




## Differential effects of elevated $p\text{CO}_2$ and warming on marine phytoplankton stoichiometry

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### Abstract

Phytoplankton stand at the base of the marine food-web, and play a major role in global carbon cycling. Rising  $\text{CO}_2$  levels and temperatures are expected to enhance growth and alter carbon:nutrient stoichiometry of marine phytoplankton, with possible consequences for the functioning of marine food-webs and the oceanic carbon pump. To date, however, the consistency of phytoplankton stoichiometric responses remains unclear. We therefore performed a meta-analysis on data from experimental studies on stoichiometric responses of marine phytoplankton to elevated  $p\text{CO}_2$  and 3–5° warming under nutrient replete and limited conditions. Our results demonstrate that elevated  $p\text{CO}_2$  increased overall phytoplankton C:N (by 4%) and C:P (by 9%) molar ratios under nutrient replete conditions, as well as phytoplankton growth rates (by 6%). Nutrient limitation amplified the  $\text{CO}_2$  effect on C:N and C:P ratios, with increases to 27% and 17%, respectively. In contrast to elevated  $p\text{CO}_2$ , warming did not consistently alter phytoplankton elemental composition. This could be attributed to species- and study-specific increases and decreases in stoichiometry in response to warming. While our observed moderate  $\text{CO}_2$ -driven changes in stoichiometry are not likely to drive marked changes in food web functioning, they are in the same order of magnitude as current and projected estimations of oceanic carbon export. Therefore, our results may indicate a stoichiometric compensation mechanism for reduced oceanic carbon export due to declining primary production in the near future.

Greenhouse gas emissions are changing our climate at an unprecedented rate. Since the industrial revolution, atmospheric  $\text{CO}_2$  partial pressure ( $p\text{CO}_2$ ) has increased by more than 40% (IPCC 2014). Relative to present day levels,  $p\text{CO}_2$  is predicted to have doubled by the end of this century (Representative Concentration Pathways 6.0 & 8.5; IPCC 2014). Without additional efforts to reduce these emissions, the global mean temperature is predicted to increase by

3–5°C (i.e., the RCP8.5 scenario in IPCC 2014). The concurrent increases in  $p\text{CO}_2$  and global mean temperature are expected to profoundly impact marine ecosystem functioning (Brierley and Kingsford 2009; Doney et al. 2012).

Marine phytoplankton are responsible for roughly 50% of the world's primary production (Field et al. 1998). By converting inorganic carbon and nutrients into organic compounds, they form the base of the marine food web. Furthermore, marine phytoplankton play a key role in the transfer of carbon from the atmosphere to the ocean's interior as part of the biological carbon pump (Legendre and Lefevre 1995; Falkowski et al. 1998). The importance of phytoplankton for marine food web functioning and carbon cycling not only depends on their productivity, but also on their elemental composition. Specifically, phytoplankton C:N:P stoichiometry is closely associated to their

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nutritional value for higher trophic levels (Sterner and Elser 2002; Hessen et al. 2013), and the relative amount of carbon that is exported to the ocean's interior (Omta et al. 2006; Tanioka and Matsumoto 2017; Kwiatkowski et al. 2018).

Although C:N:P stoichiometry of bulk seston in the ocean surface is thought to be largely stable (Redfield 1934, 1958), inter- and intraspecific variability in phytoplankton elemental composition can be substantial (Geider and La Roche 2002; Martiny et al. 2013). Phytoplankton carbon:nutrient ratios are particularly sensitive to nutrient availability, and can increase with nutrient limitation (Geider and La Roche 2002). Climate change can further increase phytoplankton carbon:nutrient ratios, and thereby alter food web structure and functioning (van de Waal et al. 2010). For instance, CO<sub>2</sub>-driven increases in phytoplankton carbon:nutrient ratios were shown to negatively affect growth and reproduction of consumers (Malzahn et al. 2010; Schoo et al. 2013; Meunier et al. 2017). Enhanced phytoplankton carbon:nutrient ratios may furthermore increase the amount of particulate carbon in the surface ocean layer that can sink to deeper waters, resulting in a higher carbon export (Riebesell et al. 2007).

Climate change can alter phytoplankton stoichiometry in different ways. Elevated *p*CO<sub>2</sub>, for example, results in higher inorganic carbon concentrations which potentially enhances net carbon fixation rates (Rost et al. 2008; Koch et al. 2013) and increases cellular carbon:nutrient ratios (Riebesell et al. 2007; Lomas et al. 2012). Warming may enhance phosphorus use efficiency of marine phytoplankton by reducing ribosomal phosphorus demand (Toseland et al. 2013), leading to increased C:P and N:P ratios. Alternatively, if warming stimulates phytoplankton growth rate, this may also lead to a decrease in cellular N:P and especially C:P ratios due to higher demands for ribosomal RNA rich in phosphorus (Growth Rate Hypothesis; Elser et al. (2003); but see Flynn et al. (2010) for a discussion on its applicability in phytoplankton).

