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Simultaneous shifts in stoichiometric and fatty acid composition of *Emiliania huxleyi* in response to environmental changes

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Abstract

Climate-driven changes in environmental conditions have significant and complex effects on marine ecosystems. Variability in phytoplankton elements and biochemicals can be important for global ocean biogeochemistry and ecological functions, while there is currently limited understanding on how elemental stoichiometry and biochemicals respond to the changing environments in key coccolithophore species such as *Emiliania huxleyi*. We investigated responses of stoichiometric C:N:P ratios, PIC:POC contents and ratios, and fatty acid (FA) composition in a strain of *E. huxleyi* under three temperatures (12, 18 and 24 ℃), three N:P supply ratios (10:1, 24:1 and 63:1 mol mol^{-1}) and two pCO_2 levels (560 and 2400 μatm). Overall, C:N:P biomass ratios showed the most pronounced response to N:P supply ratios, with low N:C and N:P biomass ratios in low N-media, and low P:C and high N:P biomass ratios in low P-media. PIC:POC ratios and polyunsaturated FA proportions strongly responded to temperature and pCO_2, both being lower under high pCO_2 and higher with warming. We observed synergistic interactions between warming and nutrient deficiency (and high pCO_2) on PIC and POC cellular contents in most cases, indicating the enhanced effect of warming on *E. huxleyi* calcification and photosynthesis under nutrient deficiency (and high pCO_2). Our results suggest differential sensitivity of elements and FAs to the changes in temperature, nutrient availability and pCO_2 in *E. huxleyi*, which is to some extent unique compared with non-calcifying algal classes. Thus, simultaneous changes of elements and FAs should be considered when predicting future roles of *E. huxleyi* in the biotic-mediated connection between biogeochemical

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cycles, ecological functions and climate change.

Key words: Coccolithophores; elements; biochemicals; warming; nutrients; CO_2

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1 Introduction

Climate change and intensive anthropogenic pressures have pronounced and diverse effects on marine ecosystems. Physical and chemical properties in marine ecosystems are changing simultaneously such as the concurrent shifts in temperature, CO_2 and oxygen concentrations, and nutrient availability (Boyd et al., 2015). These changes have altered trophic interactions in both bottom-up and top-down directions and thus result in changes in community structure and ecosystem functions (Doney et al., 2012). Phytoplankton are the base of marine food webs and major drivers of ocean biogeochemical cycling, and thus quantifying their responses to changing oceanic conditions is a major challenge in studies of food web structure and ocean biogeochemistry.

Coccolithophores are a key phytoplankton group in the ocean because of their production of calcified scales called coccoliths. They are not only important primary producers, but also play predominant roles in the oceanic calcification via releasing CO_2 to the atmosphere (Rost and Riebesell, 2004). Thus, coccolithophores have a complex and significant influence on global carbon cycle, by playing an important role in ocean-atmosphere exchange of CO_2 (Rost and Riebesell, 2004). Of all coccolithophores, *Emiliania huxleyi* is the most widely distributed and the most abundant species (Winter et al., 2014), with the capacity to form spatially extensive blooms in mid- to high-latitudes (Raitsos et al., 2006; Tyrrell and Merico, 2004). Evidence from *in situ* and satellite observations indicates that *E. huxleyi* is increasingly expanding its range poleward in both hemispheres over the last two

89 decades, and contributing factors to this poleward expansion may differ between
90 regions and hemispheres (Winter et al., 2014). For example, nutrients and dissolved
91 inorganic carbon (DIC) were positively correlated with the increase in
92 coccolithophore abundance in the subtropical North Atlantic (Krumhardt et al., 2016),
93 while temperature and irradiance were best able to explain variability in *E.*
94 *huxleyi*-dominated coccolithophore community composition and abundance across the
95 Drake Passage (Southern Ocean) (Charalampopoulou et al., 2016). Hence, empirical
96 data on the responses of *E. huxleyi* to different environmental drivers would be critical
97 for fully understanding the roles of this prominent coccolithophore species in marine
98 ecosystems.

99 Extensive experimental studies have shown highly variable responses of *E. huxleyi*
100 to rising atmospheric CO_2 (reviewed by Feng et al., 2017; Meyer and Riebesell, 2015),
101 while other studies focused on the influence of other environmental factors such as
102 temperature (Rosas-Navarro et al., 2016; Sett et al., 2014; Sorrosa et al., 2005), light
103 intensity (Nanninga and Tyrrell, 1996; Xing et al., 2015) and nutrient availability
104 (Oviedo et al., 2014; Paasche, 1998). Responses of *E. huxleyi* to the interactions
105 between these different factors have recently received more attention (De Bodt et al.,
106 2010; Feng et al., 2008; Milner et al., 2016; Perrin et al., 2016; Rokitta and Rost,
107 2012). Many of these studies above focused on the physiological, calcification and
108 photosynthetic responses of *E. huxleyi* due to its considerable role in global carbon
109 cycle. However, biogeochemical cycles of the major nutrient elements (nitrogen and
110 phosphorus) and carbon are tightly linked (Hutchins et al., 2009), and thus variability

111 in stoichiometric C:N:P ratios (cellular quotas and ratios of C, N and P) in *E. huxleyi*
112 can also be a core feature of ocean biogeochemistry. Moreover, element budgets in
113 organisms are primarily determined by the physiology and biochemistry of
114 biochemicals such as proteins and fatty acids (FAs) (Anderson et al., 2004; Sterner
115 and Elser, 2002). Thus, studying simultaneous changes of elements and biochemicals
116 enables the connection between climate change and ecosystem functions such as
117 elemental cycles; however, the role of nutrient content of food is often overlooked in
118 climate change ecology (Rosenblatt and Schmitz, 2016). Recently, Bi et al. (2017)
119 investigated responses of stoichiometric C:N:P and FAs to the interactions of three
120 environmental factors in the diatom *Phaeodactylum tricornutum* and the cryptophyte
121 *Rhodomonas* sp., showing dramatic effects of warming and nutrient deficiency, and
122 modest effects of increased pCO_2. However, for the key coccolithophore species *E.*
123 *huxleyi* much less is known about the simultaneous changes in stoichiometric C:N:P
124 ratios, calcification, photosynthesis and FAs in response to multiple environmental
125 factor changes.

126 In the present study, we conducted semi-continuous cultures of *E. huxleyi* to
127 disentangle potential effects of temperature, N:P supply ratios and pCO_2 on *E. huxleyi*
128 stoichiometric C:N:P ratios, particulate inorganic carbon (PIC) and particulate organic
129 carbon (POC) contents and their ratios, and FA composition. As the physiological (i.e.,
130 cellular) PIC and POC variations cannot directly be up scaled to total population
131 response (Matthiessen et al., 2012), responses of PIC and POC contents in our study
132 were shown both on the cellular (as pg $cell^{-1}$) and the population (as μg ml^{-1}) levels.

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133 FA data were expressed as a percentage of total fatty acids (TFAs) (FA proportion, %
134 of TFAs) to better compare our results with those in previous studies. FAs were also
135 quantified on a per unit biomass (µg mg C^{-1}), which is an ideal approach when
136 considering nutritional quality of phytoplankton for herbivores (Piepho et al., 2012).
137 Specifically, we addressed the following two questions in the present study, (i) what is
138 the sensitivity of stoichiometric C:N:P ratios, PIC:POC contents and their ratios, and
139 FAs of *E. huxleyi* to changes in temperature, N:P supply ratios and pCO_2? (ii) how do
140 stoichiometric C:N:P ratios, PIC:POC and FAs of *E. huxleyi* respond to the
141 interactions between the three environmental factors?

142 ## 2 Material and methods

143 ### 2.1 Experimental setup

144 To address our questions on how multiple environmental drivers influence
145 elemental and FA composition in *E. huxleyi*, we performed a semi-continuous culture
146 experiment crossing three temperatures (12, 18 and 24 ℃), three N:P supply ratios
147 (10:1, 24:1 and 63:1 mol mol^{-1}) and two pCO_2 levels (560 and 2400 µatm) with a
148 strain of *E. huxleyi* originating from waters off Terceira Island (Azores, North
149 Atlantic). The target values of temperature, N:P supply ratios and pCO_2 were chosen
150 to reflect a present natural regime and future ocean projections of each factor
151 (Boersma et al., 2016; Lewandowska et al., 2014; Moore et al., 2013; IPCC 2014;
152 Peñuelas et al., 2012).

