increase our current understanding of the relative importance of ecological and evolutionary changes to total phytoplankton community changes, but future research effort is needed to refine the picture.

The mechanistic understanding of ecological and evolutionary processes gained from studies in controlled microcosms, should be combined with mesocosm or field studies of increasing complexity in the future. Theoretically, the Eco-Evo assay could be applied to mesocosm experiments which run long enough to allow for evolution and communities which are naturally exposed to an environmental driver. However, such application is limited by the ability to appropriately separate the single species from within a community. Before changes in natural communities can be assessed with the Eco-Evo assay, single-species separation (e.g. by flow cytometric cell sorting) and reliable culturing methods in the laboratory must be ensured. Additionally, a method to obtain high cell densities of the separated species must be developed to allow inoculating an Eco-Evo assay from natural communities. If these requirements cannot be fulfilled, for e.g. natural communities, the variance partitioning after Lepš et al. (2011) might be applied to quantify ecological and evolutionary changes. This metrics requires to assess inter- and intraspecific trait changes along an environmental gradient and consequently partitions total mean trait changes into the underlying relative contributions of this intra and interspecific changes (Lepš et al., 2011). Thus, variance partitioning after Lepš et al. (2011) is limited to community mean traits which can be assessed *in situ*. In phytoplankton communities the applicability is thus constrained to the assessment of

changes in the master trait ‘size’ (Marañón, 2014). Size was described to be altered under climate change (Li et al., 2009; Morán et al., 2010) and can be measured *in situ*. The variance partitioning after Lepš et al. (2011) could thus allow interesting insights. On the long run, however, the effort to partition other mean traits, and moreover community properties, should increase. Since the Eco-Evo assay is the only metrics allowing to partition community property changes into its ecological and evolutionary contributions, it will be of high importance to fulfil the requirements for the assessment of natural communities in the future.

Another next step must be the quantification of ecological and evolutionary contributions to property changes expressed by different communities in response to a suite of environmental drivers. This present thesis provided novel insights, however, data are based on the responses of one single and simplified community towards one diver, CO_2. Also indicated by the findings in this study, assessing the responses of phytoplankton communities to nutrient limitation could provide valuable new insights. Overall, nutrient limitation was a potential (unintended) strong driver of genotype sorting in this study. In open ocean regions, from the tropics to mid-latitudes, nutrients are expected to decrease in the future (Behrenfeld et al., 2006; Doney, 2006), and this might strongly control plankton communities (Lewandowska et al., 2014; Moore et al., 2013). Furthermore, studying ecological and evolutionary contributions to total community changes under multiple environmental drivers could be a valuable and desirable step toward to a better understanding of changes in the ocean (Boyd et al., 2018; Riebesell and Gattuso, 2015). Climate change research currently aims to increase realism by assessing multiple environmental drivers, which simultaneously affect communities (Boyd et al., 2018; Riebesell and Gattuso, 2015). Regardless of the specific environmental driver and community context, a wide-spread use of the Eco-Evo assay could ultimately allow extracting common patterns of ecological and evolutionary changes in phytoplankton communities.

Identifying universal eco-evolutionary mechanisms in phytoplankton communities could ultimately increase our understanding of phytoplankton community changes. This process is certainly facilitated by the integration of the presented Eco-Evo assay into experimental ecology. The assay fills a gap in the existing partitioning metrics as it allows for the first time to partition (phytoplankton) community properties. This thesis clearly showed that both ecological and evolutionary changes can contribute to total phytoplankton community mean trait and property changes, this, however, depended strongly on the time scale considered. Conclusively, I want to underline the importance to integrate both ecological and evolutionary processes in empirical studies in order to understand future phytoplankton shifts in their entire complexity.

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Appendix

Chapter I

Appendix I-1: Genotype isolation and genotyping 2

All genotypes used were isolated from the same geographical region in the North Atlantic (south-east off Gran Canary, Spain, 27°59'N 15°22'W) between spring 2014-spring 2015. All genotypes of one species, with one exception, were isolated at once from a 2L seawater sample. More specifically, the *C. affinis* genotypes were isolated in December 2014, while *G. oceanica* was isolated in February 2015 and *E. huxleyi* were collected and isolated in February 2014 (except for GC22 which was isolated in February 2015). All genotypes used in this experiment will be deposited in the Roscoff Culture Collection (http://roscoff-culture-collection.org/; Table I-1-1).

All coccolithophore strains (i.e. *E. huxleyi* and *G. oceanica*) were genotyped using microsatellite (msat) analysis. For *C. affinis* the msats are still under development. We nevertheless assume that all isolated single strains belong to a different genotype. Firstly, isolation of genotypes of all species followed the same protocol. Secondly, literature has shown that isolation of the same genotype from an amount of water, like we sampled in this study, is unlikely (Evans et al., 2016; Rynearson and Armbrust, 2005).

For microsatellite analysis DNA was extracted by adding 10 µL of TE-buffer to resuspend pellets of coccolithophores. Samples were then sonificated for 3 minutes at 100% and then incubated for 1h at 56°C. Microsatellite amplification was done in the following reaction mix: 2.5µL multiplex mastermix (Qiagen), 0.25 µL 5pM forward and reverse primer (primers EHMS15b and P02E10b for *E. huxleyi* (Debora Iglesias-Rodriguez et al., 2006), primers GE06 and GE07 (Table I-1-2.) for *G. oceanica*), 1 µL Q solution (Qiagen), 0.5 µL H2O and 0.5 µL of DNA template. The PCR reaction run for msat amplification was set up as follows: an initial phase of 15min at 95°C, 30 cycles of 30sec at 94°C, 90sec at 57°C and 1min at 72°C and final step of 30 min at 60°C. 1 µL PCR products was then added to a mix of ROX and Hidi (Qiagen) of 0.25 µL and 8.75 µL respectively and incubated for 3min at 94°C to denature double stranded products. The sequencer 3130xl Genetic Analyzer (Applied Biosciences) was then used to analyse microsatellite composition of each sample and data was analysed using GeneMarker software.

Table I-1-1: All genotypes used are listed in the column "manuscript" under the name used in here and will be deposited in the Roscoff Culture Collection (RCC) under the name shown in the "RCC" column.