Numerous studies have investigated the responses of phytoplankton carbon:nutrient ratios to elevated *p*CO<sub>2</sub> and warming, reporting both positive and negative responses (Fu et al. 2007, 2008; Schaum et al. 2013). To consolidate these findings and test for potential consistency in overall responses, we summarized studies on various species in a meta-analysis to assess the net effect of elevated *p*CO<sub>2</sub> and warming on phytoplankton stoichiometry. To this end, we combined the results of single-species culture studies that documented the short-term response of marine phytoplankton C:N, C:P, and N:P ratios to *p*CO<sub>2</sub> or temperature. As climate impacts on marine phytoplankton stoichiometry can depend on nutrient availability (Li et al. 2012; Lewandowska et al. 2014; Alvarez-Fernandez et al. 2018), we separately analyzed the stoichiometric responses under nutrient-replete and -limited conditions. In our analysis, we furthermore included responses of carbon, nitrogen, and phosphorus quota as well as growth rate to assess their contribution to stoichiometric responses. In the temperature studies, we incorporated the elemental composition at the

growth optima and at 3–5°C lower temperatures to account for natural variation in organismal temperature optima.

## Materials

We compiled a database from the published literature on the C:N:P stoichiometry of phytoplankton under various *p*CO<sub>2</sub> and temperature conditions. For this purpose, a systematic literature review was carried out in Web of Science (<https://www.webofknowledge.com/>) on the 5th of March 2020 using the query (stoichiometry OR “nutrient stoichiometry” OR “CNP stoichiometry” OR “chemical composition” OR “nutritional quality” OR “nutrient composition” OR “elemental composition” OR C:N:P OR carbon:nitrogen:phosphorus) AND (phytoplankton OR algae OR microalgae OR algal OR picoplankton) AND (“climate change” OR “global warming” OR warming OR temperature OR “ocean acidification” OR CO<sub>2</sub> OR “carbon dioxide” OR “global change” OR *p*CO<sub>2</sub>), yielding 1221 publications. Further screening of the abstracts, graphs and tables of these publications rendered 139 publications that contained information on CO<sub>2</sub> and/or temperature effects on elemental composition of phytoplankton. Freshwater and macroalgal species were excluded from analysis, as well as studies on phytoplankton communities and studies where sample size was one, or not reported (Fig. S1). Data on C:N, C:P, and N:P ratios from the remaining publications were extracted from graphs, tables, supplementary material, and deposited data. Data from graphs were extracted using plot digitizing software (Mitchell et al. 1991; Huwaldt 2013). Where necessary, these data were converted to molar ratios. Standard deviations were either extracted from the publications or calculated from the data and sample size. Additionally, data on cellular carbon, nitrogen, and phosphorus quota (in mol per cell), growth rate ( $\mu$ , based on population density), experimental type (i.e., batch cultures, semi-continuous cultures or chemostat experiments), and sample size were included in the database. As light conditions could have a modulating effect on the stoichiometric responses (Rokitta and Rost 2012; García-Gómez et al. 2014), data on photosynthetically active radiation (PAR), photoperiod, and time-integrated light conditions (defined as the product of PAR and photoperiod) were included in the database as well. When studies reported on a time series, only the final data point (typically end of exponential phase) was used for further analysis. Multiple experiments described in the same paper were extracted independently. Species names were checked with AlgaeBase (Guiry and Guiry 2017) and updated to their latest nomenclature if necessary. After these steps, the data were split into a nutrient replete and a nutrient (i.e., nitrogen and phosphorus) limited dataset according to the description in the original publications. When publications reported on two of the three stoichiometric ratios (C:N, C:P, or N:P), we calculated the third ratio with its approximated standard deviation according to Larkin (2011). Similarly, when studies reported carbon, nitrogen or phosphorus quota combined with ratios, we reconstructed the

non-reported elemental data. An overview of the studies included in our dataset can be found in Supplementary Table S1.

