

Authors' response to referees: comments of the referees are in black, and responses are in blue.

Responses to comments from Reviewer #2

The authors did a good job revising the manuscript and accurately addressed my previous comments on the manuscript. I only have a few grammatical comments:

Comment 1 and comment 2

85 'the global carbon cycle'

110 'the global carbon cycle' or 'global carbon cycling'

Response:

As suggested, the word 'the' is added before 'global carbon cycle'. (See Pages 4 and 5, Lines 85 and 109)

Comment 3

187 'of' instead of 'on'

Response:

The word 'of' is used instead of 'on'. (See Page 9, Line 187)

Comment 4

324 missing 'a' before non-significant

Response:

'a' is added before 'non-significant' in several sentences. (See Page 15, Lines 324 and 330; Page 18, Line 395; Page 21, Line 448; Page 22, Lines 473)

Comment 5

435 'conditions' instead of 'condition'

Response:

The word is corrected to 'conditions'. (See Page 20, Line 437)

The list of other changes to the manuscript

Besides adjustments requested by the Reviewer, the following changes to the last version of the manuscript are also highlighted in **blue**.

1. Institutional affiliation was revised a bit. (See the title page)
2. The order of two grant numbers (41521064 and 41630966) was exchanged in Acknowledgements.
3. All significant intercepts are shown in bold in Table 1 and Table S5.

1 **Simultaneous shifts in elemental stoichiometry and fatty acids of**
2 ***Emiliania huxleyi* in response to environmental changes**

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23 **Abstract**

24 Climate-driven changes in environmental conditions have significant and complex
25 effects on marine ecosystems. Variability in phytoplankton elements and biochemicals
26 can be important for global ocean biogeochemistry and ecological functions, while
27 there is currently limited understanding on how elements and biochemicals respond to
28 the changing environments in key coccolithophore species such as *Emiliania huxleyi*.
29 We investigated responses of elemental stoichiometry and fatty acids (FAs) in a strain
30 of *E. huxleyi* under three temperatures (12, 18 and 24 °C), three N:P supply ratios
31 (molar ratios 10:1, 24:1 and 63:1) and two $p\text{CO}_2$ levels (560 and 2400 μatm). Overall,
32 C:N:P stoichiometry showed the most pronounced response to N:P supply ratios, with
33 high ratios of particulate organic carbon vs. particulate organic nitrogen (POC:PON)
34 and low ratios of PON vs. particulate organic phosphorus (PON:POP) in low N-media,
35 and high POC:POP and PON:POP in low P-media. The ratio of particulate inorganic
36 carbon vs. POC (PIC:POC) and polyunsaturated fatty acid proportions strongly
37 responded to temperature and $p\text{CO}_2$, both being lower under high $p\text{CO}_2$ and higher
38 with warming. We observed synergistic interactions between warming and nutrient
39 deficiency (and high $p\text{CO}_2$) on elemental cellular contents and docosahexaenoic acid
40 (DHA) proportion in most cases, indicating the enhanced effect of warming under
41 nutrient deficiency (and high $p\text{CO}_2$). Our results suggest differential sensitivity of
42 elements and FAs to the changes in temperature, nutrient availability and $p\text{CO}_2$ in *E.*
43 *huxleyi*, which is to some extent unique compared to non-calcifying algal classes.
44 Thus, simultaneous changes of elements and FAs should be considered when

45 predicting future roles of *E. huxleyi* in the biotic-mediated connection between
46 biogeochemical cycles, ecological functions and climate change.

47 **Key words:** Coccolithophores; elements; biochemicals; warming; nutrients; CO₂

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67 **1 Introduction**

68 Climate change and intensive anthropogenic pressures have pronounced and
69 diverse effects on marine ecosystems. Physical and chemical properties in marine
70 ecosystems are changing simultaneously such as the concurrent shifts in temperature,
71 CO₂ and oxygen concentrations, and nutrient availability (Boyd et al., 2015). These
72 changes have altered trophic interactions in both bottom-up and top-down directions
73 and thus result in changes in community structure of different trophic levels and
74 ecosystem functions (Doney et al., 2012). Phytoplankton are the base of marine food
75 webs and major drivers of ocean biogeochemical cycling, and thus quantifying their
76 responses to changing oceanic conditions is a major challenge in studies of food web
77 structure and ocean biogeochemistry.

78 Coccolithophores are a key phytoplankton group in the ocean because of their
79 production of calcified scales called coccoliths. They are not only important
80 photosynthetic producers of organic matter (causing a draw-down of CO₂ in the
81 surface layer), but also play predominant roles in the production and export of
82 calcium carbonate to deeper layers (causing a net release of CO₂ into the atmosphere)
83 (Rost and Riebesell, 2004). Owing to the determination of these two processes on
84 ocean-atmosphere exchange of CO₂, coccolithophores exhibit a complex and
85 significant influence on the global carbon cycle (Rost and Riebesell, 2004). Of all
86 coccolithophores, *Emiliania huxleyi* is the most widely distributed and the most
87 abundant species (Winter et al., 2014), with the capacity to form spatially extensive
88 blooms in mid- to high-latitudes (Raittos et al., 2006; Tyrrell and Merico, 2004).

89 Evidence from *in situ* and satellite observations indicates that *E. huxleyi* is
90 increasingly expanding its range poleward in both hemispheres over the last two
91 decades, and contributing factors to this poleward expansion may differ between
92 regions and hemispheres (Winter et al., 2014). For example, warming and freshening
93 have promoted *E. huxleyi* blooms in the Bering Sea since the late 1970s (Harada et al.,
94 2012), while temperature and irradiance were best able to explain variability in *E.*
95 *huxleyi*-dominated coccolithophore community composition and abundance across the
96 Drake Passage (Southern Ocean) (Charalampopoulou et al., 2016). Hence, empirical
97 data on the responses of *E. huxleyi* to different environmental drivers would be critical
98 for fully understanding the roles of this prominent coccolithophore species in marine
99 ecosystems.

100 Extensive experimental studies have shown highly variable responses of *E. huxleyi*
101 to rising atmospheric CO₂ (reviewed by Feng et al., 2017a; Meyer and Riebesell,
102 2015), while other studies focused on the influence of other environmental factors
103 such as temperature (Rosas-Navarro et al., 2016; Sett et al., 2014; Sorrosa et al., 2005),
104 light intensity (Nanninga and Tyrrell, 1996; Xing et al., 2015) and nutrient availability
105 (Oviedo et al., 2014; Paasche, 1998). Responses of *E. huxleyi* to the interactions
106 between these different factors have recently received more attention (De Bodt et al.,
107 2010; Feng et al., 2008; Milner et al., 2016; Perrin et al., 2016; Rokitta and Rost,
108 2012). Many of these studies above focused on the physiological, calcification and
109 photosynthetic responses of *E. huxleyi* due to its considerable role in the global carbon
110 cycle. However, biogeochemical cycles of the major nutrient elements (nitrogen and

111 phosphorus) and carbon are tightly linked (Hutchins et al., 2009), and thus variability
112 in *E. huxleyi* C:N:P stoichiometry (cellular quotas and ratios of C, N and P) can also
113 be important in ocean biogeochemistry. Moreover, elemental budgets in organisms are
114 primarily determined by the physiology and biochemistry of biochemicals such as
115 proteins and fatty acids (FAs) (Anderson et al., 2004; Sterner and Elser, 2002). Thus,
116 studying simultaneous changes of elements and biochemicals enables the connection
117 between climate change and ecosystem functions such as elemental cycles. However,
118 shifts in resource nutrient content for consumers are often overlooked in climate
119 change ecology (Rosenblatt and Schmitz, 2016). Recently, Bi et al. (2017)
120 investigated responses of C:N:P stoichiometry and FAs to the interactions of three
121 environmental factors in the diatom *Phaeodactylum tricornutum* and the cryptophyte
122 *Rhodomonas* sp., showing dramatic effects of warming and nutrient deficiency, and
123 modest effects of increased $p\text{CO}_2$. However, for the key coccolithophore species *E.*
124 *huxleyi* much less is known about the simultaneous changes in elemental
125 stoichiometry and biochemicals in response to multiple environmental 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 $p\text{CO}_2$ on *E. huxleyi*
128 elemental stoichiometry and FAs. The elevated levels of temperature and $p\text{CO}_2$ in our
129 study are within the predicted ranges of future ocean scenarios. The inter-annual
130 average temperature varied between 16 to 22 °C at the Azores
131 (<http://dive.visitazores.com/en/when-dive>; last accessed date: 22.08.2017), the source
132 region of our *E. huxleyi* strain, while annual mean sea surface temperature across the

133 North Atlantic (0 - 60 °N) is projected to reach 29.8 °C in 2100 according to the ocean
134 general circulation model (Lewandowska et al., 2014). Considerable seasonal, depth
135 and regional variations of $p\text{CO}_2$ have been observed in the present-day ocean (Joint et
136 al., 2011). In plankton-rich waters, respiration plus atmospheric CO₂-enrichment can
137 drive high regional $p\text{CO}_2$ at times today, e.g, up to 900 μatm in August, with the
138 minimum value of 192 μatm in April, in the Southern Bight of the North Sea
139 (Schiettecatte et al., 2007). In future oceans, $p\text{CO}_2$ is projected to increase with rising
140 atmospheric CO₂, being 851 - 1370 μatm by 2100 and 1371 - 2900 μatm by 2150
141 (RCP8.5 scenario of the IPCC report 2014) (IPCC, 2014). We tested the following
142 hypotheses in the present study: (i) elemental stoichiometry and FAs in *E. huxleyi*
143 show different sensitivity to considerable variations in temperature, N:P supply ratios
144 and $p\text{CO}_2$; (ii) the ratios of particulate organic carbon vs. particulate organic nitrogen
145 (POC:PON), POC vs. particulate organic phosphorus (POC:POP), and particulate
146 inorganic carbon vs. POC (PIC:POC) in *E. huxleyi* will reduce and the proportions of
147 unsaturated fatty acids will increase under projected future ocean scenarios; and (iii)
148 there are synergistic interactions between warming, nutrient deficiency and rising
149 $p\text{CO}_2$ on *E. huxleyi* elemental stoichiometry and FA composition.