153 All cultures were exposed to a light intensity of 100 µmol photons · m^{-2} · s^{-1} at a
154 16:8 h light:dark cycle in temperature-controlled rooms. The culture medium was

prepared with sterile filtered (0.2 µm pore size, Sartobran® P 300; Sartorius, Goettingen, Germany) North Sea water with a salinity of 37 psu. Macronutrients were added as sodium nitrate ($NaNO_3$) and potassium dihydrogen phosphate (KH_2PO_4) to achieve three N:P supply ratios, i.e., 35.2 µmol $\cdot L^{-1}$ N and 3.6 µmol $\cdot L^{-1}$ P (10:1 mol mol^{-1}), 88 µmol $\cdot L^{-1}$ N and 3.6 µmol $\cdot L^{-1}$ P (24:1 mol mol^{-1}) and 88 µmol $\cdot L^{-1}$ N and 1.4 µmol $\cdot L^{-1}$ P (63:1 mol mol^{-1}). Vitamins and trace metals were added based on the modified Provasoli's culture medium (Ismar et al., 2008; Provasoli, 1963). Initial pCO_2 of the culture medium was manipulated by bubbling with air containing the target pCO_2. Three replicates were set up for each treatment, resulting in 54 experimental units. Each culture was kept in a sealed cell culture flask with 920 mL culture volume. Culture flasks were carefully rotated twice per day at a set time to minimize sedimentation.

First, batch culture experiments were performed to obtain an estimate of the observed maximal growth rate (μ_{max}, day^{-1}) under three temperatures, three N:P supply ratios and two pCO_2 levels. μ_{max} was calculated based on the changes of population cell density within exponential phase (Bi et al., 2012). Once batch cultures reached the early stationary phase, semi-continuous cultures were started with the algae from batch cultures. The specific growth rate of 20% of μ_{max} (μ, day^{-1}) was applied. The equivalent daily renewal rate (D, day^{-1}) can be calculated according to the equation $D = 1- e^{-\mu t}$, where t is renewal interval (day) (here t = 1 day). The incubation water was exchanged with fresh filtered seawater enriched by macronutrients and micronutrients according to the target N:P supply ratios, as well as

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pre-acclimated to the desired pCO_2 level. To counterbalance the biological CO_2-drawdown, the required amount of CO_2-saturated seawater was also added. Renewal of the cultures was carried out at the same hour every day. The steady state in semi-continuous cultures was assessed based on the net growth rate (r). When r was zero (at steady state), µ was equivalent to D.

2.2 Sample analysis

Sampling took place at steady state for the following parameters: cell density, DIC, total alkalinity (TA), pH, total particulate carbon (TPC), POC, particulate organic nitrogen and phosphorus (PON and POP), and FAs. Cell density was counted daily in batch and semi-continuous cultures. pH measurements were conducted daily in semi-continuous cultures (Fig. S1), and the electrode was calibrated using standard pH buffers (pH 4 and pH 7; WTW, Weilheim, Germany).

DIC water samples were gently filtered using a single-use syringe filter (0.2 µm, Minisart RC25; Sartorius, Goettingen, Germany) which was connected to the intake tube of a peristaltic pump. Samples were collected into 10 ml glass vials, and all vials were immediately sealed after filling. DIC was analyzed following Hansen et al. (2013) using a gas chromatographic system (8610C; SRI-Instruments, California, USA). Samples for TA analysis were filtered through GF/F filters (Whatman GmbH, Dassel, Germany) and analyzed with the Tirino plus 848 (Metrohm, Filderstadt, Germany). The remaining carbonate parameter pCO_2 was calculated using CO2SYS (Pierrot et al., 2006) and the constants supplied by Hansson (1973) and Mehrbach et al. (1973) that were refitted by Dickson and Millero (1987) (Table S1).

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TPC, POC, PON and POP samples were filtered onto pre-combusted and pre-washed (5%~10% HCl) GF/F filters (Whatman GmbH, Dassel, Germany). For POC samples, PIC was removed by exposing filters containing TPC to fuming hydrochloric acid for 12h. Before analysis, filters were dried at 60 ℃ and stored in a desiccator. POC and PON was simultaneously determined by gas chromatography in an organic elemental analyzer (Thermo Flash 2000; Thermo Fisher Scientific Inc., Schwerte, Germany) after Sharp (1974). POP was analyzed colorimetrically by converting organic phosphorus compounds to orthophosphate (Hansen and Koroleff, 1999). PIC was determined by subtracting POC from TPC. PIC and POC production were estimated by multiplying μ with cellular PIC or POC content, respectively.

FA samples were taken on pre-combusted and hydrochloric acid-treated GF/F filters (Whatman GmbH, Dassel, Germany), and stored at -80 ℃ before measurement. FAs were measured as fatty acid methyl esters (FAMEs) using a gas chromatograph (Trace GC-Ultra; Thermo Fisher Scientific Inc., Schwerte, Germany) according to the procedure described in detail in Arndt and Sommer (2014). The FAME 19:0 was added as internal standard and 21:0 as esterification control. The extracted FAs were dissolved with n-hexane to a final volume of 100 μL. Sample aliquots (1 μL) were given into the GC by splitless injection with hydrogen as the carrier gas. Individual FAs were integrated using Chromcard software (Thermo Fisher Scientific Inc., Schwerte, Germany) and identified with reference to the standards Supelco 37 component FAME mixture and Supelco Menhaden fish oil.

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221 **2.3 Statistical analysis**

222 Generalized linear mixed models (GLMMs) were applied to test the best model
223 explaining the variations in elemental and FA composition. In our study, response
224 variables included stoichiometric C:N:P ratios (as mol mol^{-1}), PIC and POC contents
225 per cell (as pg $cell^{-1}$) and per ml (as μg ml^{-1}), PIC and POC production (as pg $cell^{-1}$
226 d^{-1}), PIC:POC ratio, FA proportion (as % of TFAs) and contents (as μg mg C^{-1}), with
227 temperature, N:P supply ratios and pCO_2 as fixed effects. Target distributions were
228 tested and link functions were consequently chosen. For all response variables, we
229 tested models containing first order effects, and second and third order interactions of
230 the three factors. The model that best predicted targets was selected based on the
231 Akaike Information Criterion corrected (AICc), i.e., a lower AICc value representing
232 a better fit of the model. Changes of 10 units or more in AICc values were considered
233 as a reasonable improvement in the fitting of GLMMs (Bolker et al., 2009). In case
234 AICc values were comparable (<10 units difference), the simpler model was thus
235 chosen, unless there were significant second or third order interactions detected.
236 Differences in AICc values between different models were more than 10 for most
237 variables, with the exception for N:P biomass ratio, cellular PIC and POC contents,
238 and saturated fatty acid (SFA) and DHA proportions, which were less than 10 between
239 different models (Table S2).

240 Two factorial ANOVA was used to test the effects of temperature and pCO_2 on μ_{max}.
241 As μ_{max} should be equal across different nutrients (Cherif and Loreau, 2010), the
242 effect of N:P supply ratios on μ_{max} was not tested. The normality of dependent

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variables was checked using the Shapiro-Wilk's *W*-test. For the significant factors, the magnitude of effect (ω^2 = (effect sum of squares – effect degree of freedom × error mean square) / (total sum of squares + error mean square)) was calculated to determine the variance in a response variable and to relate this to the total variance in the response variable (Graham and Edwards, 2001; Hughes and Stachowicz, 2009).

Nested models were applied to test whether the response pattern to one factor (a nested factor) was significant within another factor, in case significant second order interactions were detected in GLMM. Also, the nature (antagonistic, additive, or synergistic) of significant second order interactions was analysed according to Christensen et al. (2006). The observed combined effect of two factors was compared with their expected net additive effect [e.g., ($factor_1$ - control) + ($factor_2$ - control)], which was based on the sum of their individual effects. If the observed combined effect exceeded their expected additive effect, the interaction was defined as synergism. In contrast, if the observed combined effect was less than the additive effect, the interaction was defined as antagonism.

All statistical analyses were conducted using SPSS 19.0 (IBM Corporation, New York, USA). Significance level was set to $p < 0.05$ in all statistical tests.