**Response ratios**

Bias corrected log response ratios ( $RR^\Delta$ ) and their variance were calculated for paired observations from ambient and elevated  $pCO_2$ , or low and high temperatures following Lajeunesse (2015):

$$RR^\Delta = \ln\left(\frac{X_{elevated}}{X_{ambient}}\right) + \frac{1}{2} \left[ \frac{(SD_{elevated})^2}{n_{elevated} \times X_{elevated}^2} - \frac{(SD_{ambient})^2}{n_{ambient} \times X_{ambient}^2} \right] \quad (1)$$

$$var(RR^\Delta) = \frac{(SD_{elevated})^2}{n_{elevated} \times X_{elevated}^2} - \frac{(SD_{ambient})^2}{n_{ambient} \times X_{ambient}^2} + \frac{1}{2} \left[ \frac{(SD_{elevated})^4}{n_{elevated}^2 \times X_{elevated}^4} + \frac{(SD_{ambient})^4}{n_{ambient}^2 \times X_{ambient}^4} \right] \quad (2)$$

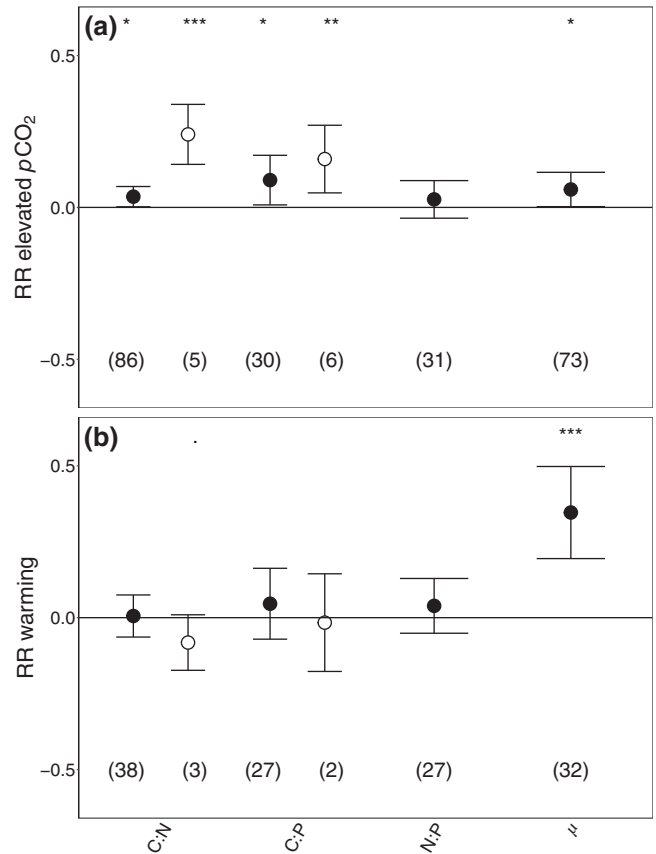
In these formulas,  $X$  denotes the mean of the fixed factor of interest (C:N, C:P, and N:P ratio,  $\mu$  and C, N and P quota), SD the standard deviation of that mean and  $n$  the sample size for ambient (control) and elevated  $pCO_2$  or temperature treatments.

For  $pCO_2$ , atmospheric levels of 300–500  $\mu atm$  were defined as the ambient control treatment, while CO<sub>2</sub> concentrations of more than 1.5 times the reported ambient CO<sub>2</sub> concentration were categorized as the elevated treatment. To avoid including CO<sub>2</sub> concentrations that were clearly outside IPCC projections, CO<sub>2</sub> levels above 2100  $\mu atm$  were excluded from the analysis. This rendered average CO<sub>2</sub> levels of  $382 \pm 30$  and  $876 \pm 271$  (mean  $\pm$  SD)  $\mu atm$  in the control and elevated treatment, respectively.

Temperature treatments were categorized using data on growth optima. For marine phytoplankton, these growth optima generally lie above the highest environmental temperatures (Thomas et al. 2012; Chen 2015). Therefore, the elevated treatment was defined as the temperature where an organism exhibited the highest growth rate in that particular study (i.e., the growth optimum), while the control treatment was then defined as 3–5°C lower (32% of temperature studies; Fig. S2a). In case the growth optimum was not provided, while showing a positive growth response (64% of temperature studies; Fig. S2b), or where growth rate was not reported (5% of temperature studies), the highest temperature was taken as the elevated temperature treatment. If studies contained several observations within our predefined ambient or elevated treatments, the lowest temperature or CO<sub>2</sub> observation within that respective treatment was selected for further analysis. This process generated a nutrient-replete and -limited dataset for single-species experiments consisting of 99 case-studies originating from 51 papers on CO<sub>2</sub> manipulation and 43 studies originating from 20 papers on temperature manipulation.

**Statistics**

All statistics were carried out in R version 4.0.2 (R Core Team 2015). To calculate the overall natural-log response ratios, mixed effect models were fitted to the study-specific response ratios and their variances for the nutrient replete dataset with the function *rma.mv* (package metafor version 2.4.0 (Viechtbauer 2010)). Reference (i.e., the publication that the data originated from), genus, and species were modeled as random effects to reflect the dependency structure of the data. To attain response ratios per phytoplankton group, we analyzed separate runs for the overall model including phytoplankton group as a moderator. To test whether the response ratios differed between nutrient-limited and nutrient-replete conditions, nutrient limitation was added to the model in an additional run as a moderator and its significance was based on the test of moderators included in the function *rma.mv*. In a separate model run, experimental type was added as a moderator to test whether response ratios differed between these experimental methods (Table S2).



