Simultaneous shifts in elemental stoichiometry and fatty acids 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 elements and biochemicals respond to the changing environments in key coccolithophore species such as *Emiliania huxleyi*. We investigated responses of elemental stoichiometry and fatty acids (FAs) in a strain of *E. huxleyi* under three temperatures (12, 18 and 24 °C), three N:P supply ratios (molar ratios 10:1, 24:1 and 63:1) and two pCO_2 levels (560 and 2400 µatm). Overall, C:N:P stoichiometry showed the most pronounced response to N:P supply ratios, with high ratios of particulate organic carbon vs. particulate organic nitrogen (POC:PON) and low ratios of PON vs. particulate organic phosphorus (PON:POP) in low N-media, and high POC:POP and PON:POP in low P-media. The ratio of particulate inorganic carbon vs. POC (PIC:POC) and polyunsaturated fatty acid 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 elemental cellular contents and docosahexaenoic acid (DHA) proportion in most cases, indicating the enhanced effect of warming 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 to 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 cycles, ecological functions and climate change.

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

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 of different trophic levels 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 photosynthetic producers of organic matter (causing a draw-down of CO_2 in the surface layer), but also play predominant roles in the production and export of calcium carbonate to deeper layers (causing a net release of CO_2 into the atmosphere) (Rost and Riebesell, 2004). Owning to the determination of these two processes on ocean-atmosphere exchange of CO_2, coccolithophores exhibit a complex and significant influence on global carbon cycle (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 decades, and contributing factors to this poleward expansion may differ between regions and hemispheres (Winter et al., 2014). For example, warming and freshening have promoted *E. huxleyi* blooms in the Bering Sea since the late 1970s (Harada et al., 2012), while temperature and irradiance were best able to explain variability in *E. huxleyi*-dominated coccolithophore community composition and abundance across the Drake Passage (Southern Ocean) (Charalampopoulou et al., 2016). Hence, empirical data on the responses of *E. huxleyi* to different environmental drivers would be critical for fully understanding the roles of this prominent coccolithophore species in marine ecosystems.

Extensive experimental studies have shown highly variable responses of *E. huxleyi* to rising atmospheric CO_2 (reviewed by Feng et al., 2017a; Meyer and Riebesell, 2015), while other studies focused on the influence of other environmental factors such as temperature (Rosas-Navarro et al., 2016; Sett et al., 2014; Sorrosa et al., 2005), light intensity (Nanninga and Tyrrell, 1996; Xing et al., 2015) and nutrient availability (Oviedo et al., 2014; Paasche, 1998). Responses of *E. huxleyi* to the interactions between these different factors have recently received more attention (De Bodt et al., 2010; Feng et al., 2008; Milner et al., 2016; Perrin et al., 2016; Rokitta and Rost, 2012). Many of these studies above focused on the physiological, calcification and photosynthetic responses of *E. huxleyi* due to its considerable role in global carbon 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 pCO_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 pCO_2 on *E. huxleyi*
128 elemental stoichiometry and FAs. The elevated levels of temperature and pCO_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 ^{o}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

North Atlantic (0 - 60 °N) is projected to reach 29.8 °C in 2100 according to the ocean general circulation model (Lewandowska et al., 2014). Considerable seasonal, depth and regional variations of pCO_2 have been observed in the present-day ocean (Joint et al., 2011). In plankton-rich waters, respiration plus atmospheric CO_2-enrichment can drive high regional pCO_2 at times today, e.g, up to 900 μatm in August, with the minimum value of 192 μatm in April, in the Southern Bight of the North Sea (Schiettecatte et al., 2007). In future oceans, pCO_2 is projected to increase with rising atmospheric CO_2, being 851 - 1370 μatm by 2100 and 1371 - 2900 μatm by 2150 (RCP8.5 scenario of the IPCC report 2014) (IPCC, 2014). We tested the following hypotheses in the present study: (i) elemental stoichiometry and FAs in *E. huxleyi* show different sensitivity to considerable variations in temperature, N:P supply ratios and pCO_2; (ii) the ratios of particulate organic carbon vs. particulate organic nitrogen (POC:PON), POC vs. particulate organic phosphorus (POC:POP), and particulate inorganic carbon vs. POC (PIC:POC) in *E. huxleyi* will reduce and the proportions of unsaturated fatty acids will increase under projected future ocean scenarios; and (iii) there are synergetic interactions between warming, nutrient deficiency and rising pCO_2 on *E. huxleyi* elemental stoichiometry and FA composition.