150 2 Material and methods

151 2.1 Experimental setup

152 To address our questions on how multiple environmental drivers influence
153 elemental and FA composition in *E. huxleyi*, we performed a semi-continuous culture
154 experiment crossing three temperatures (12, 18 and 24 °C), three N:P supply ratios

155 (molar ratios 10:1, 24:1 and 63:1) and two $p\text{CO}_2$ levels (560 and 2400 μatm). The
156 strain of *E. huxleyi* (Internal culture collection reference code: A8) was isolated from
157 waters off Terceira Island, Azores, North Atlantic (38°39'22" N 27°14'08" W).
158 Semi-continuous cultures, as a practical surrogate for fully continuous culture, have
159 been successfully used to study the responses of phytoplankton stoichiometric and
160 biochemical composition to environmental changes such as nutrient availability (Feng
161 et al., 2017a; Lynn et al., 2000; Terry et al., 1985). Our temperature range setup was
162 based on the study of Lewandowska et al. (2014), who chose a temperature increment
163 of 6 °C, according to the ocean general circulation model under the IPCC SRES A1F1
164 scenario.

165 All cultures were exposed to a light intensity of 100 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at a
166 16:8 h light:dark cycle in temperature-controlled rooms. The culture medium was
167 prepared with sterile filtered (0.2 μm pore size, Sartobran® P 300; Sartorius,
168 Goettingen, Germany) North Sea water with a salinity of 37 psu. Macronutrients were
169 added as sodium nitrate (NaNO_3) and potassium dihydrogen phosphate (KH_2PO_4) to
170 achieve three N:P supply ratios, i.e., 35.2 $\mu\text{mol} \cdot \text{L}^{-1}$ N and 3.6 $\mu\text{mol} \cdot \text{L}^{-1}$ P (10:1 mol
171 mol^{-1}), 88 $\mu\text{mol} \cdot \text{L}^{-1}$ N and 3.6 $\mu\text{mol} \cdot \text{L}^{-1}$ P (24:1 mol mol^{-1}) and 88 $\mu\text{mol} \cdot \text{L}^{-1}$ N and
172 1.4 $\mu\text{mol} \cdot \text{L}^{-1}$ P (63:1 mol mol^{-1}). Vitamins and trace metals were added based on the
173 modified Provasoli's culture medium (Ismar et al., 2008; Provasoli, 1963). Initial
174 $p\text{CO}_2$ of the culture medium was manipulated by bubbling with air containing the
175 target $p\text{CO}_2$. Three replicates were set up for each treatment, resulting in 54
176 experimental units. Each culture was kept in a sealed cell culture flask with 920 mL

177 culture volume. Culture flasks were carefully rotated twice per day at a set time to
178 minimize sedimentation.

179 First, batch culture experiments were performed to obtain an estimate of the
180 observed maximal growth rate (μ_{\max} , d^{-1}) under three temperatures, three N:P supply
181 ratios and two pCO_2 levels. μ_{\max} was calculated based on the changes of population
182 cell density within exponential phase (Bi et al., 2012). Once batch cultures reached
183 the early stationary phase, semi-continuous cultures were started with the algae from
184 batch cultures. The gross growth rate [μ (d^{-1})], resulting from the process of
185 reproduction alone due to negligible mortality in cultures lacking predators (Lampert
186 and Sommer, 2007)] was applied as 20% of μ_{\max} . Using % of μ_{\max} guarantees that the
187 strength of nutrient deficiency is equal through all temperature and pCO_2 treatments.

188 A fixed value of μ would mean weak deficiency when μ_{\max} is low, and strong
189 deficiency when it is high. Based on μ , the equivalent daily renewal rate (D , d^{-1}) can
190 be calculated according to the equation $D = 1 - e^{-\mu t}$, where t is renewal interval (here t
191 = 1 day). The volume of the daily renewal incubation water can be calculated by
192 multiplying D with the total volume of incubation water (920 mL). The incubation
193 water was exchanged with freshly made seawater medium with the target N:P supply
194 ratios, as well as pre-acclimated to the desired pCO_2 level. To counterbalance the
195 biological CO_2 -drawdown, the required amount of CO_2 -saturated seawater was also
196 added. Renewal of the cultures was carried out at the same hour every day. The steady
197 state in semi-continuous cultures was assessed based on the net growth rate [r (d^{-1})],
198 the difference between the gross growth rate and the loss rate ($r = \mu - D$]. When r

199 was zero (at steady state), μ was equivalent to D .

200 **2.2 Sample analysis**

201 Sampling took place at steady state for the following parameters: cell density,
202 dissolved inorganic carbon (DIC), total alkalinity (TA), pH, total particulate carbon
203 (TPC), POC, PON, POP and FAs. Cell density was counted daily in batch and
204 semi-continuous cultures (final cell density at steady state ranging between 1.50×10^5
205 - 17.8×10^5 cells mL^{-1} , with the average value of 7.95×10^5 cells mL^{-1}). pH
206 measurements were conducted daily in semi-continuous cultures (Fig. S1), and the
207 electrode was calibrated using standard pH buffers (pH 4 and pH 7; WTW, Weilheim,
208 Germany).

209 DIC water samples were gently filtered using a single-use syringe filter (0.2 μm ,
210 Minisart RC25; Sartorius, Goettingen, Germany) which was connected to the intake
211 tube of a peristaltic pump. Samples were collected into 10 ml glass vials, and all vials
212 were immediately sealed after filling. DIC was analyzed following Hansen et al.
213 (2013) using a gas chromatographic system (8610C; SRI-Instruments, California,
214 USA). Samples for TA analysis were filtered through GF/F filters (Whatman GmbH,
215 Dassel, Germany) and analyzed with the Tirino plus 848 (Metrohm, Filderstadt,
216 Germany). The remaining carbonate parameter $p\text{CO}_2$ was calculated using CO2SYS
217 (Pierrot et al., 2006) and the constants supplied by Hansson (1973) and Mehrbach et
218 al. (1973) that were refitted by Dickson and Millero (1987) (Table S1).

219 TPC, POC, PON and POP samples were filtered onto pre-combusted and
220 pre-washed (5% ~ 10% HCl) GF/F filters (Whatman GmbH, Dassel, Germany). For

221 POC samples, PIC was removed by exposing filters containing TPC to fuming
222 hydrochloric acid for 12h. Before analysis, filters were dried at 60 °C and stored in a
223 desiccator. POC and PON were simultaneously determined by gas chromatography
224 using an organic elemental analyzer (Thermo Flash 2000; Thermo Fisher Scientific
225 Inc., Schwerte, Germany) after Sharp (1974). POP was analyzed colorimetrically by
226 converting organic phosphorus compounds to orthophosphate (Hansen and Koroleff,
227 1999). PIC was determined by subtracting POC from TPC. PIC and POC production
228 were estimated by multiplying μ with cellular PIC and POC content, respectively. As
229 the physiological (i.e., cellular) PIC and POC variations cannot directly be up scaled
230 to total population response (Matthiessen et al., 2012), PIC and POC contents in our
231 study were shown both on the cellular (as pg cell⁻¹) and the population (as μ g ml⁻¹)
232 levels.

233 Fatty acid samples were taken on pre-combusted and hydrochloric acid-treated
234 GF/F filters (Whatman GmbH, Dassel, Germany), and stored at -80 °C before
235 measurement. FAs were measured as fatty acid methyl esters (FAMEs) using a gas
236 chromatograph (Trace GC-Ultra; Thermo Fisher Scientific Inc., Schwerte, Germany)
237 according to the procedure described in detail in Arndt and Sommer (2014). The
238 FAME 19:0 was added as internal standard and 21:0 as esterification control. The
239 extracted FAs were dissolved with n-hexane to a final volume of 100 μ L. Sample
240 aliquots (1 μ L) were given into the GC by splitless injection with hydrogen as the
241 carrier gas. Individual FAs were integrated using Chromcard software (Thermo Fisher
242 Scientific Inc., Schwerte, Germany) and identified with reference to the standards

243 Supelco 37 component FAME mixture and Supelco Menhaden fish oil. FA data were
244 expressed as a percentage of total fatty acids (TFAs) (FA proportion, % of TFAs) to
245 better compare our results with those in previous studies. FAs were also quantified on
246 a per unit biomass ($\mu\text{g mg C}^{-1}$), which is an ideal approach when considering
247 nutritional quality of phytoplankton for herbivores (Piepho et al., 2012).