**Fig. 1.** Natural-log response ratios of elevated  $pCO_2$  (a) and warming (b) on the C:N, C:P, and N:P ratios and growth rate ( $\mu$ ) of marine phytoplankton under nutrient-replete (closed circles), nitrogen- (open circles for C:N), and phosphorus-limited (open circles for C:P) conditions. Error bars represent 95% confidence intervals and sample sizes are between brackets and Significance levels are indicated as follows: \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ ,  $p < 0.10$ .

Additionally, we confirmed that the strength of responses was not influenced by the differences in CO<sub>2</sub> and temperature ranges, as we observed no correlation between the response ratios and the difference between CO<sub>2</sub> and temperature in the low and high treatments (function *lm*, Table S3). In a similar manner, we verified that no modulating effects of light conditions on the response ratios were present in the study (Table S4, Fig. S3).

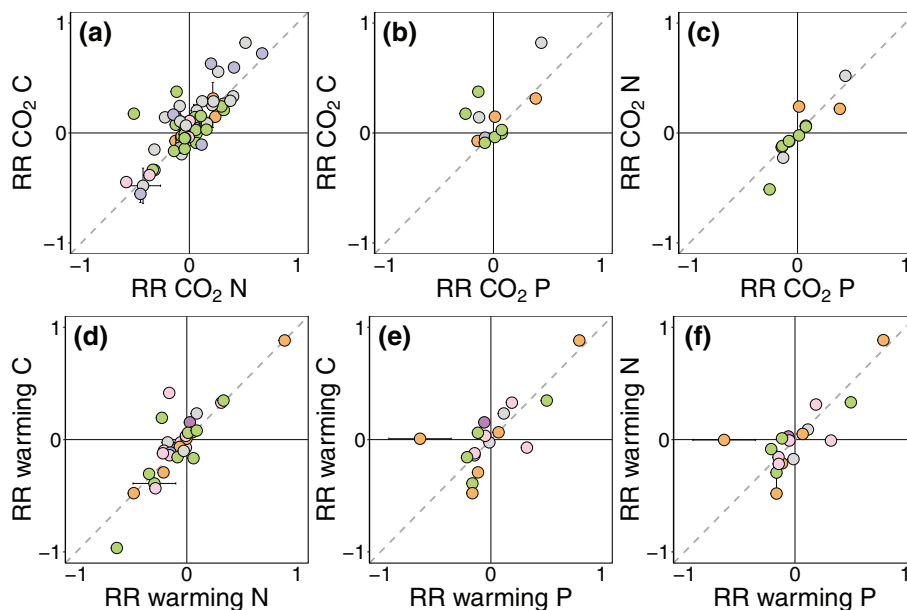
## Results

Elevated *p*CO<sub>2</sub> led to an overall increase in C:N and C:P ratios, with bias corrected response ratios ( $RR^{\Delta}$ ) of  $0.04 \pm 0.03$  and  $0.09 \pm 0.08$  (mean  $\pm$  95% CI), respectively. Elevated *p*CO<sub>2</sub> furthermore significantly enhanced growth rates ( $\mu$ ;  $RR^{\Delta} = 0.06 \pm 0.06$ ), while no overall effect of elevated *p*CO<sub>2</sub> on phytoplankton N:P ratios was observed (Fig. 1a). These shifts correspond to average increases of 3.6%, 9.4%, and 6.1% in C:N, C:P, and  $\mu$ , respectively. No interaction effects between the stoichiometric responses to elevated *p*CO<sub>2</sub> and warming were observed (Fig. S4). Furthermore, no overall changes in cellular quota were observed in response to elevated *p*CO<sub>2</sub> (Fig. S5). Nonetheless, study-specific response ratios in carbon quota to elevated *p*CO<sub>2</sub> either increased or remained unaltered compared to those of nitrogen and phosphorus (Fig. 2a–c).