2 Material and methods

2.1 Experimental setup

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

(molar ratios 10:1, 24:1 and 63:1) and two pCO_2 levels (560 and 2400 µatm). The strain of *E. huxleyi* (Internal culture collection reference code: A8) was isolated from waters off Terceira Island, Azores, North Atlantic (38°39′22″ N 27°14′08″ W). Semi-continuous cultures, as a practical surrogate for fully continuous culture, have been successfully used to study the responses of phytoplankton stoichiometric and biochemical composition to environmental changes such as nutrient availability (Feng et al., 2017a; Lynn et al., 2000; Terry et al., 1985). Our temperature range setup was based on the study of Lewandowska et al. (2014), who chose a temperature increment of 6 °C, according to the ocean general circulation model under the IPCC SRES A1F1 scenario.

All cultures were exposed to a light intensity of 100 µmol photons · m^{-2} · s^{-1} at a 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 ·L^{-1} N and 3.6 µmol ·L^{-1} P (10:1 mol mol^{-1}), 88 µmol ·L^{-1} N and 3.6 µmol ·L^{-1} P (24:1 mol mol^{-1}) and 88 µmol ·L^{-1} N and 1.4 µmol ·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

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 on 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

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, dissolved inorganic carbon (DIC), total alkalinity (TA), pH, total particulate carbon (TPC), POC, PON, POP and FAs. Cell density was counted daily in batch and semi-continuous cultures (final cell density at steady state ranging between 1.50×10^5 - 17.8×10^5 cells mL^{-1}, with the average value of 7.95×10^5 cells mL^{-1}). 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).

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 ^{o}C and stored in a desiccator. POC and PON were simultaneously determined by gas chromatography using 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 and POC content, respectively. As the physiological (i.e., cellular) PIC and POC variations cannot directly be up scaled to total population response (Matthiessen et al., 2012), PIC and POC contents in our study were shown both on the cellular (as pg cell^{-1}) and the population (as µg ml^{-1}) levels.

Fatty acid samples were taken on pre-combusted and hydrochloric acid-treated GF/F filters (Whatman GmbH, Dassel, Germany), and stored at -80 ^{o}C 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. FA data were expressed as a percentage of total fatty acids (TFAs) (FA proportion, % of TFAs) to better compare our results with those in previous studies. FAs were also quantified on a per unit biomass (μg mg C^{-1}), which is an ideal approach when considering nutritional quality of phytoplankton for herbivores (Piepho et al., 2012).

2.3 Statistical analysis

Generalized linear mixed models (GLMMs) were applied to test the best model explaining the variations in μ_{max}, elemental stoichiometry and FA composition, as this method is more appropriate for non-normal data than classical statistical procedures (Bolker et al., 2009). GLMMs combine the properties of two statistical models (linear mixed models and generalized linear models) (Bolker et al., 2009) and have been widely used in ecology (e.g., Bracewell et al., 2017; Frère et al., 2010; Jamil et al., 2014), in which data sets are often non-normally distributed. In our study, response variables included μ_{max}, elemental stoichiometry [elemental cellular contents (as pg $cell^{-1}$) and their molar ratios], POC and PIC population yield (as μg ml^{-1}) and production (as pg $cell^{-1}$ d^{-1}), FA proportion (as % of TFAs) and contents (as μg mg C^{-1}), with temperature, N:P supply ratios and pCO_2 as fixed effects. Target distributions were tested and link functions were consequently chosen. The link function is a transformation of the target that allows estimation of the model (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 link function is appropriate with any distribution except for multinomial, while logit

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

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 GLMMs. The question a nested model addresses is that, whether one factor plays a role under one (or several) configuration(s) of another factor, but not under all configurations of that factor equally. 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