		E. huxleyi		*G. oceanica*	
Manuscript	**RCC**	**Manuscript**	**RCC**	**Manuscript**	**RCC**
B13	EHGLL13B	**C48**	EHGKLC48_20	**GC31**	EHGLLGC31
B75	EHGLL57B	**C30**	EHGKLC30_20	**GC33**	EHGLLGC33
B63	EHGLL63B	**C35**	EHGKLC35_20	**GC36**	EHGLLGC36
B64	EHGLL64B	**C91**	EHGKLC91_20	**GC40**	EHGLLGC40
B67	EHGLL67B	**C96**	EHGKLC96_20	**GC58**	EHGLLGC58
B68	EHGLL68B	**C47**	EHGKLC47_20	**GC59**	EHGLLGC59
B74	EHGLL74B	**C41**	EHGKLC41_20	**GC60**	EHGLLGC60
B81	EHGLL81B	**C42**	EHGKLC42_20	**GC86**	EHGLLGC86
B82	EHGLL82B	**GC22**	EHGLLGC22	**GC89**	EHGLLGC89

Table I-1-2: Primers used for *G. oceanica* msat analysis (strains N=9). Transcriptome as basis stems from Marine Microbial 28 Eukaryote Transcriptome Sequencing Project (MMETSP; Keeling et al., 2014) and is called Gephyrocapsa-oceanica-RCC1303

Primer name	**Sequence**	**Product length (from test)**	**Msat repeat unit**	**No. Alleles**
GE06Fm	GTAATTGTCGTACGCCCG	162	(CT)17.5	8
GE06Rm	CCAGGAATAGACTTAGGCCG			
GE07Fm	TGTCTCAGAGTCTCGCGG	189	(TC)19	8
GE07Rm	GAGTTTGTGGCTGTCCTT			

Appendix I-2: Relative contribution of genotypes in mixcultures

The mixcultures consisted of all genotypes of one species that initially contributed the same number of cells/mL. The relative contribution of each genotype was therefore 11 % at the start of the experiment (Fig. I-2-1). To avoid differences in initial concentrations of the respective genotypes', the mixcultures were inoculated from a stock mixculture prepared just before the start of the experiment. As in the monoculture each experimental unit was inoculated with an initial total biovolume of 8280 $\mu m^3 * ml^{-1}$ cells, resulting in an initial concentration of 20 cells$*ml^{-1}$, 180 cells$*ml^{-1}$ and 24 cells$*ml^{-1}$ for *C. affinis*, *E. huxleyi* and *G. oceanica*, respectively.

Change in the relative contribution of genotypes in the mixcultures was not tested over the course of the experiment. While we assumed that the relative genotypic contribution changes over time (Roger et al., 2012), we, however, did not expect an actual loss of genotypes in this experiment with only one batch cycle. Personal experience with the same *E. huxleyi* genotypes showed that a significant change in relative abundance of the genotypes occurs after an experimental duration of four batch cycles while only one genotype was actually lost after this time. This suggests that genotypic exclusion in *E. huxleyi* takes longer than the experimental duration of this study. It also concurs with Lohbeck et al. (2012) demonstrating that after 160 days

(corresponding to ~ 30 batch cycles) only half of the initially present six *E. huxleyi* genotypes were lost (Lohbeck et al., 2012). The maintenance of genotypes in comparatively short-termed experiments can, however, also depend on the number of initially present genotypes. Sjöqvist and Kremp (2016) showed that all genotypes in *Skeletonema marinoi* mixcultures remained present if cultures started with lower genotypic richness (i.e. 5 genotypes). In contrast higher initial genotypic richness (i.e. 20 genotypes) enhanced exclusion (Sjöqvist and Kremp, 2016). The use of nine genotypes in this study corresponds to a midrange genotype richness compared to Sjöqvist and Kremp´s study, and thus supports that genotype exclusion is expected to be low or almost negligible in our short term experiment.

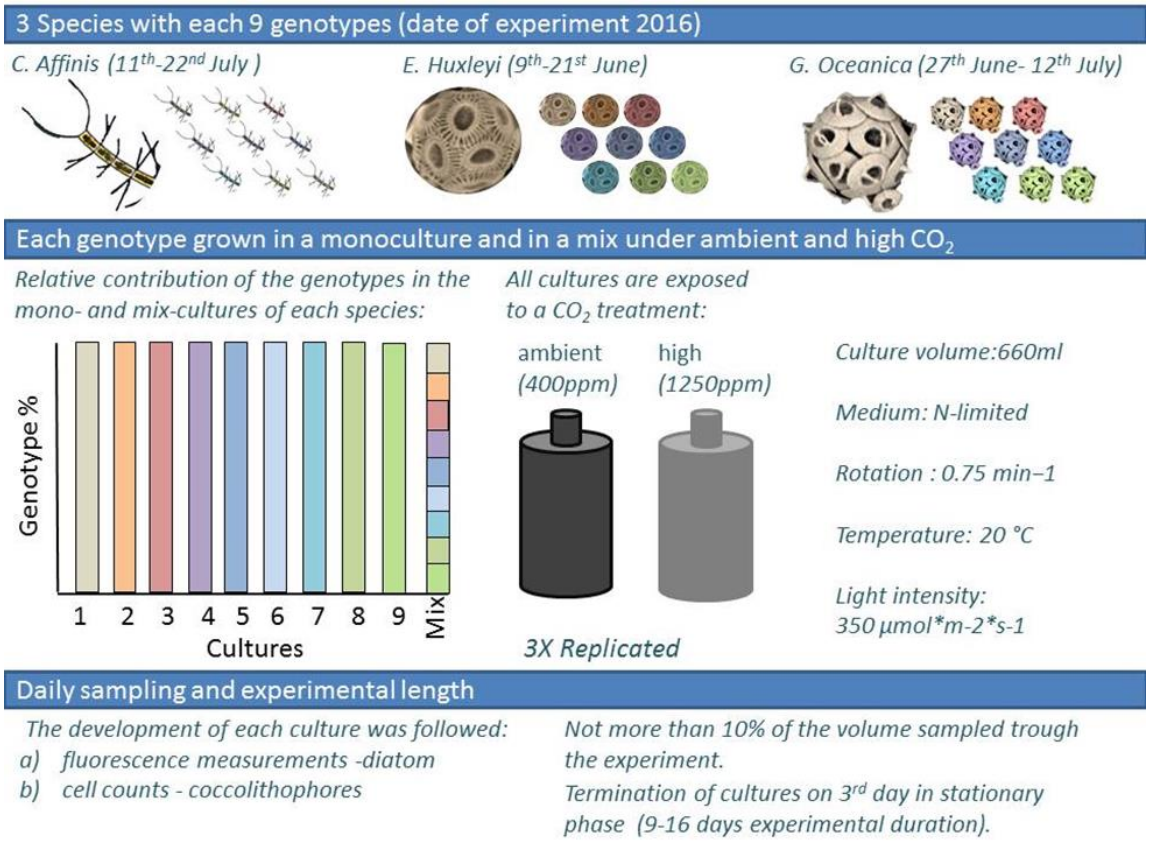

Figure I-2-1: Overview of the experimental set up. Species and genotypes at the top, followed by general information of the cultivation as well as the CO_2 treatments and genotype composition in Mono- and Mixcultures. At the bottom are information about daily sampling as well as experimental length.