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3 Results

3.1 Maximal growth rate (μ_{max})

The observed maximal growth rate of *E. huxleyi* responded significantly to temperature and the interactions between temperature and pCO_2 (Bold letters in Table 1), with temperature causing 82% of the effect magnitude. Increasing temperature stimulated μ_{max} in each pCO_2 treatment, causing μ_{max} to be two to three times higher at the highest temperature than those at the lowest temperature (Fig. 1). In contrast, the effect of elevated pCO_2 on μ_{max} showed no consistent pattern, indicating a slight positive effect at 12℃, a strong negative effect at 18℃ and a weak negative effect at 24℃ (Fig. 1). Moreover, the trend of μ_{max} to increase with increasing temperature differed between the two pCO_2 treatments, showing that at the low pCO_2, the slope of μ_{max} response to increasing temperature was higher from 12℃ to 18℃ and it became lower from 18℃ to 24℃, while the slope of μ_{max} response showed no clear difference between three temperatures at the high pCO_2 treatment.

3.2 C:N:P stoichiometry

GLMMs results showed that N:C, P:C and N:P biomass ratios responded significantly to N:P supply ratios (Bold letters in Table 2), while only N:C biomass ratios showed significant responses to temperature, with non-significant effect of pCO_2 detected. Increasing N:P supply ratios caused an increased trend in N:C biomass ratios (Fig. 2a) and a decrease in P:C biomass ratios (Fig. 2b), resulting in a positive relationship between N:P biomass ratios and N:P supply ratios (Fig. 2c). The response of N:C biomass ratios to increasing temperature was complex, showing a U-shaped

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287 response under N deficiency (N:P supply ratio = 10:1 mol mol^{-1}), and positive
288 responses under higher N:P supply ratios (Fig. 2a).

289 All three PIC-related variables (cellular PIC contents, population yields of PIC and
290 PIC production) responded significantly to temperature and pCO_2; and there were also
291 significant interactions between temperature and pCO_2 (and N:P supply ratios) on
292 cellular PIC contents, and significant effects of N:P supply ratios on population yields
293 of PIC and PIC production (Table 2). Increasing temperature and N deficiency
294 affected cellular PIC contents antagonistically, while increasing temperature and P
295 deficiency (N:P supply ratio = 63:1 mol mol^{-1}) had synergistic interactions on cellular
296 PIC contents (Table S3). As a result, cellular PIC contents showed a trend to decrease
297 slightly with increasing temperature under N deficiency and a trend to increase under
298 higher N:P supply ratios (Fig. 3a). Increasing temperature and enhanced pCO_2
299 affected cellular PIC contents synergistically (Table S3), with the negative response of
300 cellular PIC contents to enhanced pCO_2 being significantly weaker as temperature
301 increased (Fig. 3d; Nested model, $p < 0.001$). Population yields of PIC decreased
302 (under higher temperatures), but PIC production increased with increasing N:P supply
303 ratios (Fig. 3b, c), while both PIC variables increased with increasing temperature and
304 decreased with enhanced pCO_2 (Fig. 3e, f).

305 The three POC-related variables (cellular POC contents, population yields of POC
306 and POC production) responded significantly to temperature; and there were also
307 significant interactions between temperature and N:P supply ratios on cellular POC
308 contents, and significant effects of N:P supply ratios on population yields of POC and

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POC production (Table 2). For cellular POC contents, increasing temperature and N (and P) deficiency showed synergistic interactions, resulting in the highest values at the lowest temperature under lower N:P supply ratios and an increasing trend with increasing temperature under P deficiency (Fig. 4a; Table S3). Population yields of POC showed an unimodal response and POC production showed a trend to increase in response to increasing temperature and N:P supply ratios (Fig. 4b, c).

PIC:POC responded significantly to temperature and pCO_2 (Table 2), showing a clear increase with increasing temperature and a decrease with enhanced pCO_2 (Fig. 5).

3.3 Fatty acids

The most abundant FA group were polyunsaturated fatty acids (PUFAs) (33%-54% of TFAs), followed by SFAs (22%-46%) and monounsaturated fatty acids (MUFAs) (13%-27%), across the entire tested gradients of temperature, N:P supply ratios and pCO_2 (Table S4). The high proportion of PUFAs was predominantly caused by high amounts of DHA (12%-31%) and 18:4n-3 (3%-13%), and SFAs was mainly represented by 14:0 (13%-23%) and 16:0 (5%-11%). The major individual MUFA was 18:1n-9 (8%-21%).

GLMMs results showed significant effects of temperature and pCO_2 on the proportions of both MUFAs and PUFAs, and significant interactions between N:P supply ratios and pCO_2 on SFAs (Table 2). Increasing temperature caused a decrease in the proportion of MUFAs and an increase in PUFAs (Fig. 6a, b). In contrast, enhanced pCO_2 resulted in an increase in MUFAs and a decrease in PUFAs at higher

temperatures (Fig. 6d, e). Moreover, enhanced pCO_2 and N (and P) deficiency affected SFA proportion synergistically (Table S3), with the unimodal response of SFA proportion to increasing N:P supply ratios being more pronounced at the high pCO_2 (Fig. S2; Nested model, $p < 0.001$).

The proportion of major individual PUFAs (DHA) showed significant responses to temperature and N:P supply ratios, and the interactions between temperature and N:P supply ratios (and pCO_2) (Table 2). Increasing temperature caused an overall increase in DHA, and DHA also had higher values under N and P deficiency (Fig. 6c). The interactions between increasing temperature and N deficiency (and P deficiency and enhanced pCO_2) affected DHA synergistically (Table S3), and the positive effect of temperature became more pronounced at lower N:P supply ratios (Nested model, $p < 0.001$) and at the low pCO_2 (Nested model, $p < 0.001$) (Fig. 6c, f).

4 Discussion

Our study scales the impacts of temperature, N:P supply ratios and pCO_2 on elemental and FA composition of the ubiquitously important calcifier *E. huxleyi*, while accounting for their interactive effects. Overall, C:N:P biomass ratios changed markedly in response to N:P supply ratios, resulting in up to a 62% increase in N:P biomass ratios (Fig. 7). PIC:POC showed an increase of 41% with warming and a decrease of 35% under high pCO_2. PUFA proportions showed an increase of 13% with warming and a decrease of 7% with enhanced pCO_2, indicating a partial compensation by pCO_2 of a predominantly temperature-driven response. The overall

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response patterns of C:N:P stoichiometry and PUFAs in our study are consistent with those on the global scale (Martiny et al., 2013), and conform with the meta-analysis results on haptophytes (Hixson and Arts, 2016). In line with these studies, we also detected significant interactions between temperature, N:P supply ratios and pCO_2, indicating variable response patterns of elemental and FA composition in *E. huxleyi* under any given constellation of environmental factors. Our results thus underscore the importance of simultaneous consideration of multiple environmental drivers, demonstrating differential effects of the three environmental factors on elemental and FA composition of *E. huxleyi*.

4.1 Responses of maximal growth rate

Increasing temperature (12-24℃) significantly accelerated μ_{max} of *E. huxleyi* in our study (Fig. 1). This positive correlation between increasing temperature and growth rate is typical for many *E. huxleyi* strains within the range of temperature 12 to 24℃ (Feng et al., 2008; Rosas-Navarro et al., 2016; Sett et al., 2014; van Bleijswijk et al., 1994). However, the extent to which growth rate of *E. huxleyi* increases with increasing temperature varies between *E. huxleyi* strains, which may contribute to specific biogeographic distribution of different strain (Paasche, 2002). For example, growth rate of *E. huxleyi* from the Gulf of Maine (~42 ˚N) was 1.2 times higher at 26℃ than at 16℃, while growth rate of *E. huxleyi* from the Sargasso Sea (~20-35 ˚N) was 1.6 times higher at the higher temperature (Paasche, 2002). Also, our results showed that μ_{max} of *E. huxleyi* was two to three times higher at the highest temperature than at the lowest temperature, suggesting that temperature is an important environmental

375 factor in controlling the distribution of this strain of *E. huxleyi*.

376 Also, temperature showed significant interactions with pCO_2 on μ_{max} of *E. huxleyi*
377 in this study. On the one hand, elevated pCO_2 led to an increase in μ_{max} at the lowest
378 temperature (12℃) but a decrease at higher temperatures (18 and 24℃) (Fig. 1).
379 Similarly, in response to increasing pCO_2, either an increase (Feng et al., 2008) or a
380 decrease (Milner et al., 2016) in the growth of *E. huxleyi* was observed in previous
381 studies. Such a diverse response of phytoplankton growth rate to elevated pCO_2 has
382 been widely observed within functional groups such as coccolithophores and diatoms,
383 between taxa and even between strains of the same species (Dutkiewicz et al., 2015).
384 Significant interactions between pCO_2 and temperature on *E. huxleyi* growth in our
385 study suggest that experimental temperature can be an important factor resulting in
386 diverse responses of phytoplankton growth to rising pCO_2 in previous studies.