287 factors was compared with their expected net additive effect [e.g., ($factor_1$ - control) +
288 ($factor_2$ - 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 effect of N:P supply ratios and pCO_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 pCO_2 on cellular PIC content, and significant interactions between temperature and
305 pCO_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

deficiency (N:P supply ratio = 63:1 mol mol^{-1}) (Fig. 2 a-c; Nested model, $p < 0.001$). Synergistic interactions were also observed between increasing temperature and enhanced pCO_2 on cellular POP content (Table S3), showing the lowest value at low pCO_2 level and the highest one at enhanced pCO_2 in response to increasing temperature (Fig. 2g; Nested model, $p = 0.003$). For cellular PIC content, increasing temperature and N deficiency had antagonistic interactions, while increasing temperature and P deficiency showed synergistic interactions (Table S3). As a result, cellular PIC content showed a slight decreasing trend with increasing temperature under N deficiency and an increasing trend under higher N:P supply ratios (Fig. 2d; Nested model, $p = 0.030$). Increasing temperature and enhanced pCO_2 affected cellular PIC content synergistically (Table S3), with the negative response of cellular PIC content to enhanced pCO_2 being significantly weaker as temperature increased (Fig. 2h; Nested model, $p < 0.001$).

POC:PON, POC:POP and PON:POP responded significantly to N:P supply ratios (bold letters in Table 1), while only POC:PON showed significant responses to temperature, with non-significant effect of pCO_2 detected. Increasing N:P supply ratios caused a decreasing trend in POC:PON (Fig. 3a) and an increase in POC:POP (Fig. 3b), resulting in a positive relationship between PON:POP and N:P supply ratios (Fig. 3c). The response of POC:PON to increasing temperature was complex, showing a hump-shaped response under N deficiency and negative responses under higher N:P supply ratios (Fig. 3a). PIC:POC responded significantly to temperature and pCO_2, with non-significant effect of N:P supply ratios detected (Table 1). PIC:POC increased

with increasing temperature and decreased with enhanced pCO_2 (Fig. 3 d and h).

3.3 Fatty acids

The most abundant FA group was 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 (bold letters in Table 1). Increasing temperature caused a decrease in the proportion of MUFAs and an increase in PUFAs (Fig. 4 a). In contrast, enhanced pCO_2 resulted in an increase in MUFAs and a decrease in PUFAs at higher temperatures (Fig. 4 c). Moreover, enhanced pCO_2 and N (and P) deficiency affected SFA proportion synergistically (Table S3), with the unimodal response of SFA 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) (bold letters in Table 1). Increasing temperature and N:P supply ratios caused an overall increase in DHA (Fig. 4 b). The interactions between

353 increasing temperature and nutrient deficiency (and enhanced pCO_2) affected DHA
354 synergistically (Table S3), and the positive effect of temperature became more
355 pronounced at lower N:P supply ratios (Nested model, $p < 0.001$) and at the low pCO_2
356 (Nested model, $p < 0.001$) (Fig. 4 b and d).

357 ## 4 Discussion

358 Our study scales the impacts of temperature, N:P supply ratios and pCO_2 on
359 elemental stoichiometry and FA composition of the ubiquitously important calcifier *E.*
360 *huxleyi*, while accounting for their interactive effects. Overall, C:N:P stoichiometry
361 changed markedly in response to N:P supply ratios, showing a maximum of 62%
362 changes under P deficiency (Table 2). Both PIC:POC and PUFA proportion increased
363 with warming and decreased under high pCO_2, indicating a partial compensation by
364 pCO_2 of a predominantly temperature-driven response. The overall response patterns
365 of C:N:P stoichiometry in our study are consistent with those on a global scale
366 (Martiny et al., 2013), and PUFA responses conform with the meta-analysis results on
367 haptophytes (Hixson and Arts, 2016). In line with these studies, we also detected
368 significant interactions between temperature, N:P supply ratios and pCO_2 on certain
369 response variables (e.g., cellular elemental contents and DHA proportion) (Table 1),
370 indicating variable response patterns of elemental stoichiometry and FA composition
371 in *E. huxleyi* under any given constellation of environmental factors. Our results thus
372 underscore the important effects of multiple environmental drivers, demonstrating
373 differential effects of the three environmental factors on elemental stoichiometry and
374 FA composition in *E. huxleyi*.