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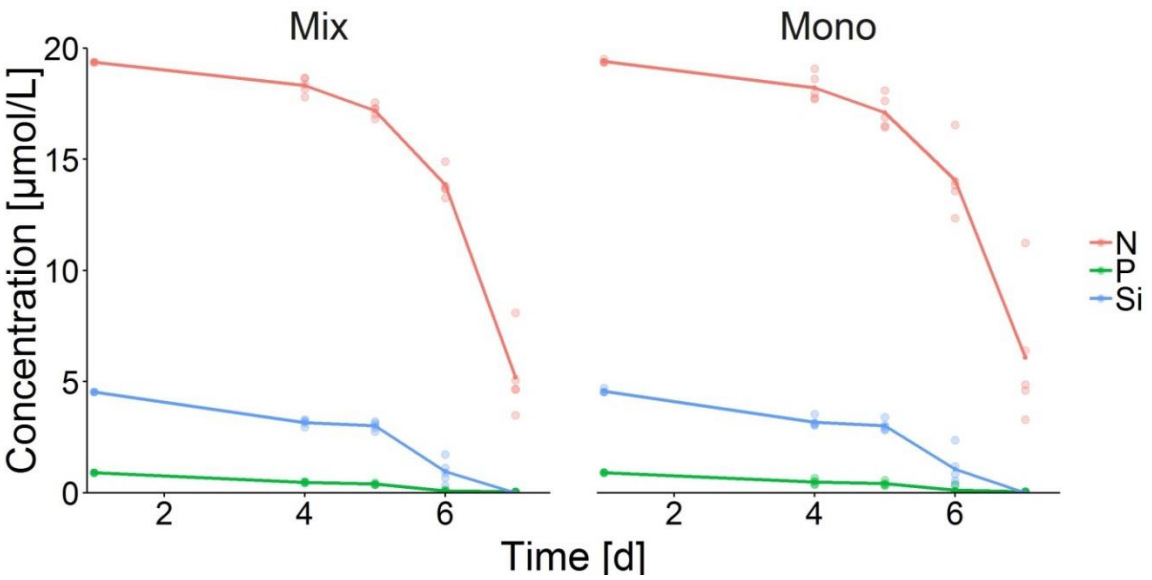
Chapter II

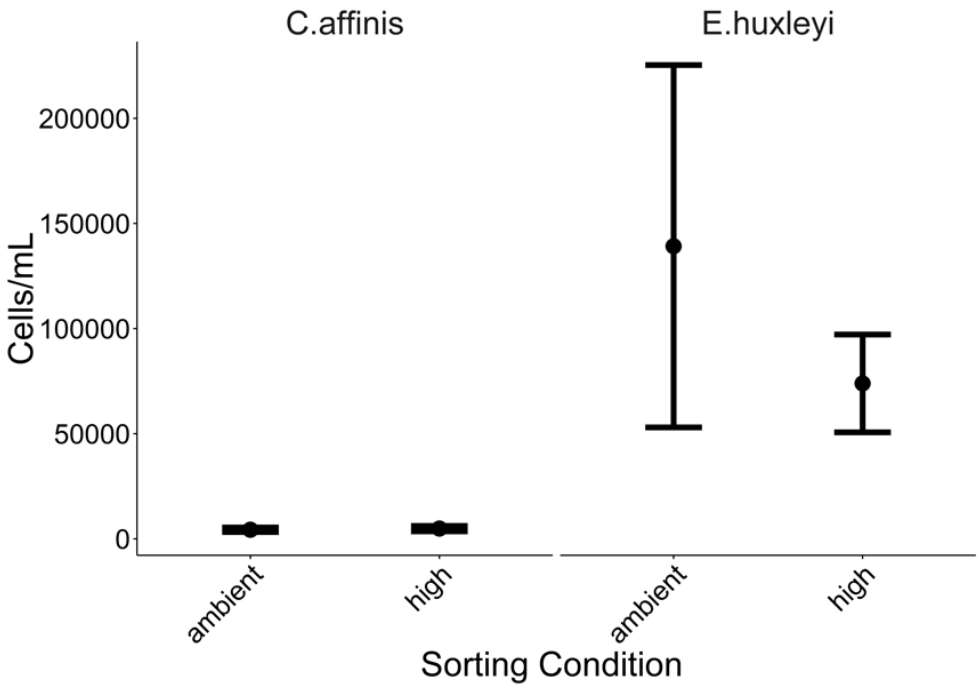
Assay treatment	Assay No	a) Genotype composition	Genotype composition from sorted community No.	*E. huxleyi* abundance in sorted community [cells/mL]	*C. affinis* abundance in sorted community [cells/mL]	*E. huxleyi* abundance in sorted community [µm³/mL]	*C. affinis* abundance in sorted community [µm³/mL]	b) Species composition	*E. huxleyi* aim relative abundance [%]	*C. affinis* aim relative abundance [%]	c) Assay environment (CO_2)	*E. huxleyi* inoculated [mL]	*C. affinis* inoculated [mL]
$Control_{ambient}$	10	ambient	3	42669	5933	1335499	3540178	ambient	0.27	0.73	ambient	1.12	1.12
$Control_{ambient}$	34	ambient	6	53291	5234	1332454	2180537	ambient	0.27	0.73	ambient	1.13	1.82
$Control_{ambient}$	29	ambient	14	46149	6455	1000889	1817473	ambient	0.27	0.73	ambient	1.50	2.18
$Control_{ambient}$	37	ambient	21	42120	5993	1018691	3021520	ambient	0.27	0.73	ambient	1.47	1.31
$Control_{ambient}$	15	ambient	24	45416	5949	928061	4260811	ambient	0.27	0.73	ambient	1.62	0.93
$Effect_{high}$	28	high	5	7104	5185	112100	4148421	high	0.06	0.94	high	2.99	1.24
$Effect_{high}$	25	high	13	14274	6730	323186	3149374	high	0.06	0.94	high	1.04	1.63
$Effect_{high}$	26	high	16	4267	5317	84592	2533010	high	0.06	0.94	high	3.96	2.03
$Effect_{high}$	8	high	23	13702	5366	362806	3586212	high	0.06	0.94	high	0.92	1.43
$Effect_{high}$	18	high	30	10249	5245	247045	3883721	high	0.06	0.94	high	1.36	1.32
Eco	19	high	5	7104	5185	112100	4148421	ambient	0.27	0.73	high	13.40	0.96
Eco	5	high	13	14274	6730	323186	3149374	ambient	0.27	0.73	high	4.65	1.26
Eco	35	high	16	4267	5317	84592	2533010	ambient	0.27	0.73	high	17.75	1.56
Eco	7	high	23	13702	5366	362806	3586212	ambient	0.27	0.73	high	4.14	1.11
Eco	33	high	30	10249	5245	247045	3883721	ambient	0.27	0.73	high	6.08	1.02
Evo	17	ambient	3	42669	5933	1335499	3540178	high	0.06	0.94	high	0.25	1.45
Evo	27	ambient	6	53291	5234	1332454	2180537	high	0.06	0.94	high	0.25	2.35
Evo	31	ambient	14	46149	6455	1000889	1817473	high	0.06	0.94	high	0.33	2.82
Evo	21	ambient	21	42120	5993	1018691	3021520	high	0.06	0.94	high	0.33	1.70
Evo	30	ambient	24	45416	5949	928061	4260811	high	0.06	0.94	high	0.36	1.20
EcoEvo	1	ambient	3	42669	5933	1335499	3540178	ambient	0.27	0.73	high	1.12	1.12