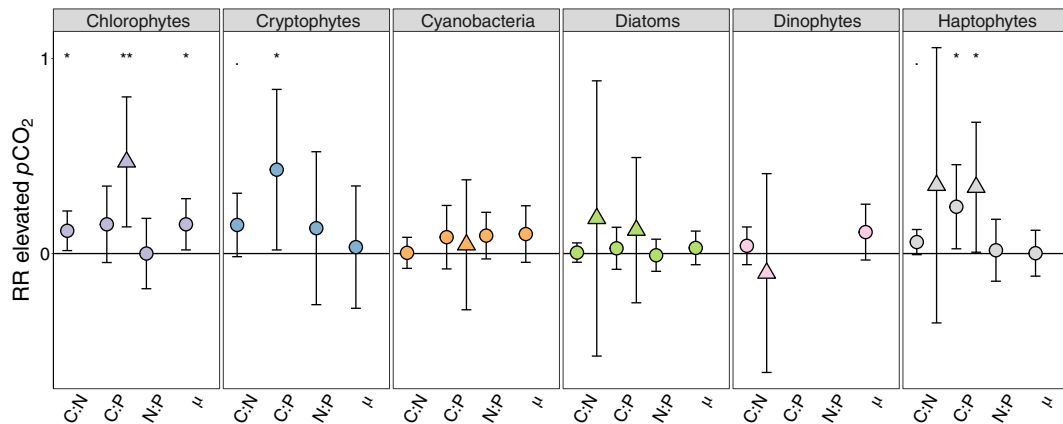
The effect of elevated *p*CO<sub>2</sub> on phytoplankton C:P ratios was stronger under phosphorus limitation (test of moderators;

QM = 8.12, df = 2,  $p < 0.05$ ), with a  $RR^{\Delta}$  of  $0.16 \pm 0.11$  (i.e., 17.3% increase). Similarly, nitrogen limitation enhanced the CO<sub>2</sub>-driven increase in C:N ratios (QM = 23.8, df = 2,  $p < 0.0001$ ), with a  $RR^{\Delta}$  of  $0.24 \pm 0.10$  (i.e., 27.2%, Fig. 1a). Group-specific C:N and C:P responses to elevated *p*CO<sub>2</sub> were never found to be negative (Fig. 3, Table S5) and almost all study-specific responses were neutral or positive (Figs. S6e,f). This indicates that the general stoichiometric responses were largely consistent over phytoplankton species and groups. Elevated *p*CO<sub>2</sub> significantly increased the C:N ratios of chlorophytes under nutrient-replete conditions (Fig. 3). Similarly, C:N ratios of cryptophytes and haptophytes tended to be enhanced with elevated *p*CO<sub>2</sub>, though this relationship was not significant (i.e.,  $p = 0.08$ ). No significant group-specific changes in C:N ratios were observed under nitrogen-limited conditions. C:P ratios of haptophytes increased in response to elevated *p*CO<sub>2</sub> under both nutrient replete and P-limited conditions. Additionally, C:P ratios increased for cryptophytes under replete nutrient conditions and for chlorophytes under P-limitation (Fig. 3). No stoichiometric responses to elevated *p*CO<sub>2</sub> were available for raphidophytes, nor for C:P and N:P responses of dinophytes.

With experimental warming of 3–5°C, growth rate significantly increased with a  $RR^{\Delta}$  of  $0.35 \pm 0.15$  (i.e., a 41.4% increase; Fig. 1b). No overall effect of warming on phytoplankton C:N:P stoichiometry under nutrient-replete or phosphorus-limiting conditions (Fig. 1b), nor on carbon, nitrogen, and phosphorus quota (Fig. S5) was observed. Under



**Fig. 2.** Relationship between natural-log response ratios on C, N and P quota of elevated *p*CO<sub>2</sub> (a–c) and warming (d–f) under nutrient replete conditions. Error bars represent variance of the response ratio (see Materials). Algal groups are indicated by different colors, with chlorophytes in lilac, cryptophytes in blue, cyanobacteria in orange, diatoms in green, dinophytes in pink and haptophytes in gray. The dashed line is a 1:1 relationship and indicates a similar relative response of the specific elemental quota and no subsequent change in elemental ratio. Significance levels are indicated as follows: \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ ,  $p < 0.10$ .



**Fig. 3.** Summary of group specific natural-log response ratios (mean ± 95% confidence interval) of elevated *p*CO<sub>2</sub> on the C:N, C:P, and N:P ratios and growth rate ( $\mu$ ) of marine phytoplankton under nutrient-replete (circles), as well as nitrogen- and phosphorus-limited (triangles; to the right of the replete response) conditions. Sample size for the specific groups can be found in Table S5. Significance levels are indicated as follows: \*\*\**p* < 0.001, \*\**p* < 0.01, \**p* < 0.05,  $\cdot$  *p* < 0.10.