248 **2.3 Statistical analysis**

249 Generalized linear mixed models (GLMMs) were applied to test the best model
250 explaining the variations in μ_{max} , elemental stoichiometry and FA composition, as this
251 method is more appropriate for non-normal data than classical statistical procedures
252 (Bolker et al., 2009). GLMMs combine the properties of two statistical models (linear
253 mixed models and generalized linear models) (Bolker et al., 2009) and have been
254 widely used in ecology (e.g., Bracewell et al., 2017; Frère et al., 2010; Jamil et al.,
255 2014), in which data sets are often non-normally distributed. In our study, response
256 variables included μ_{max} , elemental stoichiometry [elemental cellular contents (as pg
257 cell^{-1}) and their molar ratios], POC and PIC population yield (as $\mu\text{g ml}^{-1}$) and
258 production (as $\text{pg cell}^{-1} \text{d}^{-1}$), FA proportion (as % of TFAs) and contents (as $\mu\text{g mg C}^{-1}$),
259 with temperature, N:P supply ratios and $p\text{CO}_2$ as fixed effects. Target distributions
260 were tested and link functions were consequently chosen. The link function is a
261 transformation of the target that allows estimation of the model
262 (https://www.ibm.com/support/knowledgecenter/SSLVMB_21.0.0/com.ibm.spss.statistics.help/idh_glmm_target.htm; last accessed date: 14.08.2017). For example, identity
264 link function is appropriate with any distribution except for multinomial, while logit

265 can be used only with the binomial or multinomial distribution. For all response
266 variables, we tested models containing first order effects, and second and third order
267 interactions of the three factors. The model that best predicted targets was selected
268 based on the Akaike Information Criterion corrected (AICc), i.e., a lower AICc value
269 representing a better fit of the model. Changes of 10 units or more in AICc values
270 were considered as a reasonable improvement in the fitting of GLMMs (Bolker et al.,
271 2009). In case AICc values were comparable (< 10 units difference), the simpler
272 model was thus chosen, unless there were significant second or third order
273 interactions detected. According to differences in AICc values, models containing
274 only first order effects of the three factors were selected as the best models for most
275 response variables, while those also containing second order interactions were chosen
276 for cellular POC, PON, POP and PIC contents, and the proportions of saturated fatty
277 acid (SFA) and docosahexaenoic acid (22:6n-3; DHA) (bold letters in Table S2).
278 Models containing third order interactions were not selected for any response
279 variable.

280 Nested models were applied to test whether the response pattern to one factor (a
281 nested factor) was significant within another factor, in case significant second order
282 interactions were detected in GLMMs. The question a nested model addresses is that,
283 whether one factor plays a role under one (or several) configuration(s) of another
284 factor, but not under all configurations of that factor equally. Also, the nature
285 (antagonistic, additive, or synergistic) of significant second order interactions was
286 analysed according to Christensen et al. (2006). The observed combined effect of two

287 factors was compared with their expected net additive effect [e.g., (factor₁ - control) +
288 (factor₂ - control)], which was based on the sum of their individual effects. If the
289 observed combined effect exceeded their expected additive effect, the interaction was
290 defined as synergism. In contrast, if the observed combined effect was less than the
291 additive effect, the interaction was defined as antagonism.

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

294 **3 Results**

295 **3.1 Maximal growth rate (μ_{\max})**

296 We observed a highly significant effect of temperature (bold letters in Table 1) and
297 non-significant **effects** of N:P supply ratios and $p\text{CO}_2$ on μ_{\max} in *E. huxleyi*. Increasing
298 temperature stimulated μ_{\max} , causing μ_{\max} to be two to three times higher at the highest
299 temperature than those at the lowest temperature (Fig. 1).

300 **3.2 Elemental stoichiometry**

301 GLMMs results showed that cellular contents of POC, PON, POP and PIC
302 responded significantly to temperature and the interactions between temperature and
303 N:P supply ratios (bold letters in Table 1). Moreover, there were significant effects of
304 $p\text{CO}_2$ on cellular PIC content, and significant interactions between temperature and
305 $p\text{CO}_2$ on cellular POP and PIC contents. For cellular contents of POC, PON and POP,
306 increasing temperature and nutrient deficiency showed synergistic interactions (Table
307 S3), resulting in lower values at higher temperatures under N deficiency (N:P supply
308 ratio = 10:1 mol mol⁻¹) and increasing values with increasing temperature under P

309 deficiency (N:P supply ratio = 63:1 mol mol⁻¹) (Fig. 2a-c; Nested model, $p < 0.001$).
310 Synergistic interactions were also observed between increasing temperature and
311 enhanced $p\text{CO}_2$ on cellular POP content (Table S3), showing the lowest value at low
312 $p\text{CO}_2$ level and the highest one at enhanced $p\text{CO}_2$ in response to increasing
313 temperature (Fig. 2g; Nested model, $p = 0.003$). For cellular PIC content, increasing
314 temperature and N deficiency had antagonistic interactions, while increasing
315 temperature and P deficiency showed synergistic interactions (Table S3). As a result,
316 cellular PIC content showed a slight decreasing trend with increasing temperature
317 under N deficiency and an increasing trend under higher N:P supply ratios (Fig. 2d;
318 Nested model, $p = 0.030$). Increasing temperature and enhanced $p\text{CO}_2$ affected
319 cellular PIC content synergistically (Table S3), with the negative response of cellular
320 PIC content to enhanced $p\text{CO}_2$ being significantly weaker as temperature increased
321 (Fig. 2h; Nested model, $p < 0.001$).

322 POC:PON, POC:POP and PON:POP responded significantly to N:P supply ratios
323 (bold letters in Table 1), while only POC:PON showed significant responses to
324 temperature, with a non-significant effect of $p\text{CO}_2$ detected. Increasing N:P supply
325 ratios caused a decreasing trend in POC:PON (Fig. 3a) and an increase in POC:POP
326 (Fig. 3b), resulting in a positive relationship between PON:POP and N:P supply ratios
327 (Fig. 3c). The response of POC:PON to increasing temperature was complex, showing
328 a hump-shaped response under N deficiency and negative responses under higher N:P
329 supply ratios (Fig. 3a). PIC:POC responded significantly to temperature and $p\text{CO}_2$,
330 with a non-significant effect of N:P supply ratios detected (Table 1). PIC:POC

331 increased with increasing temperature and decreased with enhanced $p\text{CO}_2$ (Fig. 3d
332 and h).

333 **3.3 Fatty acids**

334 The most abundant FA group was polyunsaturated fatty acids (PUFAs) (33% - 54%
335 of TFAs), followed by SFAs (22% - 46%) and monounsaturated fatty acids (MUFAs)
336 (13% - 27%), across the entire tested gradients of temperature, N:P supply ratios and
337 $p\text{CO}_2$ (Table S4). The high proportion of PUFAs was predominantly caused by high
338 amounts of DHA (12% - 31%) and 18:4n-3 (3% - 13%), and SFAs was mainly
339 represented by 14:0 (13% - 23%) and 16:0 (5% - 11%). The major individual MUFA
340 was 18:1n-9 (8% - 21%).

341 GLMMs results showed significant effects of temperature and $p\text{CO}_2$ on the
342 proportions of both MUFAs and PUFAs, and significant interactions between N:P
343 supply ratios and $p\text{CO}_2$ on SFAs (bold letters in Table 1). Increasing temperature
344 caused a decrease in the proportion of MUFAs and an increase in PUFAs (Fig. 4a). In
345 contrast, enhanced $p\text{CO}_2$ resulted in an increase in MUFAs and a decrease in PUFAs
346 at higher temperatures (Fig. 4c). Moreover, enhanced $p\text{CO}_2$ and N (and P) deficiency
347 affected SFA proportion synergistically (Table S3), with the unimodal response of
348 SFA to increasing N:P supply ratios being more pronounced at the high $p\text{CO}_2$ (Fig. S2;
349 Nested model, $p < 0.001$).

350 The proportion of major individual PUFAs (DHA) showed significant responses to
351 temperature and N:P supply ratios, and the interactions between temperature and N:P
352 supply ratios (and $p\text{CO}_2$) (bold letters in Table 1). Increasing temperature and N:P

353 supply ratios caused an overall increase in DHA (Fig. 4b). The interactions between
354 increasing temperature and nutrient deficiency (and enhanced $p\text{CO}_2$) affected DHA
355 synergistically (Table S3), and the positive effect of temperature became more
356 pronounced at lower N:P supply ratios (Nested model, $p < 0.001$) and at the low $p\text{CO}_2$
357 (Nested model, $p < 0.001$) (Fig. 4b and d).