387 On the other hand, we observed different slopes of μ_{max} in response to increasing
388 temperature at two pCO_2 levels, showing a decrease in the slope with increasing
389 temperature at the low pCO_2 and a relatively constant slope at the high pCO_2 (Fig. 1).
390 Our results are consistent with a conceptual graph proposed by Sett et al. (2014). The
391 graph showed a clear increase in metabolic rates from low to intermediate temperature
392 and a slight increase from intermediate to high temperature at the low pCO_2 (~560
393 μatm), while and the changes of metabolic rates are similar from low to intermediate
394 temperature and from intermediate to high temperature at the high pCO_2 (~2400 μatm)
395 (Sett et al., 2014). While the physiological mechanisms governing adaptation of
396 phytoplankton to rising pCO_2 are still unclear, one possible explanation is that

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increasing temperature may modulate the balance between a fertilizing effect of ocean carbonation and a metabolic repression by ocean acidification (Bach et al., 2011; Sett et al., 2014).

4.2 Responses of C:N:P biomass ratios

4.2.1 Effects of N:P supply ratios

N:P supply ratios showed highly significant effects on N:C, P:C and N:P biomass ratios in *E. huxleyi* in this study, contributing up to 62% changes in C:N:P biomass ratios (Table 2; Fig. 7). The strong contribution of nutrient availability to the elemental ratios of marine phytoplankton community biomass was also found on the global scale (Daines et al., 2014; Martiny et al., 2013), with nitrate concentration as a proxy of nutrient availability explaining 36% of variation in N:P ratio and 42% of variation in C:P ratio (Martiny et al., 2013). Similarly, previous lab experiments reported that nutrient availability prevailed the governing effect on stoichiometric ratios in *E. huxleyi* (Skau, 2015).

Overall, N deficiency caused low N:C and N:P biomass ratios, while P deficiency resulted in low P:C biomass ratios and high N:P biomass ratios in *E. huxleyi* in this and most previous studies (Langer et al., 2013; Leonardos and Geider, 2005b; Perrin et al., 2016). An important biogeochemical question is the extent to which N:P biomass ratios change under N and P deficiency, respectively. Our results showed that changes in N:P biomass ratios of *E. huxleyi* were mostly due to P deficiency (a 62% increase), with smaller changes (a 36% decrease) induced by N deficiency (Fig. 7). This observation is consistent with the high variability of P:C in response to changes

in phosphate and the less variable N:C to changes in nitrate based on global suspended particle measurements (Galbraith and Martiny, 2015). Indeed, conflicting response patterns of C:N:P biomass ratios of *E. huxleyi* to nutrient deficiency were observed between different studies (Borchard and Engel, 2012; Matthiessen et al., 2012; Perrin et al., 2016), which could be due to strain-specific responses and interactions between nutrients and other environmental drivers. Nevertheless, the nutrient-dependence of C:N:P biomass ratios of *E. huxleyi* in our study is consistent with responses of marine plankton on the global scale, which may reflect the capacity of this species to thrive under a wide range of environmental conditions. This capacity was largely revealed by a pan-genome assessment, which distributed genetic traits variably between strains and showed a suit of core genes for the uptake of inorganic nitrogen and N-rich compounds such as urea (Read et al., 2013). In spite of strain diversity within *E. huxleyi*, a recent study suggested that the global physiological response of this species to nutrient environments is highly conserved across strains and may underpin its success under a variety of marine environments (Alexander, 2016).

4.2.2 Effects of temperature

Temperature had a weaker effect (5-8% changes) than N:P supply ratios on variation of N:P and P:C ratios (Table 2; Fig. 7), consistent with results in marine plankton communities on the global scale (Martiny et al., 2013). While both N:C and P:C biomass ratios increased with increasing temperature in our study, the changes in N:C ratios (8%) were larger than those in P:C ratios (5%). As a result, the observed

N:P ratios also increased in response to warming, in accordance with the positive relationship between temperature and N:P ratios of marine phytoplankton sampled from different biogeochemical provinces (Martiny et al., 2016; Toseland et al., 2013; Yvon-Durocher et al., 2015). These responses are consistent with proposed physiological mechanisms (Toseland et al., 2013), which showed that eukaryotic phytoplankton at higher temperatures required less P-rich ribosomes and thus produced higher N:P ratios.

4.2.3 Effects of pCO_2

Partial CO_2 pressure showed a non-significant effect on *E. huxleyi* C:N:P biomass ratios in our study (Table 2), being consistent with the previous findings of the less important effect of pCO_2 than nutrients and temperature on *E. huxleyi* (Boyd et al., 2010; Feng, 2015). Several experimental studies also showed non-significant changes in *E. huxleyi* C:N:P stoichiometry in response to rising pCO_2 (Engel et al., 2014; Lefebvre et al., 2012; Olson et al., 2017); however, some studies reported clear changes in C:N or C:P ratio (Engel et al., 2005; Leonardos and Geider, 2005a; Matthiessen et al., 2012). Both experimental and model studies have suggested that rising pCO_2 seems to change phytoplankton stoichiometry under specific conditions, e.g., at high light condition (Feng et al., 2008) and at low nutrient loads (Leonardos and Geider, 2005a; Verspagen et al., 2014). Moreover, the sensitivity of phytoplankton to CO_2 may also depend on culture systems, either transient or sufficiently stable, influencing optimal allocation of energy and resources (Engel et al., 2014).

Taken together, our results indicate that N:P supply ratios are reflected in elemental

make-up of cell biomass in *E. huxleyi*, across different temperatures and pCO_2 levels, showing the absence of significant interactions between the three environmental factors. However, for two algal species from non-calcifying classes (the diatom *P. tricornutum* and the cryptophyte *Rhodomonas* sp.) temperature had the most consistent significant effect on N:C and P:C biomass ratios and showed significant interactions with N:P supply ratios and pCO_2 in our previous work (Bi et al., 2017). Both temperature and N:P supply ratios were also ranked as important factors in regulating phytoplankton stoichiometry in previous studies (Boyd et al., 2010; Feng, 2015). Differential C:N:P responses to environmental drivers between phytoplankton groups suggest that taxonomic composition may explain the variable C:N:P ratios in surface phytoplankton community (Martiny et al., 2013).

4.3 Responses of PIC:POC contents and ratios

Both partial CO_2 pressure and temperature had highly significant effects on PIC:POC in our study, with enhanced pCO_2 resulting in an overall 49% decrease in PIC:POC and warming resulting in a 41% increase in PIC:POC, while N:P supply ratios showed no significant effect (Table 2; Fig. 7). This result is in agreement with rankings of the importance of environmental drivers on PIC:POC in a Southern Hemisphere strain of *E. huxleyi*, showing the order of pCO_2 (negative effect) > temperature (positive effect) and non-significant effect of nitrate or phosphate (Feng, 2015).

The negative effect of enhanced pCO_2 on PIC:POC was also observed for different strains of *E. huxleyi* in most previous studies (Meyer and Riebesell, 2015 and

485 references therein). Negative responses of PIC:POC to increasing pCO_2 in our study
486 were driven by the significant decrease in the three PIC-related response variables
487 (calcification) and no significant change in all three variables of POC (photosynthesis)
488 (Table 2; Fig. 7). To date, studies and reviews also showed a greater impact of ocean
489 acidification on calcification than on photosynthesis in coccolithophores (De Bodt et
490 al., 2010; Feng et al., 2017; Meyer and Riebesell, 2015). Feng et al. (2017) suggested
491 that the decreased calcification in *E. huxleyi* may be caused by the increased
492 requirement of energy to counteract intracellular acidification. And the increased
493 activity of carbonic anhydrase (CA) at low pCO_2 may explain the lack of a significant
494 effect of pCO_2 on the photosynthetic or growth rate (Feng et al., 2017), as
495 up-regulation of CA at low DIC was previously observed (Bach et al., 2013).