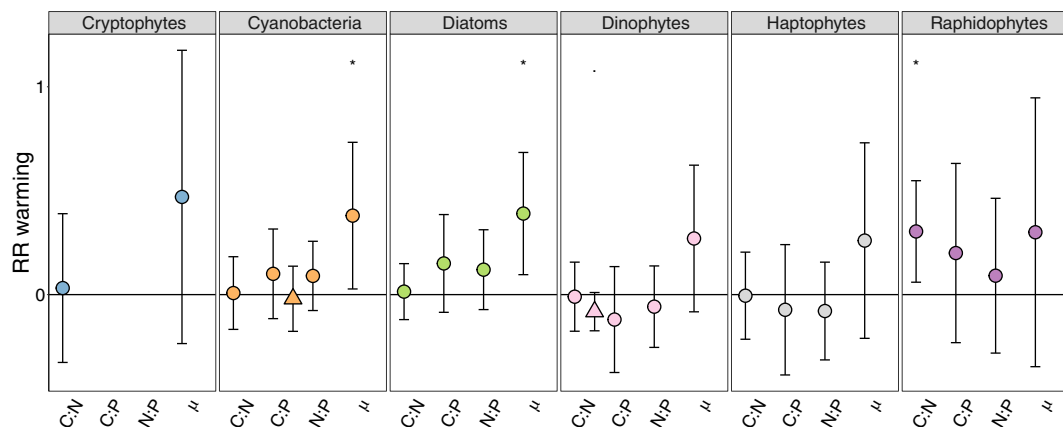
nitrogen limiting conditions, warming tended to reduce C:N ratios ( $RR^{\Delta} = -0.08 \pm 0.09$ ; *p* = 0.08), though this relationship was not significant and sample size was low (Fig. 1b). No overall effects of warming on the C:N, C:P, and N:P stoichiometry of specific phytoplankton groups were found either (Fig. 4, Table S5), except from an increase in C:N ratio for one tested raphidophyte and a marginally significant reduction in dinophyte C:N ratios under N-limitation.

From all 38 studies, increases in carbon quota with experimental warming were only observed in 6 studies, while 10 studies reported a significant decrease (Fig. S7a). Similarly, also positive and negative responses of nitrogen and phosphorus quota were observed (Fig. S7b,c). Response ratios of C, N and P quota to experimental warming showed negative, neutral and positive responses explaining the inconsistent shifts in C:N, C:P, and N:P stoichiometry

(Fig. 2). Thus, the absence of an overall effect of warming does not imply that there are no temperature-dependent changes in phytoplankton stoichiometry, but rather demonstrates contrasting study-specific responses.

**Discussion**

In this meta-analysis we aimed to determine the effect of elevated *p*CO<sub>2</sub> and warming on phytoplankton C:N:P stoichiometry. Synthesizing 99 published case-studies, we observed that elevated *p*CO<sub>2</sub> increased overall phytoplankton C:N (by 4%) and C:P (by 9%) molar ratios under nutrient replete conditions, as well as phytoplankton growth rates (by 6%). Nutrient limitation amplified the CO<sub>2</sub> effect on C:N and C:P ratios, with increases of 27% and 17%, respectively. The 43 case-studies on the effects of environmental warming,



**Fig. 4.** Summary of group specific natural-log response ratios (mean ± 95% confidence interval) of warming on the C:N, C:P, and N:P stoichiometry and growth rate ( $\mu$ ) of marine phytoplankton under nutrient-replete (circles), as well as nitrogen- and phosphorus-limited (triangles; to the right of the replete response) conditions. Sample size for the specific groups can be found in Table S5. Significance levels are indicated as follows: \*\**p* < 0.01, \**p* < 0.05,  $\cdot$  *p* < 0.10.

however, did not consistently show altered phytoplankton elemental composition. The possible implications of these findings are discussed below.

### Stoichiometric responses to elevated pCO<sub>2</sub>

We observed a general increase in phytoplankton C:N and C:P ratios with elevated pCO<sub>2</sub>, which was consistent across most phytoplankton groups (Fig. 3). The changes in carbon:nutrient ratios with elevated pCO<sub>2</sub> may result from either increases in cellular carbon quota, decreases in nutrient quota, or a combination, though this was not immediately clear from the meta-analytic results on the elemental quota (Fig. S5). Unfortunately, not all papers included in our meta-analysis contained information on elemental quota, preventing us to directly link alterations of C:N:P stoichiometry to changes in C, N, and P quota of the tested phytoplankton species. Limited availability of raw data is a frequent issue in meta-analytic studies, which can be prevented by data publication in repositories. In ecological literature, this is becoming a more adopted practice (Culina et al. 2020; O'Dea et al. 2021). In our analysis, study-specific response ratios did nonetheless indicate that carbon quota responded considerably stronger than nitrogen and phosphorus quota (Fig. 2). Aside from investing this excess carbon into population growth, phytoplankton cells may also become larger in response to elevated pCO<sub>2</sub> (Fu et al. 2007). Furthermore, they may accumulate the excess carbon as carbon-rich macromolecules such as carbohydrates or fatty acids (Geider and La Roche 2002). Almost all marine phytoplankton groups have evolved carbon concentrating mechanisms (CCMs) to optimize CO<sub>2</sub> fixation (Reinfelder 2011). CCMs can be flexible and be down-regulated with elevated pCO<sub>2</sub> (Beardall and Giordano 2002; Van de Waal et al. 2019). Such a down-regulation of costly CCMs has been hypothesized to allow reallocation of energy or elements to, for instance, nutrient acquisition (Van de Waal and Litchman 2020). Apparently, this reallocation does not necessarily lead to disproportional increases in net nutrient assimilation and could be accompanied by comparable or even stronger increases in the net assimilation of carbon, as is evident from the increased carbon but not nitrogen quota in our analysis (Fig. 2a).