358 **4 Discussion**

359 Our study scales the impacts of temperature, N:P supply ratios and $p\text{CO}_2$ on
360 elemental stoichiometry and FA composition of the ubiquitously important calcifier *E.*
361 *huxleyi*, while accounting for their interactive effects. Overall, C:N:P stoichiometry
362 changed markedly in response to N:P supply ratios, showing a maximum of 62%
363 changes under P deficiency (Table 2). Both PIC:POC and PUFA proportion increased
364 with warming and decreased under high $p\text{CO}_2$, indicating a partial compensation by
365 $p\text{CO}_2$ of a predominantly temperature-driven response. The overall response patterns
366 of C:N:P stoichiometry in our study are consistent with those on a global scale
367 (Martiny et al., 2013), and PUFA responses conform with the meta-analysis results on
368 haptophytes (Hixson and Arts, 2016). In line with these studies, we also detected
369 significant interactions between temperature, N:P supply ratios and $p\text{CO}_2$ on certain
370 response variables (e.g., cellular elemental contents and DHA proportion) (Table 1),
371 indicating variable response patterns of elemental stoichiometry and FA composition
372 in *E. huxleyi* under any given constellation of environmental factors. Our results thus
373 underscore the important effects of multiple environmental drivers, demonstrating
374 differential effects of the three environmental factors on elemental stoichiometry and

375 FA composition in *E. huxleyi*.

376 **4.1 Responses of maximal growth rate**

377 Increasing temperature significantly accelerated μ_{\max} of *E. huxleyi* in our study (Fig.

378 1; Table 1). This positive correlation between increasing temperature and growth rate

379 is typical for many *E. huxleyi* strains within the range of temperature 12 to 24 °C used

380 in our study (Feng et al., 2008; Rosas-Navarro et al., 2016; Sett et al., 2014; van

381 Bleijswijk et al., 1994). However, the extent to which growth rate of *E. huxleyi*

382 increases with increasing temperature varies between *E. huxleyi* strains, which may

383 contribute to specific biogeographic distribution of different strains (Paasche, 2002).

384 For example, growth rate of *E. huxleyi* from the Gulf of Maine (~ 42 °N) was 1.2

385 times higher at 26 °C than that at 16 °C, while growth rate of *E. huxleyi* from the

386 Sargasso Sea (~ 20 - 35 °N) was 1.6 times higher at the higher temperature (Paasche,

387 2002). In our study, μ_{\max} of *E. huxleyi* (from the Azores, ~ 38 °N) was two to three

388 times higher at the highest temperature than that at the lowest temperature, showing a

389 similar change pattern with that in the *E. huxleyi* strain from the Sargasso Sea. The

390 results above suggest that the biogeographic origin of an *E. huxleyi* strain is important

391 for their growth response to temperature.

392 **4.2 Responses of C:N:P stoichiometry**

393 N:P supply ratios showed highly significant effects on C:N:P stoichiometry (up to a

394 62% increase in PON:POP under P deficiency) in *E. huxleyi* in our study, with a

395 weaker effect of warming (a 6% decrease in POC:PON) and a non-significant effect

396 of $p\text{CO}_2$ observed (Table 1; Table 2). Similarly, previous lab experiments also

397 reported that nutrient availability played a more important role than temperature and
398 $p\text{CO}_2$ for C:N:P stoichiometry in different strains of *E. huxleyi* such as those from
399 outer Oslofjord (Skau, 2015) and from the Chatham Rise, east of New Zealand (Feng
400 et al., 2017b). Also, for marine phytoplankton community biomass on a global scale
401 nitrate concentration as a proxy of nutrient availability explained 36% and 42% of
402 variation in N:P and C:P, respectively, with the less variation explained by
403 temperature (33% and 38% of the variation in N:P and C:P, respectively) (Martiny et
404 al., 2013).

405 N deficiency caused overall high POC:PON and low PON:POP, while P deficiency
406 resulted in high POC:POP and PON:POP in *E. huxleyi* in this and most previous
407 studies (Langer et al., 2013; Leonardos and Geider, 2005b; Perrin et al., 2016). An
408 important biogeochemical question is the extent to which C:N:P stoichiometry
409 changes in response to N and P deficiency. We found that the high percent change in
410 PON:POP (a 62% increase) under P deficiency was mainly due to a 60% increase in
411 POC:POP, associated with the higher percent change in cellular POC content (a 50%
412 increase) and the lower percent change in cellular POP content (a 8% decrease) (Table
413 2). Under N deficiency, the 36% decrease in PON:POP was driven by a 33% increase
414 in POC:PON and a 15% decrease in POC:POP, along with similar percent changes in
415 cellular elemental contents (32% to 53% decrease). The more variable POC:POP
416 under P deficiency and the less variable POC:PON under N deficiency in our study
417 are consistent with the findings in global suspended particle measurements, which
418 showed the high variability of P:C in response to changes in phosphate and the less

419 variable N:C to changes in nitrate (Galbraith and Martiny, 2015). The consistence of
420 C:N:P stoichiometric responses in our study with those on a global scale may reflect
421 the capacity of *E. huxleyi* to thrive under a wide range of environmental conditions.
422 This capacity was largely revealed by a pan-genome assessment, which distributed
423 genetic traits variably between strains and showed a suit of core genes for the uptake
424 of inorganic nitrogen and N-rich compounds such as urea (Read et al., 2013). In spite
425 of strain diversity within *E. huxleyi*, a recent study suggested that the global
426 physiological response of this species to nutrient environments is highly conserved
427 across strains and may underpin its success under a variety of marine environments
428 (Alexander, 2016).

429 Warming resulted in a significant, but slight decrease in POC:PON (-6%),
430 associated with a 8% decrease in cellular POC content and a 5% increase in cellular
431 PON content, while non-significant responses of POC:POP or PON:POP were
432 observed in *E. huxleyi* (Table 2). In the literature, variable changes of POC:PON to
433 warming were observed in *E. huxleyi*, showing positive (Borchard and Engel, 2012),
434 negative (Feng et al., 2008; Matson et al., 2016), and U-shaped responses
435 (Rosas-Navarro et al., 2016). Similar to our study, Borchard and Engel (2012) also
436 found that increasing temperature caused a stronger change in POC:PON than that in
437 POC:POP at higher P [conditions](#) in the strain PML B92/11 from Bergen, Norway. The
438 mechanism behind the stronger change in POC:PON compared to POC:POP with
439 warming may be explained by the temperature-dependent physiology hypothesis,
440 which shows that organisms in warmer conditions require fewer P-rich ribosomes,

441 relative to N-rich proteins (Toseland et al., 2013).

442 The single effects of nutrient availability and temperature described above can be
443 modulated by their interactions. We observed synergistic interactions between
444 warming and nutrient deficiency on cellular contents of POC, PON and POP, and
445 between warming and enhanced $p\text{CO}_2$ on cellular POP content (Table 1; Table S3).

446 An overall synergistic effect was also observed across 171 studies on the responses of
447 marine and coastal systems to multiple stressors (Crain et al., 2008). Furthermore,
448 although a 29% change emerged in cellular POP content with rising $p\text{CO}_2$, we found a
449 non-significant single effect of $p\text{CO}_2$ on *E. huxleyi* C:N:P stoichiometry. Previous
450 studies showed that rising $p\text{CO}_2$ seems to change phytoplankton stoichiometry under
451 specific conditions, e.g. at high light intensity ($400 \text{ }\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) (Feng et
452 al., 2008) and low nutrient loads ($500 \text{ }\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at N:P supply ratio ≤ 15
453 or N:P supply ratio ≥ 30) (Leonardos and Geider, 2005a). In our study, we used
454 relatively lower light intensity ($100 \text{ }\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) than that in previous
455 studies, and did not investigate irradiance effects. Additional research is required to
456 assess the effects of other environmental factors such as irradiance and their
457 interactions on C:N:P stoichiometry in our *E. huxleyi* strain.

458 Taken together, our results indicate that C:N:P stoichiometry in *E. huxleyi* largely
459 reflected the changes in N:P supply ratios, across different temperatures and $p\text{CO}_2$
460 levels. However, for two algal species from non-calcifying classes (the diatom *P.*
461 *tricornutum* and the cryptophyte *Rhodomonas* sp.) temperature had the most
462 consistent significant effect on stoichiometric ratios in our previous work (Bi et al.,

463 2017). The results above are consistent with the ranking of environmental control
464 factors in Boyd et al. (2010), which showed that temperature, nitrogen and
465 phosphorus were ranked as important factors for major phytoplankton groups.