Appendix II-1:

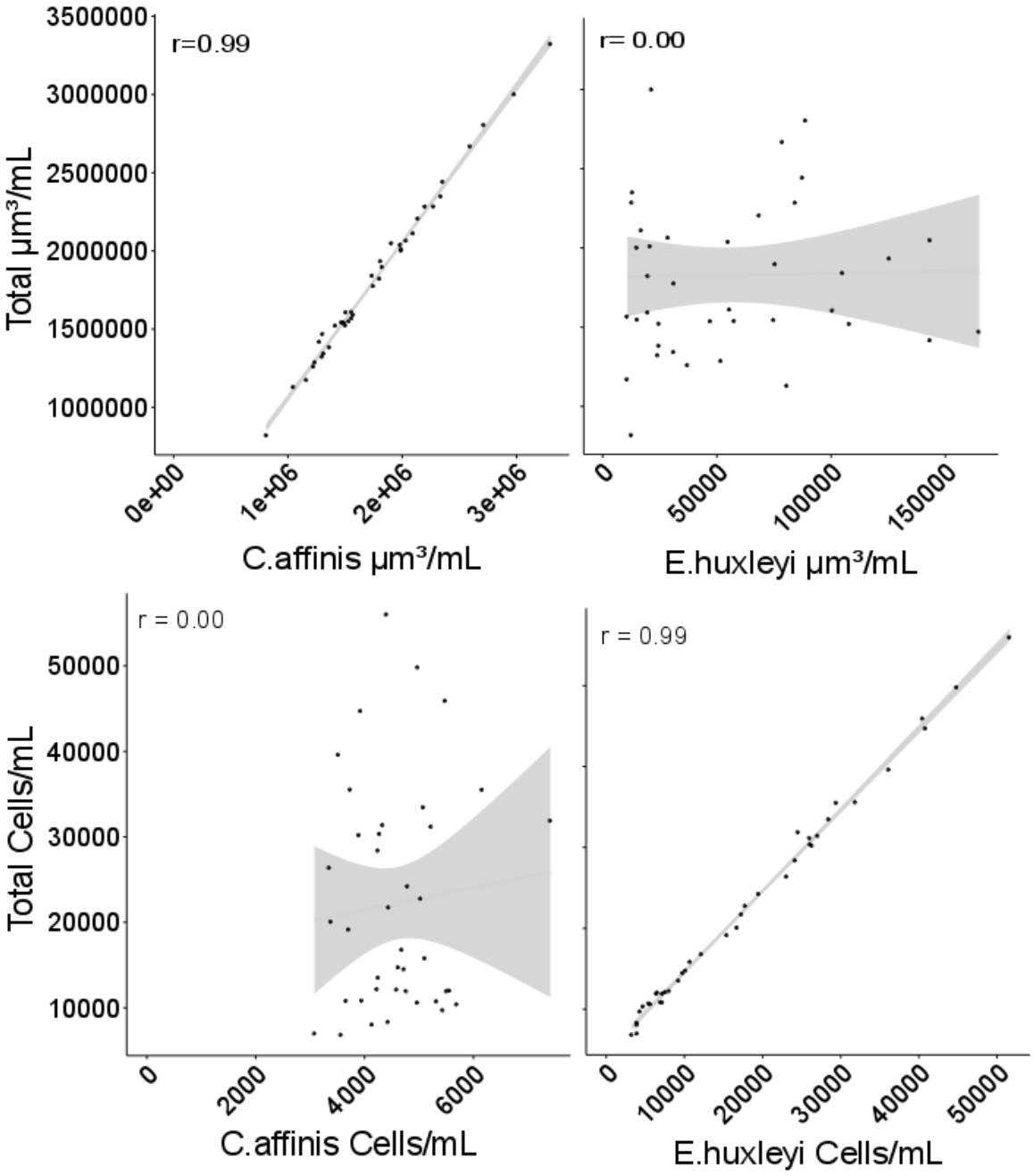
Table with details on how the assay communities were combined and inoculated.
E. huxleyi inoculated [ml] = (1/ *E. huxleyi* abundance in sorted community [µm3/mL])*(to transfer Volume of 5.5million * *E. huxleyi* aim relative abundance [%])
C. affinis inoculated [ml] = (1/ *C. affinis* abundance in sorted community [µm3/mL])*(to transfer Volume of 5.5million * *C. affinis* aim relative abundance [%]).



Appendix II-2: Figure showing an exemplified nutrient uptake (nitrate, phosphate and silicate) by a community of *C. affinis* and *E. huxleyi* in the Mix and Mono cultures of *C. affinis*. In batch cycle 11 running simultaneously to the Eco-Evo Assay, nutrient samples of each 3 replicates were taken at day 4, 5, 6 and 7, sterile filtered, stored in the freezer until analysis with a SAN++ System from Skalar. At the start of each batch nutrients were added to the final concentrations of 19.59 ± 0.65 µmol/L nitrate, 0.97 ± 0.09 µmol/L phosphate and 3.81 ± 0.55 µmol/L silicate.



Appendix II-3: Figure displaying an example abundance of *C. affinis* and *E. huxleyi* in Mono cultures, including the same genotypes and being exposed to the same laboratory conditions. Data from batch cycle 11 running simultaneously to the Eco-Evo Assay. N=5; Mean and 95 % CI.