### Stoichiometric responses to elevated temperature

The growth optima of marine phytoplankton generally lie well above the highest environmental temperatures (Thomas et al. 2012; Chen 2015) and a recent meta-analysis showed enhanced phytoplankton growth rates with warming (Seifert et al. 2020). It is highly conceivable that global warming will generally enhance phytoplankton growth rates, at least in polar and temperate regions (Thomas et al. 2012; Boyd et al. 2013; Brandenburg et al. 2019). Our results indeed showed significantly enhanced growth with warming, which was a direct consequence of the selection for temperature ranges between the growth optimum and 3–5°C lower temperatures (*see* Materials

and Fig. S2). The increased growth rate with warming did not co-occur with consistent changes in phytoplankton C:N, C:P, and N:P stoichiometry. The results presented in our analysis are in contrast with work from Yvon-Durocher and colleagues, who observed enhanced C:P ratios with increased temperatures (Yvon-Durocher et al. 2015). Possibly, this discrepancy could be a result of warming-induced increases in phytoplankton growth rates, for which we standardized the growth response to temperature in our analysis. As far as we can tell, the growth response to temperature increases was allowed to vary in the analysis of Yvon-Durocher et al. (2015). Some species in our dataset showed an increase in their phosphorus quota (Fig. S7c), possibly following enhanced phosphorus demands associated with higher growth rates (Elser et al. 2003), other species showed reduced phosphorus quota possibly suggesting an enhanced phosphorus-use efficiency (Toseland et al. 2013). Warming generally affects a wide range of cellular mechanisms, including carbon and nutrient assimilation rates (Hancke et al. 2008), as well as photosynthetic and respiration rates (Cabrerizo et al. 2014). Recent work of Barton and colleagues shows that long-term exposure to experimental warming can lead to altered photosynthetic and respiration ratios (Barton et al. 2020). However, the amount of compensation in carbon fixation rates for short-term exposure to warming differed between the phytoplankton species studied. Overall, the interplay of multiple temperature-dependent changes in cellular processes may differ between and within species and complicates our mechanistic understanding on the effects of warming on phytoplankton stoichiometry. More research is needed to better understand the effects of global warming on the complex physiology of marine phytoplankton.

### Stoichiometric responses toward multiple global change factors

Global change involves shifts in multiple factors that all act together. Besides elevated pCO<sub>2</sub> and warming, oceans also experience shifts in other factors like nutrient concentrations, salinity, and light availability (Doney 2006; Boyd and Hutchins 2012; Seifert et al. 2020). A relationship between light availability and the stoichiometric responses to climate change could have been expected as CO<sub>2</sub>-driven increases in photosynthesis and cellular growth can be limited by light availability (Li et al. 2021), with possible consequences for the stoichiometric responses of phytoplankton to environmental changes (Feng et al. 2008; Kranz et al. 2010). We did not, however, observe a consistent modulating effect of light availability on marine phytoplankton C:N:P stoichiometry in our analyses (Fig. S3), suggesting that our results are not biased by experimental light conditions.

Large parts of present-day oceans experience nutrient limitation (Moore et al. 2013). Moreover, nutrient availability in the oceans is expected to be reduced as a result of increased thermal stratification by global warming (Behrenfeld et al. 2006; Polovina et al. 2008; Boyce et al. 2010), as well as

climate-driven shifts in global-scale nutrient distribution (Moore et al. 2018). In our analysis, we demonstrate that the impacts of elevated  $p\text{CO}_2$  are stronger under nutrient limitation as compared to nutrient replete conditions. We note, however, that the number of studies testing the combined effects of elevated  $p\text{CO}_2$  and nutrient limitation on phytoplankton stoichiometry were limited, thereby revealing an important knowledge gap. Although warming did not have a consistent direct effect on phytoplankton C:N, C:P, and N:P stoichiometry, it may indirectly magnify the effects of CO<sub>2</sub> on phytoplankton C:N and C:P ratios through enhanced nutrient limitation.