466 **4.3 Responses of PIC:POC**

467 Both $p\text{CO}_2$ and temperature had highly significant effects on PIC:POC in our study,
468 with enhanced $p\text{CO}_2$ and warming resulting in an overall 49% decrease and a 41%
469 increase in PIC:POC, respectively, while N:P supply ratios showed no significant
470 effect (Table 1; Table 2). This result is in agreement with rankings of the importance
471 of environmental drivers on PIC:POC in a Southern Hemisphere strain of *E. huxleyi*
472 (isolated from the Chatham Rise), showing the order of $p\text{CO}_2$ (negative effect) >
473 temperature (positive effect) and a non-significant effect of nitrate or phosphate (Feng
474 et al., 2017b).

475 The negative effect of enhanced $p\text{CO}_2$ on PIC:POC has been widely observed for
476 different strains of *E. huxleyi* (Meyer and Riebesell, 2015 and references therein). The
477 negative response of PIC:POC to rising $p\text{CO}_2$ in our study was driven by the
478 significant decrease in cellular PIC content (calcification), with cellular POC content
479 (photosynthesis) showing non-significant changes (Table 1; Table 2). Previous studies
480 also showed a greater impact of ocean acidification on calcification than on
481 photosynthesis in coccolithophores (De Bodt et al., 2010; Feng et al., 2017a; Meyer
482 and Riebesell, 2015). Feng et al. (2017a) suggested that the decreased calcification in
483 *E. huxleyi* may be caused by the increased requirement of energy to counteract
484 intracellular acidification. The increased activity of carbonic anhydrase (CA) at low

485 $p\text{CO}_2$ may explain the lack of a significant effect of $p\text{CO}_2$ on the photosynthetic or
486 growth rate (Feng et al., 2017a), as up-regulation of CA at low DIC was previously
487 observed (Bach et al., 2013).

488 Warming causes diverse responses of calcification and photosynthesis within *E.*
489 *huxleyi* species (Rosas-Navarro et al., 2016 and references therein; the present study).
490 Overall, our study showed that the increase in PIC:POC at high temperatures was
491 driven by a markedly increased cellular PIC content (28%) and a decreased cellular
492 POC content (-8%) (Table 1; Table 2), consistent with the responses of PIC:POC to
493 warming in other *E. huxleyi* strains such as the strain PML B92/11 (Sett et al., 2014)
494 and the strain CCMP3266 from the Tasman Sea (Matson et al., 2016). The positive
495 response of PIC:POC to increasing temperature may be explained by the allocation of
496 carbon to calcification rather than photosynthesis at high temperatures (Sett et al.,
497 2014).

498 Significant interactions were observed between temperature and N:P supply ratios,
499 and between temperature and $p\text{CO}_2$ on cellular particulate carbon contents in our
500 study (Table 1). For example, the negative relationship between cellular PIC content
501 and enhanced $p\text{CO}_2$ became weaker at higher temperatures (Fig. 2h). This result is in
502 agreement with the modulating effect of temperature on the CO_2 sensitivity of key
503 metabolic rates in cocolithophores, due to the shift of the optimum CO_2
504 concentration for key metabolic processes towards higher CO_2 concentrations from
505 intermediate to high temperatures (Sett et al., 2014). Specifically, the interactions
506 between warming and nutrient deficiency (and high $p\text{CO}_2$) synergistically affected

507 both PIC and POC cellular contents in most cases in our study (Table S3), indicating
508 that nutrient deficiency and high $p\text{CO}_2$ are likely to enhance the effect of warming on
509 *E. huxleyi* calcification and photosynthesis efficiency.

510 In summary, our results showed an overall reduced PIC:POC in *E. huxleyi* under
511 future ocean scenarios of warming and higher $p\text{CO}_2$ (Fig. 3h; Table 2), consistent with
512 the reduced ratio of calcium carbon production to organic carbon during the *E. huxleyi*
513 bloom in previous mesocosm experiments (Delille et al., 2005; Engel et al., 2005). It
514 is worth noting that cellular PIC and POC contents are a measure for physiological
515 response and cannot be directly used to infer population response, as different
516 responses between cellular and population yields of PIC (and POC) (as $\mu\text{g ml}^{-1}$) to
517 environmental changes were evident in previous work (Matthiessen et al., 2012) and
518 the present study (Table S5, S6; Fig. S3, S4). Thus, scaling our results up to
519 coccolithophores carbon export should consider these uncertainties.

520 **4.4 Responses of fatty acids**

521 Our study provides one of the first experimental demonstrations of the relative
522 importance of temperature, N:P supply ratios and $p\text{CO}_2$ on *E. huxleyi* FA composition.
523 Both temperature and $p\text{CO}_2$ had significant effects on the proportions of MUFA and
524 PUFA, with warming causing larger changes in MUFA and PUFA than rising $p\text{CO}_2$,
525 while significant effects of N:P supply ratios were only observed for DHA proportion
526 (Table 1; Table 2).

527 Increasing temperature caused a 20% decline in MUFA proportion and a 13%
528 increase in PUFA proportion in our study (Table 2). This result is consistent with the

529 negative response of MUFA proportion and positive response of PUFA proportion to
530 warming in other haptophytes based on a meta-analysis on 137 FA profiles (Hixson
531 and Arts, 2016), showing an opposite response to general patterns of phytoplankton
532 FAs to warming. Although warming is expected to have a negative effect on the
533 degree of fatty acid unsaturation to maintain cell membrane structural functions
534 (Fuschino et al., 2011; Guschina and Harwood, 2006; Sinensky, 1974), variable FA
535 responses to warming were widely observed in different phytoplankton groups (Bi et
536 al., 2017; Renaud et al., 2002; Thompson et al., 1992). Contradictory findings were
537 even reported in meta-analyses on large FA profiles such as the absence (Galloway
538 and Winder, 2015) or presence (Hixson and Arts, 2016) of the negative correlation
539 between temperature and the proportion of long-chain EFAs in freshwater and marine
540 phytoplankton. While the underlying mechanisms of variable FA responses are still
541 unclear, it is known that both phylogeny and environmental conditions determine
542 phytoplankton FA composition (Bi et al., 2014; Dalsgaard et al., 2003; Galloway and
543 Winder, 2015). In our study, we found significant interactions between temperature
544 and $p\text{CO}_2$ (and N:P supply ratios) on the individual FA component DHA, showing that
545 $p\text{CO}_2$ and nutrient availability may alter the effect of warming on *E. huxleyi* FA
546 composition.

547 Enhanced $p\text{CO}_2$ led to an overall 7% increase in MUFAs and a 7% decrease in
548 PUFA (Table 2), consistent with FA response patterns in the *E. huxleyi* strain PML
549 B92/11 (Riebesell et al., 2000) and the strain AC472 from Western New Zealand,
550 South Pacific (Fiorini et al., 2010). Also in a natural plankton community (Raunefjord,

551 southern Norway), PUFA proportion was reduced at high $p\text{CO}_2$ level in the nano-size
552 fraction, suggesting a reduced Haptophyta (dominated by *E. huxleyi*) biomass and a
553 negative effect of high $p\text{CO}_2$ on PUFA proportion (Bermúdez et al., 2016). To date,
554 several mechanisms have been suggested to explain the reduced PUFAs at high $p\text{CO}_2$
555 in green algae (Pronina et al., 1998; Sato et al., 2003; Thompson, 1996), with much
556 less work conducted in other phytoplankton groups. One possible mechanism was
557 demonstrated in the study on *Chlamydomonas reinhardtii*, showing that the repression
558 of the CO_2 -concentrating mechanisms (CCMs) was associated with reduced FA
559 desaturation at high CO_2 concentration (Pronina et al., 1998). Our observed decrease
560 in the proportion and content of PUFAs at higher $p\text{CO}_2$ (Table S6) fits well with the
561 mechanism proposed by Pronina et al. (1998), which may be attributed to the
562 repression of CCMs at high $p\text{CO}_2$ in *E. huxleyi*.

563 N and P deficiency caused no significant changes in the proportions of MUFAAs and
564 PUFAs, while a 14% to 22% increase in DHA proportion was observed (Table 2).
565 While nutrients often play a major role on phytoplankton lipid composition (Fields et
566 al., 2014; Hu et al., 2008), the less pronounced effects of nutrient deficiency in our
567 study indicate a unique lipid biosynthesis in *E. huxleyi*. Indeed, Van Mooy et al. (2009)
568 suggested that *E. huxleyi* used non-phosphorus betaine lipids as substitutes for
569 phospholipids in response to P scarcity. Genes are also present in the core genome of
570 *E. huxleyi* for the synthesis of betaine lipids and unusual lipids used as
571 nutritional/feedstock supplements (Read et al., 2013). Therefore, the lack of
572 significant nutrient effects on most FA groups in *E. huxleyi* in our study may be

573 caused by the functioning of certain lipid substitutions under nutrient deficiency.

574 In summary, our study showed stronger effects of $p\text{CO}_2$ and temperature, and a
575 weaker effect of N:P supply ratios on the proportions of unsaturated FAs in *E. huxleyi*.