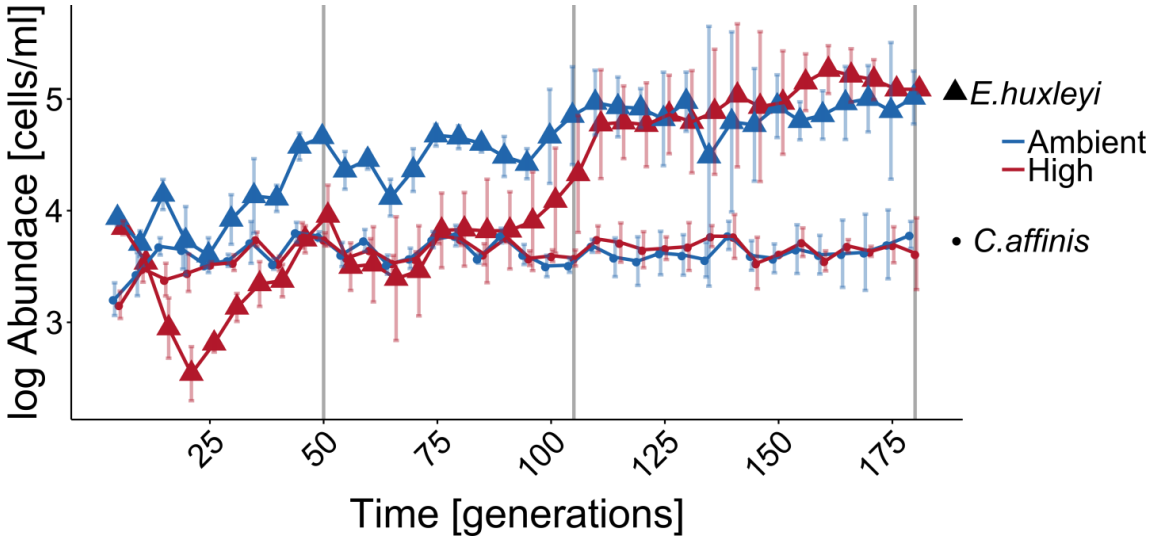


Appendix II-4: Figure shows correlations of total biovolume (µm³/mL) to the biovolume of *C. affinis* (µm³/mL) and *E. huxleyi* (µm³/mL) separately (shown in the top left and right, respectively). Lower graphs show the correlation of total cell numbers (Cells/ml) to the abundance of *C. affinis* and *E. huxleyi* (cells/mL; left and right, respectively). Data are taken from the end of the assay of all 40 experimental units; strength of correlation are shown at the top left of each graph.

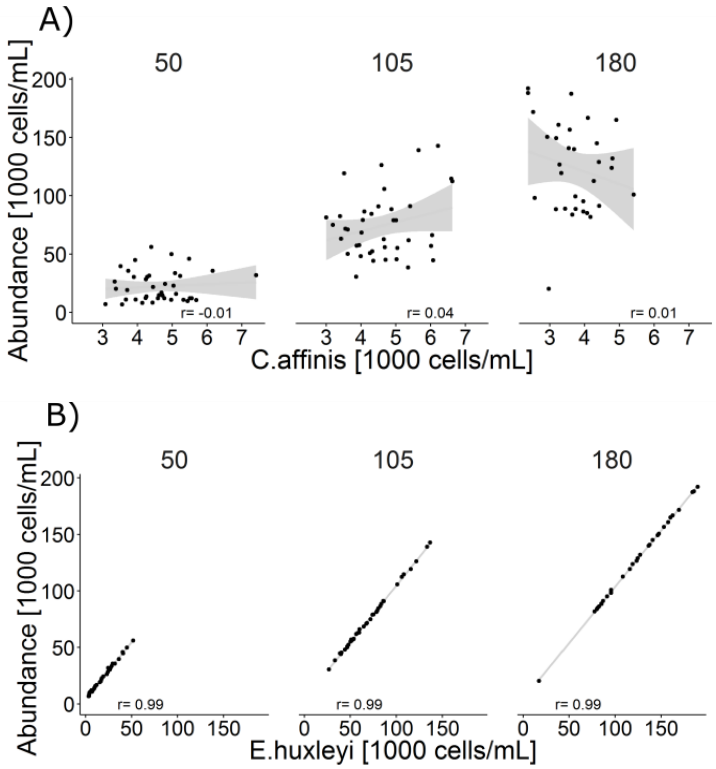
Appendix II-5: Table with the extension of the Eco-Evo Assay, which includes Plasticity.

	$Control_{ambient}$	$Effect_{novel}$	Plasticity	Eco	Evo	EcoEvo+ Plasticity
Plasticity	ambient	novel	ambient	novel	novel	ambient
Genotype composition	ambient	novel	novel	novel	ambient	ambient
Species composition	ambient	novel	novel	ambient	novel	ambient
Assay environment	ambient	novel	novel	novel	novel	novel