The effects of temperature and  $p\text{CO}_2$  on phytoplankton C:N:P stoichiometry may be affected by other parameters, so that the combined effect of environmental change in future oceans may be non-additive. For example, a recent meta-analysis showed that while higher temperature and light availability generally led to enhanced phytoplankton growth rates, these responses were strongly reduced when combined with elevated  $p\text{CO}_2$  (Seifert et al. 2020). Similarly, temperature driven increases in primary productivity disappeared when combined with elevated  $p\text{CO}_2$  (Gao et al. 2017). The current number of studies testing the interactive effects of warming and  $p\text{CO}_2$  on the C:N:P stoichiometry of marine phytoplankton, however, is rather limited. A preliminary analysis using our dataset did not reveal an interaction effect of warming and  $p\text{CO}_2$  on phytoplankton stoichiometry (Fig. S4). We note that the response ratios in this analysis on interactive effects deviated from the overall results on single-stressor studies. Therefore, these studies are not representative for the overall dataset, and more work is required to fully elucidate the potential interactive effects of elevated  $p\text{CO}_2$  and warming on phytoplankton C:N:P stoichiometry.

### Potential implications for trophic interactions and carbon cycling

Climate-driven shifts in phytoplankton stoichiometry may have consequences for the marine food web, as well as for oceanic carbon export. CO<sub>2</sub>-driven changes in phytoplankton elemental stoichiometry can affect the growth and development of zooplankton grazers, as for instance shown for marine copepods (Schoo et al. 2013; Cripps et al. 2016). On the other hand, CO<sub>2</sub>-driven increases in the carbon:nutrient ratio of marine algae can lead to enhanced ingestion rates of amphipods (Duarte et al. 2016), suggesting potential compensatory feeding of these animals to cope with lowered nutritional quality of the algae. Anticipated changes in carbon:nutrient ratios, as shown by our meta-analysis, are relatively small (4–9% under nutrient replete conditions), though study-specific responses varied greatly (Fig. S6d,e). As higher trophic levels typically experience consequences of stoichiometric mismatches with increases in C:N and C:P of over 100% (e.g., Schoo et al. 2013), it is less known how zooplankton grazers will respond to moderate changes in elemental

stoichiometry. Therefore, the implications of our observed CO<sub>2</sub>-driven changes in producer stoichiometry on zooplankton life history may vary.

The observed changes in phytoplankton carbon:nutrient ratios could also have implications for the biological carbon pump, which has been estimated to export 6 Pg C per year to the deep ocean (Siegel et al. 2014). This carbon export is dependent both on phytoplankton production as well as their elemental composition. Net primary production is expected to decrease in response to enhanced nutrient limitation caused by temperature-driven increases in thermal stratification, particularly in tropical and subtropical ocean waters (Behrenfeld et al. 2006). This decline in net primary production is estimated to reduce global carbon export by up to 17% (Kwiatkowski et al. 2018). Assuming all else being equal, such a loss in carbon export may be compensated by increased phytoplankton carbon:nutrient ratios, as for the same nutrient availability a higher amount of carbon can be fixed and enhance the carbon flux into the ocean's interior. The observed CO<sub>2</sub>-driven increase in phytoplankton C:P ratio of 4–27% in our meta-analysis is in the same order of magnitude as current and projected estimations of oceanic carbon export (Siegel et al. 2014; Kwiatkowski et al. 2018), and may indicate a stoichiometric compensation mechanism for reduced oceanic carbon export due to declining primary production. This is in line with literature, for instance by recent work of Matsumoto and colleagues, who showed that adding flexible C:N:P stoichiometry to an ocean carbon cycle model could act as a buffer against warming-induced changes in carbon export (Matsumoto et al. 2020). Similarly, Oschlies et al. (2008) observed increased carbon export production with variable phytoplankton C:N ratios in model simulations. Future in-depth modeling studies on the potential implications of climate-driven changes in phytoplankton stoichiometry on marine carbon cycling are required to further understand the potential consequences of changes in phytoplankton elemental composition on marine biogeochemistry.

### Conclusion

Our meta-analysis demonstrates that marine phytoplankton C:N and C:P stoichiometry is expected to increase in response to elevated  $p\text{CO}_2$ , particularly in combination with nutrient limitation. The effects of experimental warming on phytoplankton stoichiometry are not consistent across studies, indicating context dependency. Observed differences between species' stoichiometric responses can inform ocean carbon and food web models to derive more realistic projections on climate change impacts. Importantly, the observed increases in carbon:nutrient ratios may enhance carbon export from the euphotic zone and may thereby at least partly compensate for the reduced primary production in nutrient depleted waters of the future ocean.

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#### Conflict of Interest

None declared.

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