576 It should be noted that using different units to quantify FA composition may cause
577 contradictory results, e.g., an increase in PUFA proportion (% of TFAs) but
578 non-significant changes in PUFA contents per biomass ($\mu\text{g mg C}^{-1}$) with increasing
579 temperature in our study (Table S5, S6). Moreover, PUFA contents per biomass in two
580 species of non-calcifying classes (*P. tricornutum* and *Rhodomonas* sp.) showed a
581 different response pattern from that observed in *E. huxleyi* in our study, i.e., a
582 significant negative effect of enhanced $p\text{CO}_2$ on PUFA contents in *E. huxleyi* (Table
583 S6), but a non-significant effect of $p\text{CO}_2$ on PUFA contents in *P. tricornutum* and
584 *Rhodomonas* sp. (Bi et al., 2017). This different response between phytoplankton
585 groups is in agreement with findings in mesocosm studies (Bermúdez et al., 2016;
586 Leu et al., 2013), suggesting that changes in taxonomic composition can cause
587 different relationships between PUFAs and $p\text{CO}_2$ in natural phytoplankton
588 community.

589 **4.5 Implications for marine biogeochemistry and ecology**

590 We observed an overall increase in POC:PON (with warming and N deficiency)
591 and POC:POP (with N and P deficiency) in *E. huxleyi*, while enhanced $p\text{CO}_2$ showed
592 no significant effect (Table 2). This result indicates that nitrogen and phosphorus
593 requirements in *E. huxleyi* are likely to reduce under projected future changes in
594 temperature and nutrient deficiency, and show minor changes in response to higher

595 $p\text{CO}_2$. Likewise, Hutchins et al. (2009) suggested negligible or minor effects of
596 projected future changes in $p\text{CO}_2$ on most phytoplankton phosphorus requirements.
597 Moreover, the overall low PIC:POC under future ocean scenarios (warming and
598 enhanced $p\text{CO}_2$) indicates that carbon production by the strain *E. huxleyi* in our study
599 acts as a carbon sink. This argument is consistent with the findings of the decreased
600 calcification with increasing $p\text{CO}_2$ in most coccolithophores (Beaufort et al., 2011;
601 Hutchins and Fu, 2017), which may reduce vertical exported fluxes of sinking
602 calcium carbonate and minimize calcification as a carbon source term, ultimately
603 downsizing the ocean's biological carbon cycle (Hutchins and Fu, 2017).

604 Besides the overall increase in POC:PON and POC:POP, we found an overall
605 increase in the proportions of PUFAs (with warming and enhanced $p\text{CO}_2$) and DHA
606 (with warming, N and P deficiency and enhanced $p\text{CO}_2$) in *E. huxleyi* (Table 2), but a
607 decrease in PUFA and DHA contents per biomass with enhanced $p\text{CO}_2$ (Table S6).
608 The relationship between changes in stoichiometry and FA composition in
609 phytoplankton varies in a complex way with environmental conditions and algal
610 taxonomy (Bi et al., 2014; Pedro Cañavate et al., 2017; Sterner and Schulz, 1998). For
611 example, the correlation between PON:POC and PUFA contents per biomass was
612 negative in *Rhodomonas* sp. and positive in *P. tricornutum* under N deficiency (Bi et
613 al., 2014). Our findings thus indicate that elemental composition responses may be
614 coupled with responses in essential FA composition in the strain of *E. huxleyi* studied
615 under certain configurations of environmental drivers. Such a linkage between
616 stoichiometric and FA composition is important in studies of food web dynamics, as

617 the C:N and C:P stoichiometry and PUFAs both have been used as indicators of
618 nutritional quality of phytoplankton, with high POC:PON (and POC:POP) and low
619 contents in certain PUFAs often constraining zooplankton production by reducing
620 trophic carbon transfer from phytoplankton to zooplankton (Hessen, 2008; Jónasdóttir
621 et al., 2009; Müller-Navarra et al., 2000; Malzahn et al., 2016). In addition, other
622 factors such as the cell size of phytoplankton and nutritional requirements of
623 consumers can also influence trophic transfer efficiency (Anderson and Pond, 2000;
624 Sommer et al., 2016). Nevertheless, studies on plant-herbivore interactions reported
625 that changes in elemental and biochemical composition in phytoplankton can translate
626 to higher trophic levels (Kamya et al., 2017; Malzahn et al., 2010; Rossoll et al., 2012)
627 and refer to direct effects of environmental changes on low trophic level consumers,
628 which can be modified by indirect bottom-up driven impacts through the primary
629 producers (Garzke et al., 2016; Garzke et al., 2017).

630 **5 Conclusions**

631 Our study shows that N:P supply ratios had the strongest effect on C:N:P
632 stoichiometry, while temperature and $p\text{CO}_2$ played more influential roles on PIC:POC
633 and PUFA proportions in *E. huxleyi*. The specific response patterns of elemental ratios
634 and FAs have important implications for understanding biogeochemical and
635 ecological functioning of *E. huxleyi*. The observations presented here suggest
636 differential responses of elements and FAs to rising temperature, nutrient deficiency
637 and enhanced $p\text{CO}_2$ in *E. huxleyi*, being to some extent unique compared to algal
638 species from non-calcifying classes. Thus, the role of multiple environmental drivers

639 under the biodiversity context should be considered to truly estimate the future
640 functioning of phytoplankton in the changing marine environments.

641 **Data availability:** data sets are available upon request by contacting Meixun Zhao
642 (maxzhao@ouc.edu.cn and maxzhao04@yahoo.com).

643 **Author contribution:** R. Bi, S. Ismar, U. Sommer and M. Zhao designed the
644 experiments and R. Bi carried them out. R. Bi prepared the manuscript with
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647

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1025 **Fig. 1** Responses of the observed maximal growth rate (μ_{\max} ; mean \pm SE) to
1026 temperature, N:P supply ratios and $p\text{CO}_2$ in *Emiliania huxleyi*. The selected model
1027 contains only the first order effects of the three environmental factors, with the results
1028 of AICc shown in Table S2.

1029 **Fig. 2** Responses of cellular contents of (a, e) particulate organic carbon (POC), (b, f)
1030 particulate organic nitrogen (PON), (c, g) particulate organic phosphorus (POP) and
1031 (d, h) particulate inorganic carbon (PIC) (mean \pm SE) to temperature, N:P supply
1032 ratios and $p\text{CO}_2$ in *Emiliania huxleyi*. The selected models contain the first order
1033 effects, and second order interactions of the three environmental factors for the four
1034 response variables, with the results of AICc shown in Table S2.

1035 **Fig. 3** The ratios of (a, e) particulate organic carbon vs. particulate organic nitrogen
1036 (POC:PON), (b, f) POC vs. particulate organic phosphorus (POC:POP), (c, g) PON vs.
1037 POP (PON:POP) and (d, h) particulate inorganic carbon vs. POC (PIC:POC) (mean \pm
1038 SE) in response to temperature, N:P supply ratios and $p\text{CO}_2$ in *Emiliania huxleyi*. The
1039 selected models contain only the first order effects of the three environmental factors
1040 for the four response variables, with the results of AICc shown in Table S2.

1041 **Fig. 4** Responses of the proportions of (a, c) monounsaturated fatty acids (MUFA)
1042 and polyunsaturated fatty acids (PUFA), and (b, d) docosahexaenoic acid (DHA)
1043 (mean \pm SE) to temperature, N:P supply ratios and $p\text{CO}_2$ in *Emiliania huxleyi*. For
1044 MUFA and PUFA proportions, the selected models contain only the first order effects
1045 of the three environmental factors, and that for DHA proportion contains also second
1046 order interactions, with the results of AICc shown in Table S2.

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1051 Table 1. Results of the selected GLMMs testing for the effects of temperature, N:P
 1052 supply ratios and $p\text{CO}_2$ on the observed maximal growth rate (μ_{max}), elemental
 1053 stoichiometry and fatty acid proportions in *Emiliania huxleyi*. Significant p values are
 1054 shown in bold; T: temperature; N:P: N:P supply ratios; TFA: total fatty acid; SFA:
 1055 saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty
 1056 acid; DHA: docosahexaenoic acid. Results of AICc are shown in Table S2.