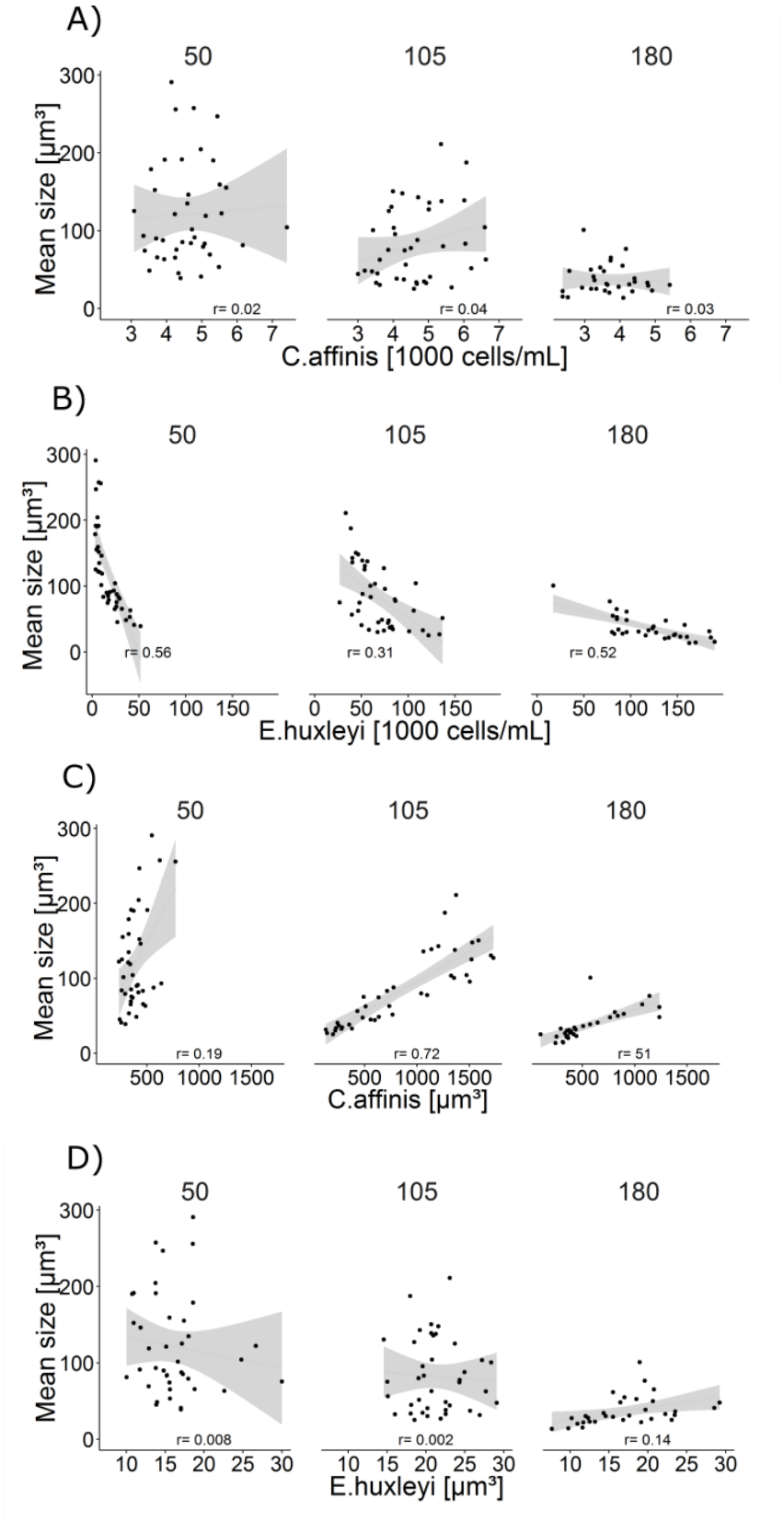
Chapter III



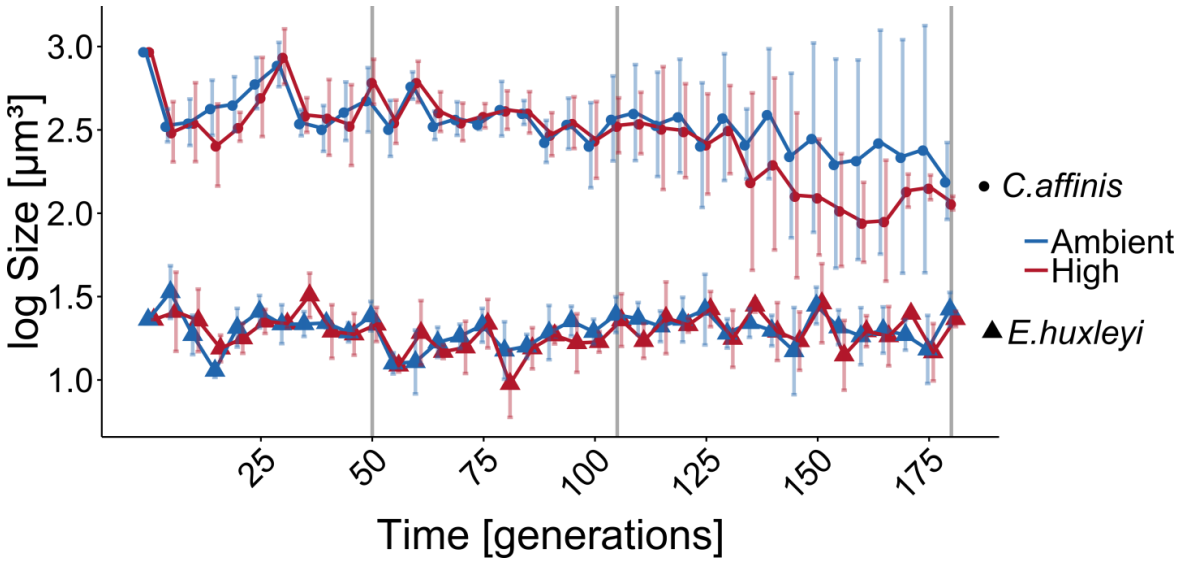
Appendix III-1: Graphical display of the abundance of *C. affinis* and *E. huxleyi* in the communities held under ambient and high CO_2 over time (mean and 95 % CI). To be able to see fluctuations in both species, with quite diverging abundance, the log to the basis of 10 is shown.



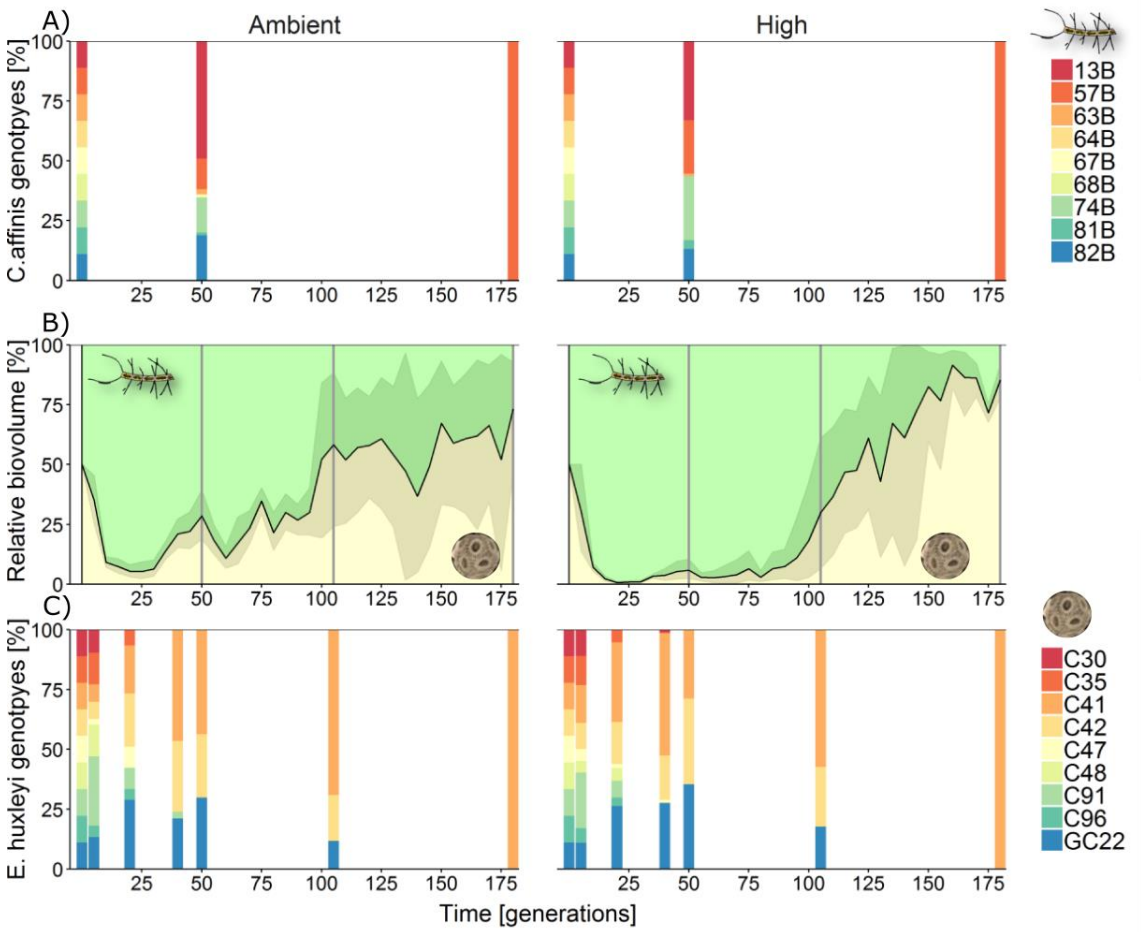
Appendix III-2: Figure showing the correlation of total abundance with *C. affinis* abundance (A) and *E. huxleyi* abundance (B) and in the Eco-Evo Assay with communities sorted for 50, 105 and 180 generations.



Appendix III-3: Graphical display of the correlation of mean community size with *C. affinis* abundance (A) and cell size (C) and *E. huxleyi* abundance (B) and cell size (D) in the Eco-Evo Assay with communities sorted for 50, 105 and 180 generations.