496 A positive response or no clear change in PIC:POC was also observed for *E.*
497 *huxleyi* in response to high pCO_2 in previous studies (Feng et al., 2017), with the
498 influence of other environmental drivers proposed as a potential driver (De Bodt et al.,
499 2010; Feng et al., 2017; Feng et al., 2008). Indeed, we observed that the negative
500 relationship between cellular PIC contents and enhanced pCO_2 became weaker at the
501 highest temperature (Fig. 3d). This result is in agreement with the modulating effect
502 of temperature on the CO_2 sensitivity of key metabolic rates in coccolithophores, due
503 to the shift of the optimum CO_2 concentration for key metabolic processes towards
504 higher CO_2 concentrations from intermediate to high temperatures (Sett et al., 2014).

505 Temperature causes diverse responses of calcification and photosynthesis within *E.*
506 *huxleyi* species in the literature (Rosas-Navarro et al., 2016 and references therein)

and the present study. Overall, our study showed that the positive response of PIC:POC to increasing temperature was driven by a marked increased PIC (28%~161%), and by a less pronounced change in POC (-8%~68%) (Table 2; Fig. 7). The overall responses of PIC:POC contents and ratios to increasing temperature was consistent with those in other *E. huxleyi* strains (Matson et al., 2016; Sett et al., 2014), indicating carbon allocation to calcification rather than photosynthesis at high temperatures (Sett et al., 2014). Specifically, we observed that the interactions of warming and nutrient deficiency (and high pCO_2) synergistically affected both PIC and POC cellular contents in most cases, suggesting that nutrient deficiency and high pCO_2 are likely to enhance the effect of warming on *E. huxleyi* calcification and photosynthesis efficiency.

In summary, our results showed an overall reduced PIC:POC in *E. huxleyi* under future ocean scenarios of warming and higher CO_2 (Fig. 5b), consistent with the reduced ratio of calcium carbon production to organic carbon during the *E. huxleyi* bloom in previous mesocosm experiments (Delille et al., 2005; Engel et al., 2005). It is worth noting that cellular PIC and POC contents are a measure for physiological response and cannot be used to infer population response and thus potential carbon export (Matthiessen et al., 2012), as different responses between cellular and population yields of PIC (and POC) to environmental changes were evident in previous work (Matthiessen et al., 2012) and the present study (Fig. 7). Moreover, significant interactions between temperature and pCO_2 (and N:P supply ratios) on cellular particulate carbon contents suggest that the effects of multiple environmental

529 drivers should be tested to better generalize the dynamic of particulate carbon from
530 laboratory observations to that in natural conditions.

531 **4.4 Responses of fatty acids**

532 **4.4.1 Effects of temperature**

533 Temperature had highly significant effects on the proportions of MUFAs, PUFAs
534 and DHA, with increasing temperature causing a 20% decline in MUFAs and a 13%
535 increase in PUFAs in our study (Table 2; Fig. 7). This result is consistent with the
536 negative response of MUFA proportions and positive response of PUFAs to warming
537 in other haptophytes based on a meta-analysis on 137 FA profiles (Hixson and Arts,
538 2016), showing an opposite response to general patterns of phytoplankton FAs to
539 warming. Although warming is expected to have a negative effect on the degree of
540 fatty acid unsaturation to maintain cell membrane structural functions (Fuschino et al.,
541 2011; Guschina and Harwood, 2006; Sinensky, 1974), variable FA responses to
542 warming were widely observed in different phytoplankton groups (Bi et al., 2017;
543 Renaud et al., 2002; Thompson et al., 1992). Contradictory findings were even
544 reported in meta-analyses on large FA profiles such as the absence (Galloway and
545 Winder, 2015) or presence (Hixson and Arts, 2016) of the negative correlation
546 between temperature and the proportion of long-chain EFAs in freshwater and marine
547 phytoplankton. While the underling mechanisms of variable FA responses are still
548 unclear, it is known that both phylogeny and environmental conditions determine
549 phytoplankton FA composition (Bi et al., 2014; Dalsgaard et al., 2003; Galloway and
550 Winder, 2015). In our study, we found significant interactions between temperature

and pCO_2 (and N:P supply ratios) on the individual FA component DHA, showing that pCO_2 and nutrient availability may alter the effect of warming on *E. huxleyi* FA composition.

4.4.2 Effects of pCO_2

Partial CO_2 pressure had significant effects on the proportion of MUFAs and PUFAs in *E. huxleyi* in our study, with enhanced pCO_2 causing an overall 7% increase in MUFAs and a 7% decrease in PUFAs (Table 2; Fig. 7), consistent with FA response patterns in another strain of *E. huxleyi* (Riebesell et al., 2000) and other coccolithophores (Fiorini et al., 2010). Also in a natural plankton community (Raunefjord, southern Norway), PUFA proportion was reduced at high pCO_2 level in the nano-size fraction, suggesting a reduced Haptophyta (dominated by *E. huxleyi*) biomass and a negative effect of high pCO_2 on PUFA proportion (Bermúdez et al., 2016). To date, several mechanisms have been suggested to explain the reduced PUFAs at high pCO_2 in green algae (Pronina et al., 1998; Sato et al., 2003; Thompson, 1996), with much less work conducted in other phytoplankton groups. One possible mechanism was demonstrated in the study on *Chlamydomonas reinhardtii*, showing that the repression of the CO_2-concentrating mechanisms (CCMs) was associated with reduced FA desaturation at high CO_2 concentration (Pronina et al., 1998). Similarly in our study, both the proportion and contents of PUFAs decreased at high pCO_2 (Fig. 7), which may be attributed to the repression of CCMs at high pCO_2 in *E. huxleyi*.

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573 **4.4.3 Effects of N:P supply ratios**

574 Significant effects of N:P supply ratios was only observed for the proportion of
575 DHA in this study (Table 2), with N and P deficiency causing a 14% and 22%
576 increase in DHA proportion, respectively (Fig. 7). While nutrients often play a major
577 role on phytoplankton lipid composition (Fields et al., 2014; Hu et al., 2008), the less
578 pronounced effects of nutrient deficiency in our study indicate a unique lipid
579 biosynthesis in *E. huxleyi*. Indeed, Van Mooy et al. (2009) suggested that *E. huxleyi*
580 used non-phosphorus betaine lipids as substitutes for phospholipids in response to P
581 scarcity. Genes are also present in the core genome of *E. huxleyi* for the synthesis of
582 betaine lipids and unusual lipids used as nutritional/feedstock supplements (Read et
583 al., 2013). Therefore, the lack of significant nutrient effects on most FA groups in *E.*
584 *huxleyi* in our study may be caused by the functioning of certain lipid substitutions
585 under nutrient deficiency.

586 In summary, this study showed the strongest effects of temperature and pCO_2, a
587 weaker effect of N:P supply ratios, and non-significant interactions between the three
588 environmental factors, on the proportions of unsaturated fatty acids in *E. huxleyi*.
589 Specifically, we observed that PUFA proportions in *E. huxleyi* responded positively to
590 warming and negatively to enhanced pCO_2. It should be noted that using different
591 units to quantify FA composition may cause contradictory results, e.g., an increase in
592 PUFA proportion (% of TFAs) but an overall decline in PUFA contents per biomass
593 (μg mg C^{-1}) with increasing temperature in our study (Fig. 7; Table S4, S5). Such
594 biochemical quality differences can translate to higher trophic levels (Rossoll et al.,

595 2012) and refer to direct effects of environmental changes on low trophic level
596 consumers, which can be modified by indirect bottom-up driven impacts through the
597 primary producers (Garzke et al., 2016; Garzke et al., 2017). Similar to *E. huxleyi*,
598 PUFA contents in two species of non-calcifying classes (*P. tricornutum* and
599 *Rhodomonas* sp.) responded negatively to warming and positively to N (and P)
600 deficiency (Bi et al., 2017). However, differential responses were also observed, e.g.,
601 a significant negative effect of enhanced pCO_2 on PUFA contents in *E. huxleyi*, but a
602 non-significant effect of pCO_2 on PUFA contents in *P. tricornutum* and *Rhodomonas*
603 sp. (Bi et al., 2017). This different response between phytoplankton groups is in
604 agreement with findings in mesocosm studies (Bermúdez et al., 2016; Leu et al.,
605 2013), suggesting that changes in taxonomic composition can cause different
606 relationship between PUFAs and pCO_2 in natural phytoplankton community.