Variable	Factor	Coefficient \pm SE	t	p
μ_{max} (d^{-1})	Intercept	-1.368 \pm 0.225	-6.075	<0.001
	T	0.074 \pm 0.010	7.082	<0.001
	$p\text{CO}_2$	<0.001 \pm <0.001	-0.472	0.644
	N:P	<0.001 \pm 0.002	-0.162	0.873
POC cellular content (pg cell $^{-1}$)	Intercept	3.683 \pm 0.377	9.779	<0.001
	T	-0.089 \pm 0.020	-4.577	<0.001
	$p\text{CO}_2$	<0.001 \pm <0.001	-0.929	0.358
	N:P	-0.008 \pm 0.008	-0.996	0.324
	T \times $p\text{CO}_2$	<0.001 \pm <0.001	1.886	0.066
	T \times N:P	0.001 \pm <0.001	3.477	0.001
	$p\text{CO}_2$ \times N:P	<0.001 \pm <0.001	-0.359	0.721
PON cellular content (pg cell $^{-1}$)	Intercept	1.208 \pm 0.491	2.458	0.018
	T	-0.083 \pm 0.026	-3.259	0.002
	$p\text{CO}_2$	<0.001 \pm <0.001	-0.873	0.387
	N:P	-0.008 \pm 0.011	-0.709	0.482
	T \times $p\text{CO}_2$	<0.001 \pm <0.001	1.549	0.128
	T \times N:P	0.001 \pm 0.001	2.802	0.007
	$p\text{CO}_2$ \times N:P	<0.001 \pm <0.001	0.165	0.870
POP cellular content (pg cell $^{-1}$)	Intercept	-0.564 \pm 0.468	-1.206	0.234
	T	-0.091 \pm 0.024	-3.751	<0.001
	$p\text{CO}_2$	<0.001 \pm <0.001	-1.656	0.104
	N:P	-0.018 \pm 0.010	-1.840	0.072
	T \times $p\text{CO}_2$	<0.001 \pm <0.001	2.396	0.021
	T \times N:P	0.001 \pm <0.001	2.410	0.020
	$p\text{CO}_2$ \times N:P	<0.001 \pm <0.001	0.572	0.570
PIC cellular content (pg cell $^{-1}$)	Intercept	3.293 \pm 0.406	8.122	<0.001
	T	-0.067 \pm 0.021	-3.193	0.003
	$p\text{CO}_2$	-0.001 \pm <0.001	-5.519	<0.001
	N:P	-0.003 \pm 0.009	-0.292	0.772
	T \times $p\text{CO}_2$	<0.001 \pm <0.001	4.584	<0.001
	T \times N:P	0.001 \pm <0.001	2.340	0.024

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POC:PON (mol mol ⁻¹)	$p\text{CO}_2 \times \text{N:P}$	$<0.001 \pm <0.001$	0.111	0.912
	Intercept	2.741 ± 0.081	33.823	<0.001
	T	-0.008 ± 0.004	-2.169	0.035
	$p\text{CO}_2$	$<0.001 \pm <0.001$	0.153	0.879
	N:P	-0.004 ± 0.001	-5.430	<0.001
POC:POP (mol mol ⁻¹)	Intercept	5.423 ± 0.128	42.300	<0.001
	T	-0.007 ± 0.006	-1.242	0.220
	$p\text{CO}_2$	$<0.001 \pm <0.001$	0.069	0.945
	N:P	0.012 ± 0.001	9.617	<0.001
PON:POP (mol mol ⁻¹)	Intercept	2.702 ± 0.145	18.590	<0.001
	T	0.001 ± 0.007	0.157	0.876
	$p\text{CO}_2$	$<0.001 \pm <0.001$	-0.169	0.866
	N:P	0.016 ± 0.001	11.200	<0.001
PIC:POC	Intercept	0.460 ± 0.066	7.010	<0.001
	T	0.025 ± 0.003	8.184	<0.001
	$p\text{CO}_2$	$<0.001 \pm <0.001$	-12.837	<0.001
	N:P	$<0.001 \pm 0.001$	-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
	$p\text{CO}_2$	$<0.001 \pm <0.001$	-0.238	0.813
	N:P	-0.004 ± 0.003	-1.248	0.218
	$T \times p\text{CO}_2$	$<0.001 \pm <0.001$	1.816	0.076
	$T \times \text{N:P}$	$<0.001 \pm <0.001$	1.657	0.104
	$p\text{CO}_2 \times \text{N:P}$	$<0.001 \pm <0.001$	-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
	$p\text{CO}_2$	$0.001 \pm <0.001$	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
	$p\text{CO}_2$	-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
	$p\text{CO}_2$	$<0.001 \pm <0.001$	1.874	0.067
	N:P	0.010 ± 0.004	2.735	0.009
	$T \times p\text{CO}_2$	$<0.001 \pm <0.001$	-2.946	0.005
	$T \times \text{N:P}$	$-0.001 \pm <0.001$	-2.898	0.006
	$p\text{CO}_2 \times \text{N:P}$	$<0.001 \pm <0.001$	1.249	0.218

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1061 Table 2. The changes in cellular elemental contents (as pg cell⁻¹), elemental molar
 1062 ratios and the proportions of major fatty acid groups and docosahexaenoic acid (DHA)
 1063 (as % of total fatty acids) in response to warming, N and P deficiency and enhanced
 1064 $p\text{CO}_2$ in *Emiliania huxleyi*. Here, only significant changes are shown based on
 1065 GLMM results in Table 1. Red and blue arrows indicate a mean percent increase and
 1066 decrease in a given response, respectively.

Response	Effect				
	Warming	-N	-P	Enhanced $p\text{CO}_2$	Interactions
POC cellular content	⬇ -8%	⬇ -39%	⬆ 50%	-	T×N:P supply
PON cellular content	⬆ 5%	⬇ -53%	⬆ 52%	-	T×N:P supply
POP cellular content	⬆ 9%	⬇ -32%	⬇ -8%	⬆ 29%	T×N:P supply T×CO ₂
PIC cellular content	⬆ 28%	⬇ -31%	⬆ 65%	⬇ -36%	T×N:P supply T×CO ₂
POC:PON	⬇ -6%	⬆ 33%	-	-	
POC:POP	-	⬇ -15%	⬆ 60%	-	
PON:POP	-	⬇ -36%	⬆ 62%	-	
PIC:POC	⬆ 41%	-	-	⬇ -49%	
SFA proportion	-	⬇ -7%	⬇ -15%	⬆ 7%	N:P supply×CO ₂
MUFA proportion	⬇ -20%	-	-	⬆ 7%	
PUFA proportion	⬆ 13%	-	-	⬇ -7%	
DHA proportion	⬆ 16%	⬆ 14%	⬆ 22%	⬇ -7%	T×N:P supply T×CO ₂

1067 Changes $\geq 25\%$ Changes $< 25\%$ - No significant change

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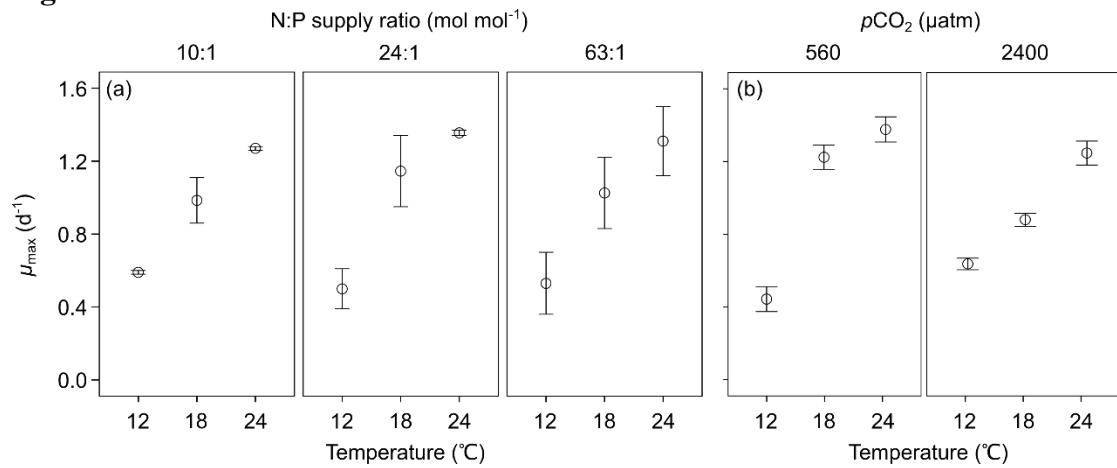
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Fig. 1