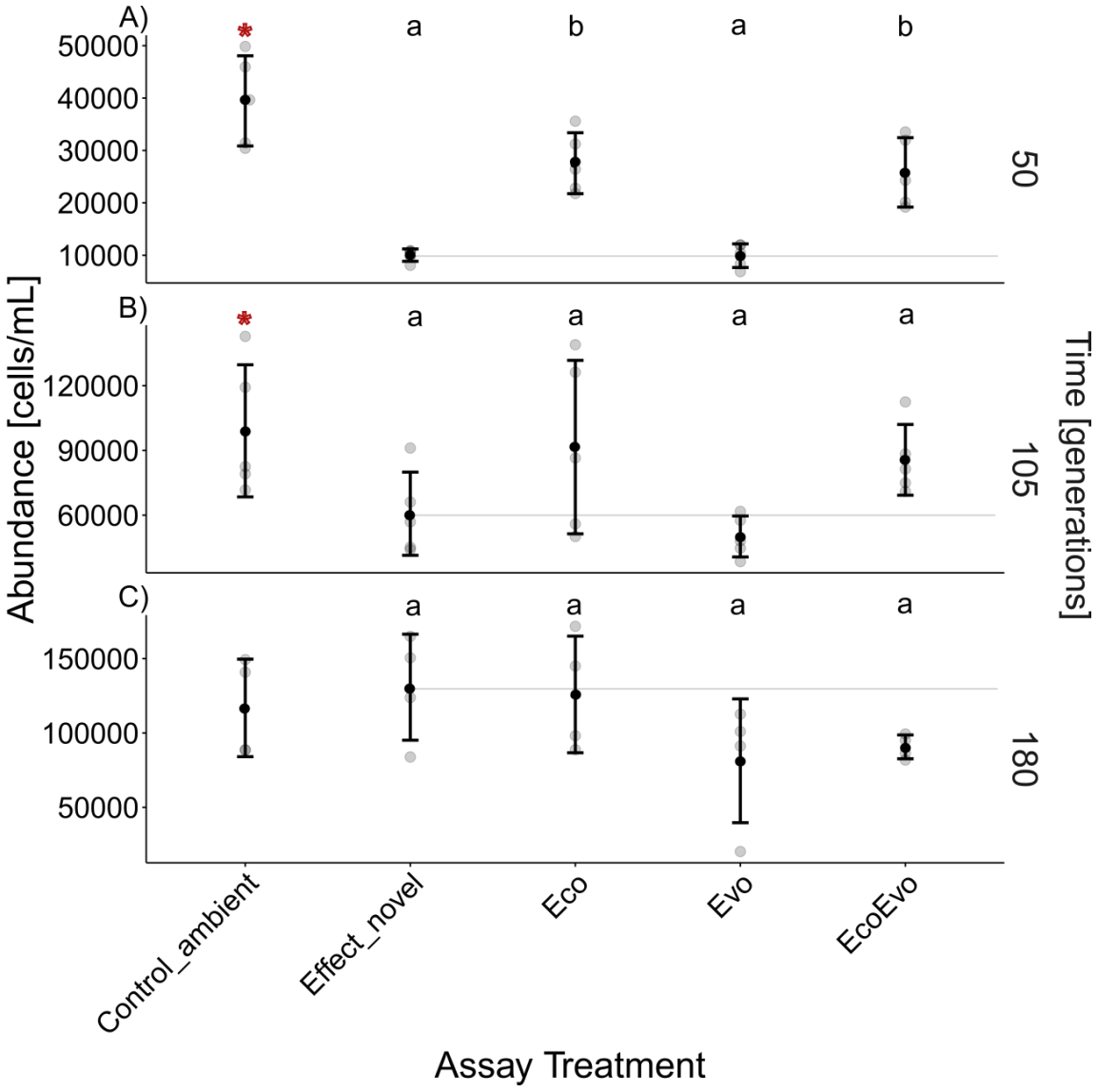
Appendix III-4: Graphical display of the size of *C. affinis* and *E. huxleyi* in the communities held under ambient and high CO_2 over time (mean and 95 % CI). To be able to see fluctuations in both species, with quite diverging size, the log to the basis of 10 is shown.



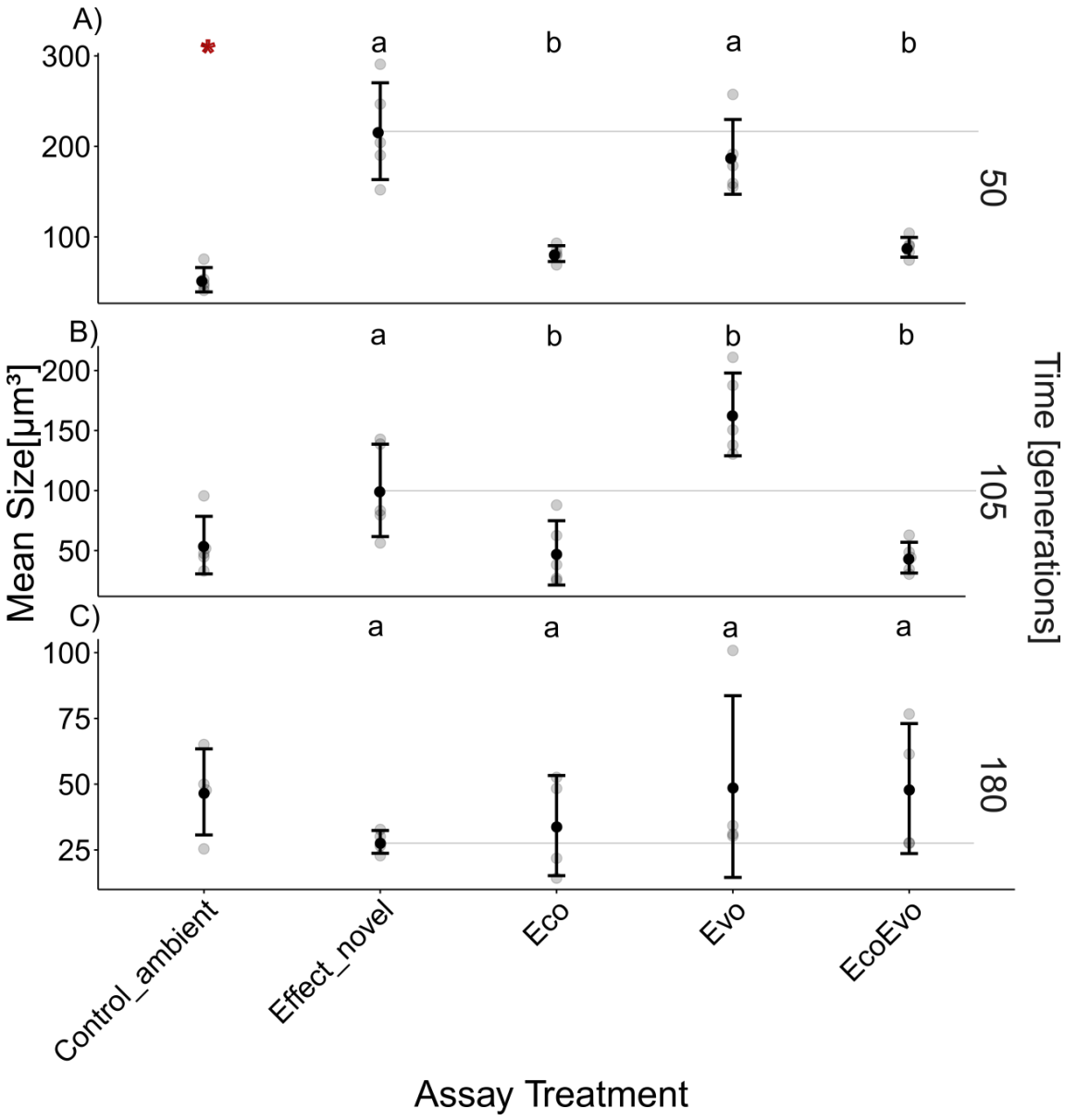
Appendix III-5: Graphical display of the community shifts over time in response to ambient and high seawater CO_2 concentration in the sorting phase (plots on left and right hand side, respectively). Relative shifts in biovolume of the two species in the community are shown in the middle (B; mean and 95 % CI), top and bottom plots show respective changes of *C. affinis* (A) and *E. huxleyi* (B) genotypes (mean). n = 5 until generation 135 in high and generation 165 in ambient then n = 4. Genotypes frequency shifts were analysed by isolating 20 individuals per replicate and species after 5, 20, 40, 50, 105 and 180 generations. Isolated cells were grown for two weeks to reach a sufficient density for subsequent microsatellite analysis (N ranged between 33 and 88; see Appendix III-6 for details).