607 ## 5 Conclusions

608 Our study showed that N:P supply ratios had the strongest effect on C:N:P biomass
609 ratios, while pCO_2 and temperature played more influential roles on PIC:POC and
610 PUFA proportions in *E. huxleyi*. The specific response patterns of elemental ratios and
611 PUFAs have important implications for understanding biogeochemical and ecological
612 functioning of *E. huxleyi*. The overall low PIC:POC under future ocean scenarios
613 (warming and enhanced pCO_2) indicates that carbon production by the strain *E.*
614 *huxleyi* in our study acts as a carbon sink. This argument is consistent with the finding
615 that the calcification of coccolithophores decreases with increasing pCO_2 in the
616 present ocean and over the past forty thousand years (Beaufort et al., 2011). It should

617 be noted that carbon production discussed so far are based on responses of cellular or
618 population PIC:POC in a single strain of *E. huxleyi*, and thus it cannot be used to infer
619 potential carbon flux between surface ocean and the atmosphere. From the ecological
620 point of view, the contradictory response patterns of N:C and P:C biomass ratios (an
621 overall increase) and PUFA contents (an overall decrease) under future ocean
622 scenarios in our study make the extraction of a generic response signal of nutritional
623 food quality difficult. Nonetheless, the observations presented here suggest
624 differential responses of elements and FAs to rising temperature, enhanced pCO_2 and
625 nutrient deficiency in *E. huxleyi*, being to some extent unique compared with algal
626 species from non-calcifying classes. Thus, the role of multiple environmental drivers
627 under the biodiversity context should be considered to truly estimate the future
628 functioning of phytoplankton in the changing marine environments.
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Data availability: data sets are available upon request by contacting Meixun Zhao (maxzhao@ouc.edu.cn and maxzhao04@yahoo.com).

Author contribution: R. Bi, S. Ismar, U. Sommer and M. Zhao designed the experiments and R. Bi carried them out. R. Bi prepared the manuscript with contributions from all co-authors.

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1023 Table 1. Results of ANOVA testing for the responses of the observed maximum
1024 growth rate to temperature and pCO_2 in *Emiliania huxleyi*. Significant *p* values are
1025 shown in bold.

Factor	df	*F*	*p*	ω^2
Temperature	2	89.842	**<0.001**	0.82
pCO_2	1	3.808	0.075	
Temperature × pCO_2	2	10.638	**0.002**	0.09

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1044 Table 2. Results of the selected GLMMs testing for the effects of temperature, N:P
1045 supply ratios and pCO_2 on stoichiometric C:N:P biomass ratios, PIC and POC
1046 contents and their ratios, PIC and POC production, and fatty acid proportions in
1047 *Emiliania huxleyi*. Significant *p* values are shown in bold; T: temperature; N:P: N:P
1048 supply ratio; TFA: total fatty acid; SFA: saturated fatty acid; MUFA: monounsaturated
1049 fatty acid; PUFA: polyunsaturated fatty acid; DHA: docosahexaenoic acid (22:6n-3).

Variable	Factor	Coefficienct ± SE	*t*	*p*
N:C biomass ratio (mol mol^{-1})	Intercept	0.062 ± 0.007	8.659	<0.001
	T	0.001 ± <0.001	2.641	**0.011**
	pCO_2	<0.001 ± <0.001	-0.240	0.811
	N:P	<0.001 ± <0.001	4.717	**<0.001**
P:C biomass ratio (mmol mol^{-1})	Intercept	4.481 ± 0.451	9.946	<0.001
	T	0.016 ± 0.021	0.773	0.443
	pCO_2	<0.001 ± <0.001	0.628	0.553
	N:P	-0.037 ± 0.005	-8.154	**<0.001**
N:P biomass ratio (mol mol^{-1})	Intercept	2.702 ± 0.145	18.590	<0.001
	T	0.001 ± 0.007	0.157	0.876
	pCO_2	<0.001 ± <0.001	-0.169	0.866
	N:P	0.016 ± 0.001	11.200	**<0.001**
PIC (pg cell^{-1})	Intercept	3.293 ± 0.406	8.122	<0.001
	T	-0.067 ± 0.021	-3.193	**0.003**
	pCO_2	-0.001 ± <0.001	-5.519	**<0.001**
	N:P	-0.003 ± 0.009	-0.292	0.772
	T × pCO_2	<0.001 ± <0.001	4.584	**<0.001**
	T × N:P	0.001 ± <0.001	2.340	**0.024**
	pCO_2 × N:P	<0.001 ± <0.001	0.111	0.912
PIC (μg ml^{-1})	Intercept	6.922 ± 0.968	7.149	<0.001
	T	0.201 ± 0.045	4.442	**<0.001**
	pCO_2	-0.002 ± <0.001	-8.955	**<0.001**
	N:P	-0.034 ± 0.010	-3.404	**0.001**
PIC production (pg cell^{-1} d^{-1})	Intercept	-0.689 ± 0.105	-6.581	<0.001
	T	0.047 ± 0.005	9.589	**<0.001**
	pCO_2	<0.001 ± <0.001	-5.294	**<0.001**
	N:P	0.007 ± 0.001	6.339	**<0.001**
POC (pg cell^{-1})	Intercept	3.683 ± 0.377	9.779	<0.001
	T	-0.089 ± 0.020	-4.577	**<0.001**
	pCO_2	<0.001 ± <0.001	-0.929	0.358
	N:P	-0.008 ± 0.008	-0.996	0.324

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	T × pCO_2	<0.001 ± <0.001	1.886	0.066
	T × N:P	0.001 ± <0.001	3.477	**0.001**
	pCO_2 × N:P	<0.001 ± <0.001	-0.359	0.721
POC (µg ml^{-1})	Intercept	13.456 ± 1.007	13.360	<0.001
	T	-0.096 ± 0.047	-2.045	**0.046**
	pCO_2	<0.001 ± <0.001	-0.361	0.719
	N:P	-0.035 ± 0.010	-3.436	**0.001**
POC production (pg $cell^{-1}$ d^{-1})	Intercept	-0.261 ± 0.101	-2.587	0.013
	T	0.023 ± 0.005	4.895	**<0.001**
	pCO_2	<0.001 ± <0.001	1.631	0.109
	N:P	0.007 ± 0.001	6.899	**<0.001**
PIC:POC	Intercept	0.460 ± 0.066	7.010	<0.001
	T	0.025 ± 0.003	8.184	**<0.001**
	pCO_2	<0.001 ± <0.001	-12.837	**<0.001**
	N:P	<0.001 ± 0.001	-0.166	0.869
SFA proportion (% of TFAs)	Intercept	3.506 ± 0.145	24.178	<0.001
	T	-0.012 ± 0.008	-1.538	0.131
	pCO_2	<0.001 ± <0.001	-0.238	0.813
	N:P	-0.004 ± 0.003	-1.248	0.218
	T × pCO_2	<0.001 ± <0.001	1.816	0.076
	T × N:P	<0.001 ± <0.001	1.657	0.104
	pCO_2 × N:P	<0.001 ± <0.001	-2.487	**0.016**
MUFA proportion (% of TFAs)	Intercept	30.259 ± 1.344	22.518	<0.001
	T	-0.579 ± 0.063	-9.240	**<0.001**
	pCO_2	0.001 ± <0.001	2.269	**0.028**
	N:P	-0.014 ± 0.014	-1.050	0.299
PUFA proportion (% of TFAs)	Intercept	32.264 ± 2.300	14.028	<0.001
	T	0.638 ± 0.107	5.949	**<0.001**
	pCO_2	-0.002 ± 0.001	-2.769	**0.008**
	N:P	0.034 ± 0.023	1.453	0.152
DHA proportion (% of TFAs)	Intercept	2.204 ± 0.185	11.887	<0.001
	T	0.054 ± 0.010	5.611	**<0.001**
	pCO_2	<0.001 ± <0.001	1.874	0.067
	N:P	0.010 ± 0.004	2.735	**0.009**
	T × pCO_2	<0.001 ± <0.001	-2.946	**0.005**
	T × N:P	-0.001 ± <0.001	-2.898	**0.006**
	pCO_2 × N:P	<0.001 ± <0.001	1.249	0.218

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Fig. 1 Responses of the observed maximal growth rate (μ_{max}; mean ± SE) to temperature and pCO_2 in *Emiliania huxleyi*.

Fig. 2 Responses of (a) N:C, (b) P:C and (c) N:P biomass ratios (mean ± SE) to temperature and N:P supply ratios in *Emiliania huxleyi*.

Fig. 3 Responses of PIC content (a, d) per cell and (b, e) per ml and (c, f) PIC production (mean ± SE) to temperature, N:P supply ratios and pCO_2 in *Emiliania huxleyi*.