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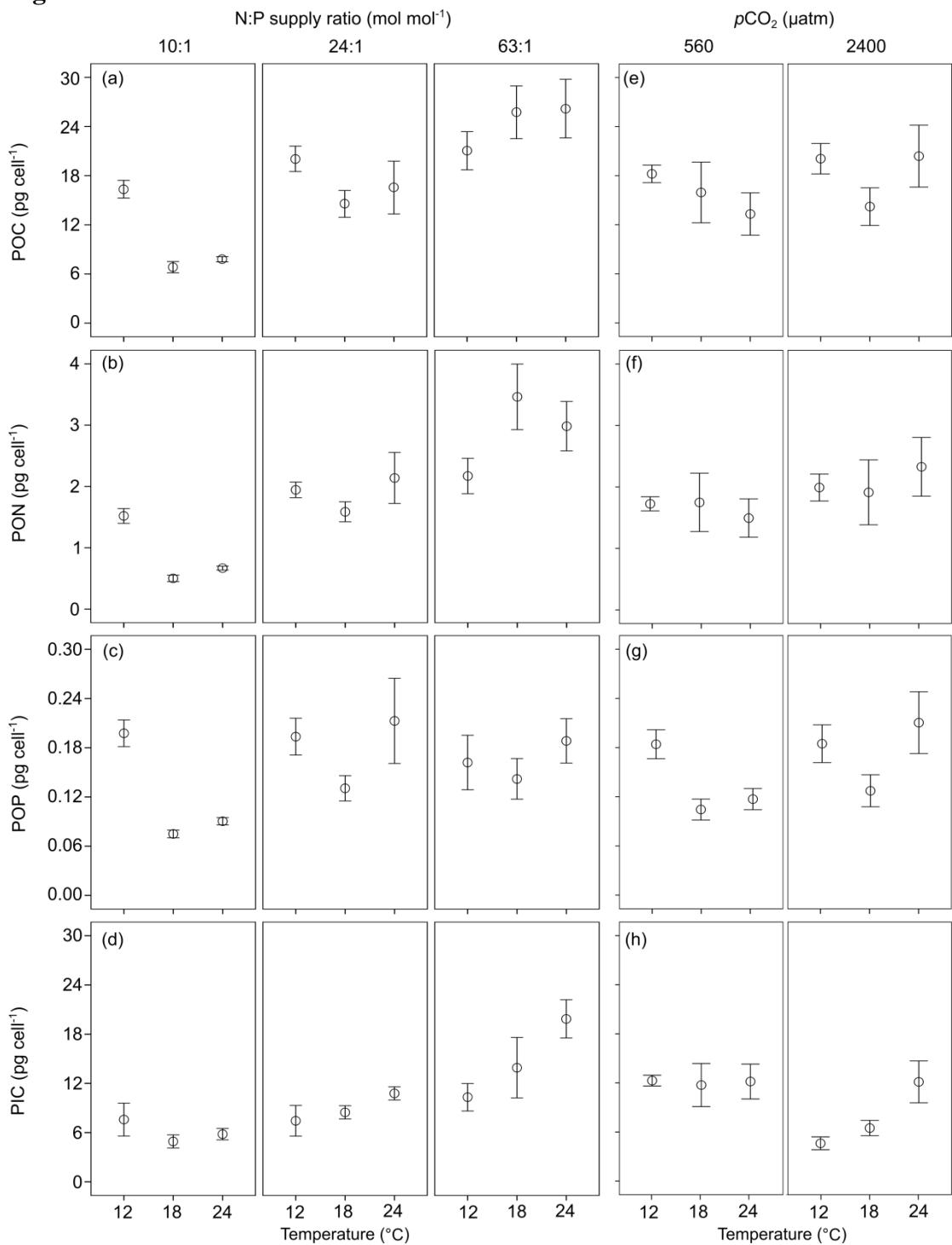
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Fig. 2

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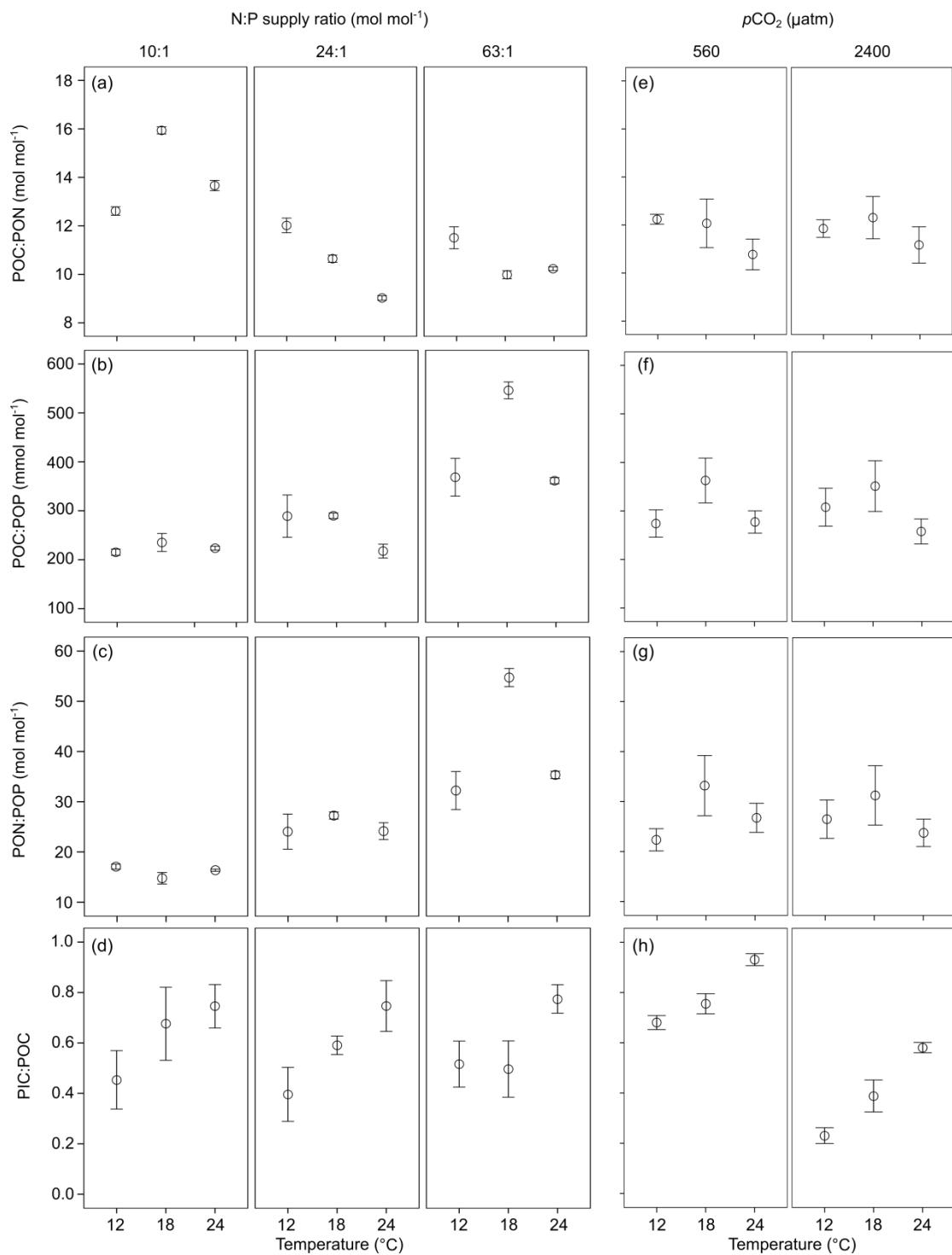
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Fig. 3

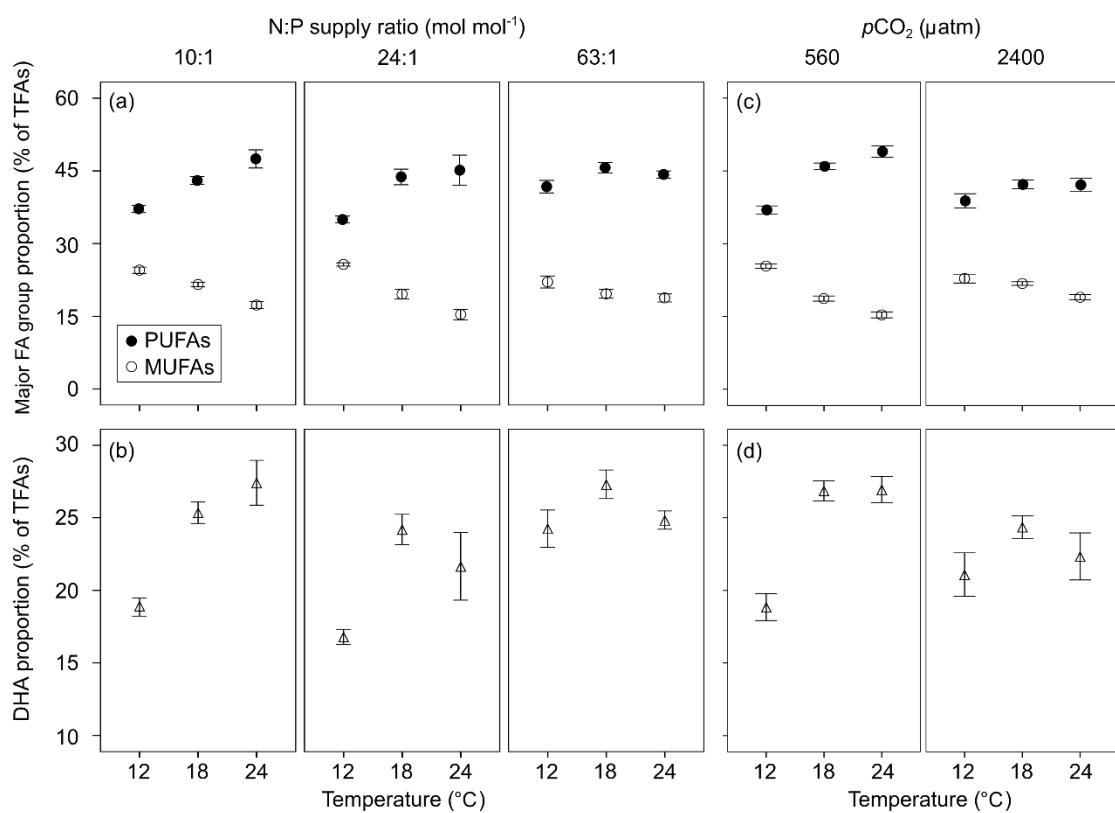
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Fig. 4

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