Appendix III-6: Table listing the number of Individuals (N) that have been genotyped at the different point in the selection phase for *E. huxleyi* and *C. affinis* from communities sorted under ambient and high CO_2 condition, respectively.

Time [generation]	*N E. huxleyi*		*N C. affinis*	
	Ambient CO_2	High CO_2	Ambient CO_2	High CO_2
5	83	82	0	0
20	45	57	0	0
40	71	76	0	0
50	68	38	82	88
105	33	36	0	0
180	57	59	54	62



Appendix III-7: Figure showing the total abundance of the Eco-Evo Assay communities assessed using communities sorted for 50 (A), 105 (B) and 180 (C) generations (mean und standard deviation). Red star shows significant total change between ambient and high CO_2 sorted communities measured in $Control_{ambient}$ and $Effect_{novel}$ in the Eco-Evo assay. Grey line indicates comparison of the Eco, Evo and EcoEvo treatment effects to the $Effect_{novel}$ and lower case letters indicate significant differences found in the Anova using the $Effect_{novel}$ as intercept. n=5 until generation 105 then n=4.



Appendix III-8: Figure showing mean size of the Eco-Evo Assay communities assessed using communities sorted for 50 (A), 105 (B) and 180 (C) generations (mean und standard deviation). Red star shows significant total change between ambient and high CO_2 sorted communities measured in $Control_{mbient}$ and $Effect_{novel}$ in the Eco-Evo assay. Grey line indicates comparison of the Eco, Evo and EcoEvo treatment effects to the $Effect_{novel}$ and lower case letters indicate significant differences found in the Anova using the $Effect_{novel}$ as intercept. n=5 until generation 105 then n=4.

Curriculum Vitae

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Education

Since 2015 PhD student the GEOMAR in Kiel, Germany; thesis title: *"Eco-evolutionary importance in competing marine phytoplankton communities."*

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Publications

2018 Pansch, C., Hattich, G.S.I., Hindrichs, M.E., Pansch, A., Zagrodzka, Z., Havenhand, J.N., 2018. *Long-term exposure to acidification disrupts reproduction in a marine invertebrate.* Plos One 13, 1–17.

2017 Hattich, G.S.I., Listmann, L., Raab, J., Ozod-Seradj, D., Reusch, T.B.H., Matthiessen, B., 2017. *Inter- and intraspecific phenotypic plasticity of three phytoplankton species in response to ocean acidification.* Biology Letters 13, 1–4.

2017 Mayor, D.J., Gray, N.B., Hattich, G.S.I., Thornton, B., 2017. *Detecting the presence of fish farm-derived organic matter at the seafloor using stable isotope analysis of phospholipid fatty acids*. Scientific Reports 7. 5146.

2016 Huhn, M., Hattich, G.S.I., Zamani, N.P., Juterzenka, K. Von, Lenz, M., 2016. *Tolerance to stress differs between Asian green mussels Perna viridis from the impacted Jakarta Bay and from natural habitats along the coast of West Java.* Marine Pollution Bulletin. 110, 757-766.

Description of Author contributions

Chapter I: Inter- and intra-specific phenotypic plasticity of three phytoplankton species in response to ocean acidification

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Contributions: Study designed by GH, LL, TBHR, BM; Lab work carried out by GH, LL, JR, DO; data analysis by GH, LL; GH and LL wrote the manuscript with input from all other authors. GH und LL equally contributed to this manuscript.

Chapter II: Experimentally decomposing total phytoplankton community change into ecological and evolutionary components

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Under preparation to be submitted in L&O Methods

Contributions: Eco-Evo assay designed by BM and GH; Study testing the assay designed by GH, LL, BM; Lab work carried out by GH, LL, CP; data analysis by GH; GH wrote the manuscript and all other authors revised the manuscript.

Chapter III: Both ecology and evolution contribute to phytoplankton community change

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Under preparation to be submitted in Nature Ecology and Evolution

Contributions: Study designed by GH, LL, BM and TR. Lab work carried out by GH, LL; data analyses by GH; GH wrote the manuscript and all other authors revised the manuscript.

Eidesstattliche Erklärung

Hiermit erkläre ich, Giannina Saskia Isabelle Hattich, dass die vorgelegte Dissertation:

"Eco-evolutionary importance in competing marine phytoplankton communities"

von mir, unter Beratung meiner Betreuer, selbständig verfasst wurde, nach Inhalt und Form meine eigene Arbeit ist und keine weiteren Quellen und Hilfsmittel als angegeben verwendet wurden.

Die Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden.

Diese Arbeit wurde an keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt und ist mein erstes und einziges Promotionsverfahren. Kapitel I dieser Arbeit wurde in der wissenschaftlichen Fachzeitschrift Biology Letters in 2017 publiziert. Der Anteil der Autoren an den Manuskripten wird im Abschnitt „Author contributions" erläutert (Seite 117).

Mir wurde noch kein akademischer Grad entzogen.

Kiel, den 27. November 2018 ……

Giannina Hattich