Fig. 4 Responses of POC content (a, d) per cell and (b, e) per ml and (c, f) POC production (mean ± SE) to temperature, N:P supply ratios and pCO_2 in *Emiliania huxleyi*.

Fig. 5 Responses of PIC:POC ratio (mean ± SE) to temperature, N:P supply ratios and pCO_2 in *Emiliania huxleyi*.

Fig. 6 Responses of the proportions of (a, d) monounsaturated fatty acids (MUFAs), (b, e) polyunsaturated fatty acids (PUFAs) and (c, f) docosahexaenoic acid (DHA) (mean ± SE) to temperature, N:P supply ratios and pCO_2 in *Emiliania huxleyi*.

Fig. 7 The changes in C:N:P stoichiometry, PIC and POC contents and their ratios, PIC and POC production, and the proportions and contents of major fatty acid groups and DHA in response to warming, N and P deficiency and enhanced pCO_2 in *Emiliania huxleyi*. Here, not only significant effects are depicted, but also non-significant and substantial effects on response variables. Significant interactions are presented based on GLMM results in Table 2. Red and blue arrows indicate a mean percent increase and decrease in a given response, respectively.

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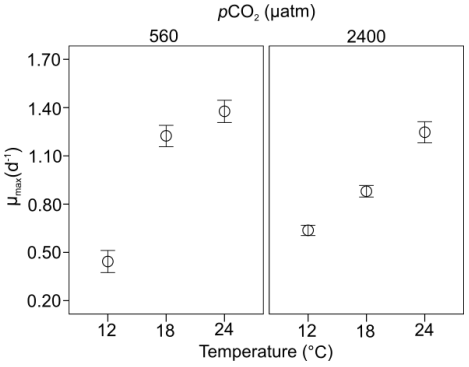
1085 **Fig. 1**



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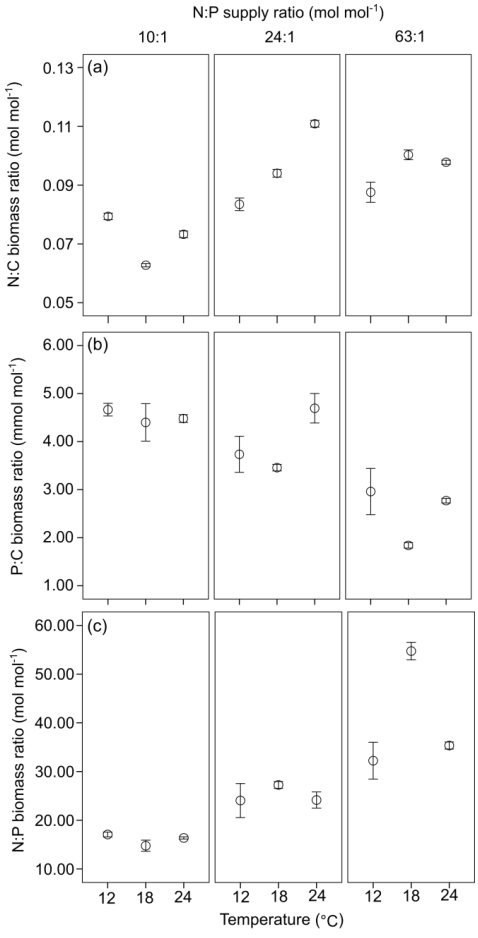
1103 **Fig. 2**



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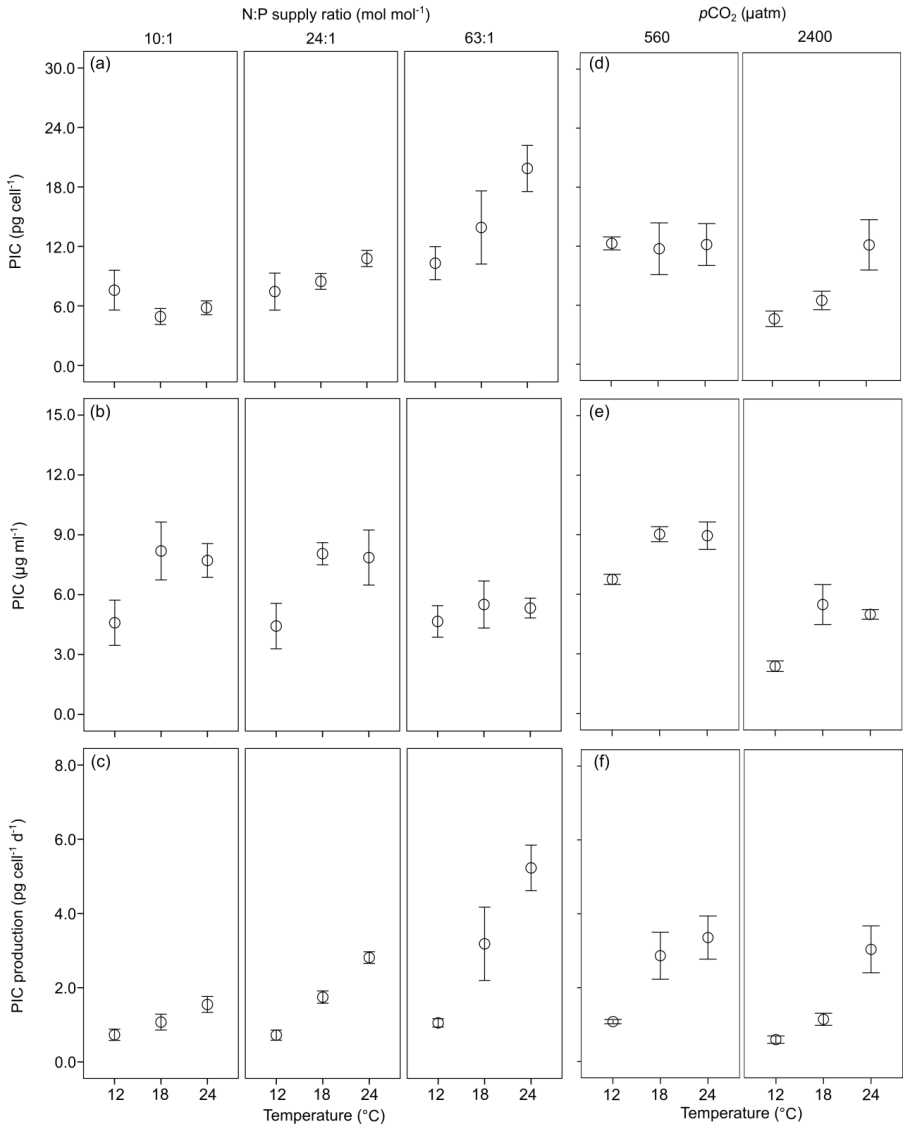
1112 **Fig. 3**



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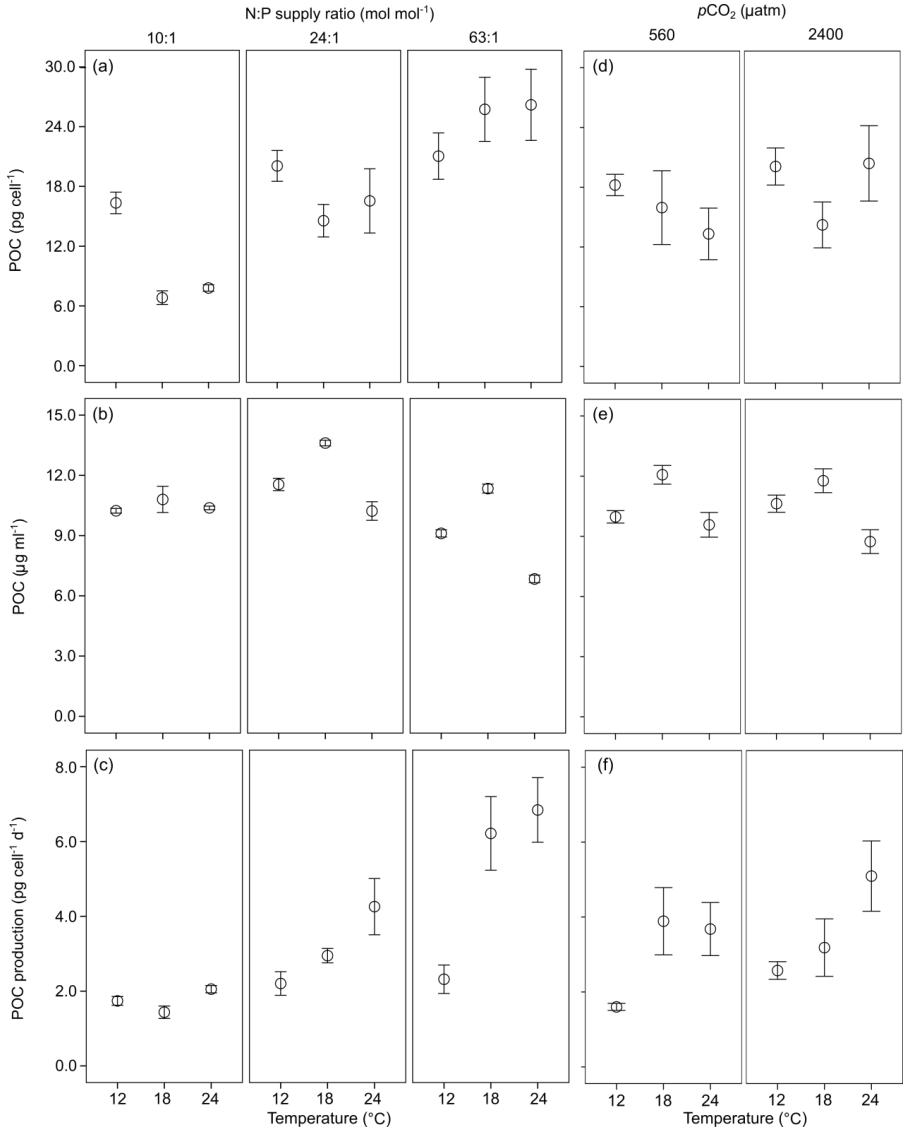
1118 **Fig. 4**



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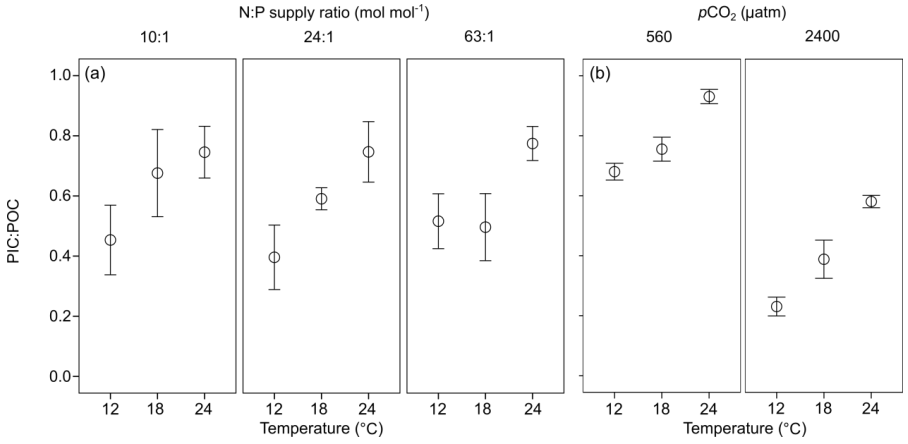
1124 **Fig. 5**



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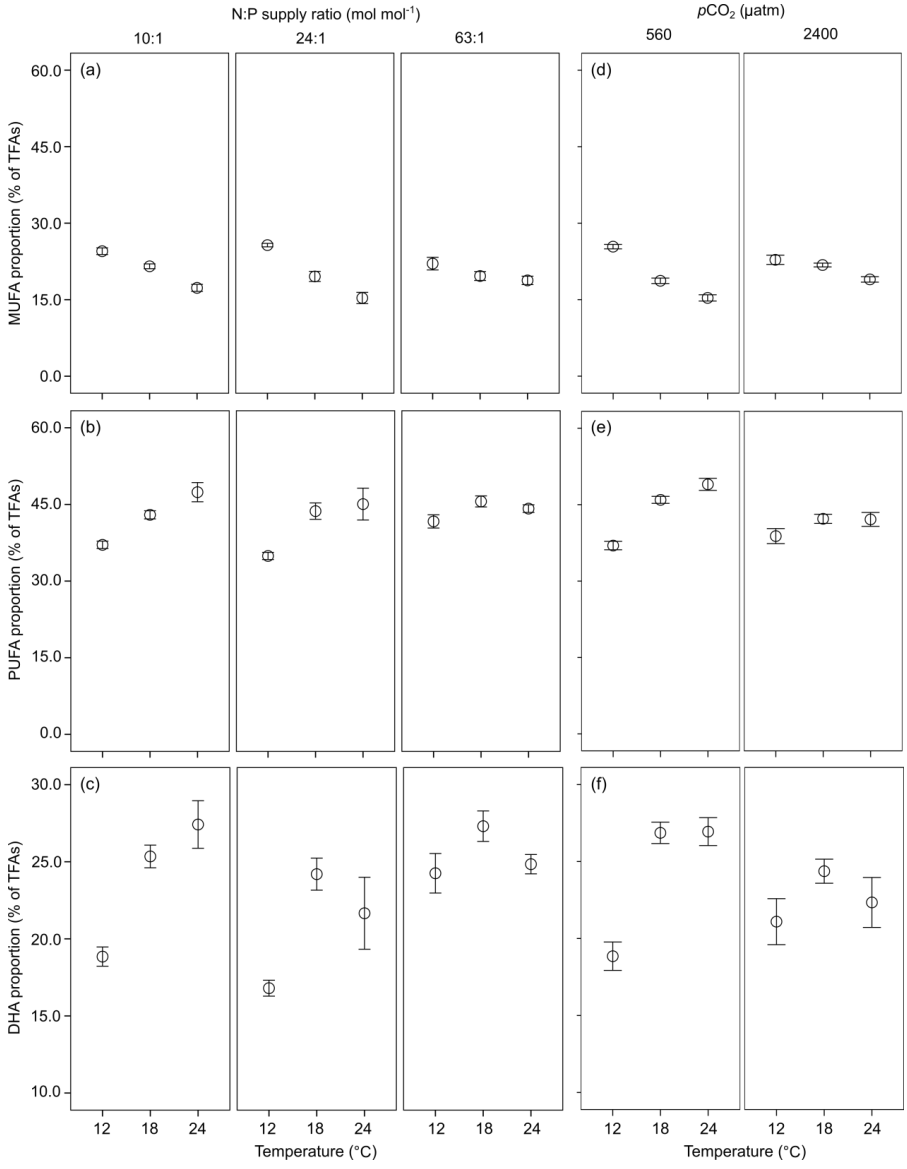
1140 **Fig. 6**



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1145 **Fig. 7**

Response	Effect				
	Warming	-N	-P	Enhanced pCO_2	Interactions
N:C biomass ratio	↑ 8%	↓ -25%	–	–	
P:C biomass ratio	↑ 5%	↑ 14%	↓ -36%	–	
N:P biomass ratio	↑ 5%	↓ -36%	↑ 62%	–	
PIC content (per cell)	↑ 28%	↓ -31%	↑ 65%	↓ -36%	T×N:P supply T×CO_2
PIC content (per ml)	↑ 36%	↓ -48%	↑ 1%	↓ -24%	
PIC production	↑ 161%	↓ -37%	↑ 79%	↓ -35%	
POC content (per cell)	↓ -8%	↓ -39%	↑ 50%	–	T×N:P supply
POC content (per ml)	↓ -6%	↓ -11%	↓ -23%	–	
POC production	↑ 68%	↓ -44%	↑ 63%	–	
PIC:POC ratio	↑ 41%	–	–	↓ -49%	
SFAs (% of TFAs)	↑ 5%	↓ -7%	↓ -15%	↑ 7%	N:P supply×CO_2
MUFAs (% of TFAs)	↓ -20%	–	–	↑ 7%	
PUFAs (% of TFAs)	↑ 13%	–	–	↓ -7%	
DHA (% of TFAs)	↑ 16%	↑ 14%	↑ 22%	↓ -7%	T×N:P supply T×CO_2
SFAs (per biomass)	↓ -21%	↑ 36%	↑ 11%	↓ -14%	
MUFAs (per biomass)	↓ -35%	↑ 56%	↑ 35%	↓ -11%	
PUFAs (per biomass)	↓ -9%	↑ 44%	↑ 29%	↓ -24%	
DHA (per biomass)	↓ -5%	↑ 53%	↑ 42%	↓ -24%	

1146 ↑↓ Changes ≥ 25% ↑↓ Changes < 25% – No clear changes

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