4.1 Responses of maximal growth rate

Increasing temperature significantly accelerated μ_{max} of *E. huxleyi* in our study (Fig. 1; Table 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 °C used in our study (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 strains (Paasche, 2002). For example, growth rate of *E. huxleyi* from the Gulf of Maine (~ 42 °N) was 1.2 times higher at 26 °C than that at 16 °C, while growth rate of *E. huxleyi* from the Sargasso Sea (~ 20 - 35 °N) was 1.6 times higher at the higher temperature (Paasche, 2002). In our study, μ_{max} of *E. huxleyi* (from the Azores, ~ 38 °N) was two to three times higher at the highest temperature than that at the lowest temperature, showing a similar change pattern with that in the *E. huxleyi* strain from the Sargasso Sea. The results above suggest that the biogeographic origin of an *E. huxleyi* strain is important for their growth response to temperature.

4.2 Responses of C:N:P stoichiometry

N:P supply ratios showed highly significant effects on C:N:P stoichiometry (up to a 62% increase in PON:POP under P deficiency) in *E. huxleyi* in our study, with a weaker effect of warming (a 6% decrease in POC:PON) and non-significant effect of pCO_2 observed (Table 1; Table 2). Similarly, previous lab experiments also reported that nutrient availability played a more important role than temperature and pCO_2 for

C:N:P stoichiometry in different strains of *E. huxleyi* such as those from outer Oslofjord (Skau, 2015) and from the Chatham Rise, east of New Zealand (Feng et al., 2017b). Also, for marine phytoplankton community biomass on a global scale nitrate concentration as a proxy of nutrient availability explained 36% and 42% of variation in N:P and C:P, respectively, with the less variation explained by temperature (33% and 38% of the variation in N:P and C:P, respectively) (Martiny et al., 2013).

N deficiency caused overall high POC:PON and low PON:POP, while P deficiency resulted in high POC:POP and PON:POP 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 C:N:P stoichiometry changes in response to N and P deficiency. We found that the high percent change in PON:POP (a 62% increase) under P deficiency was mainly due to a 60% increase in POC:POP, associated with the higher percent change in cellular POC content (a 50% increase) and the lower percent change in cellular POP content (a 8% decrease) (Table 2). Under N deficiency, the 36% decrease in PON:POP was driven by a 33% increase in POC:PON and a 15% decrease in POC:POP, along with similar percent changes in cellular elemental contents (32% to 53% decrease). The more variable POC:POP under P deficiency and the less variable POC:PON under N deficiency in our study are consistent with the findings in global suspended particle measurements, which showed the high variability of P:C in response to changes in phosphate and the less variable N:C to changes in nitrate (Galbraith and Martiny, 2015). The consistence of C:N:P stoichiometric responses in our study with those on a global scale may reflect

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

427 Warming resulted in a significant, but slight decrease in POC:PON (-6%),
428 associated with a 8% decrease in cellular POC content and a 5% increase in cellular
429 PON content, while non-significant responses of POC:POP or PON:POP were
430 observed in *E. huxleyi* (Table 2). In the literature, variable changes of POC:PON to
431 warming were observed in *E. huxleyi*, showing positive (Borchard and Engel, 2012),
432 negative (Feng et al., 2008; Matson et al., 2016), and U-shaped responses
433 (Rosas-Navarro et al., 2016). Similar to our study, Borchard and Engel (2012) also
434 found that increasing temperature caused a stronger change in POC:PON than that in
435 POC:POP at higher P condition in the strain PML B92/11 from Bergen, Norway. The
436 mechanism behind the stronger change in POC:PON compared to POC:POP with
437 warming may be explained by the temperature-dependent physiology hypothesis,
438 which shows that organisms in warmer conditions require fewer P-rich ribosomes,
439 relative to N-rich proteins (Toseland et al., 2013).

440 The single effects of nutrient availability and temperature described above can be

441 modulated by their interactions. We observed synergistic interactions between
442 warming and nutrient deficiency on cellular contents of POC, PON and POP, and
443 between warming and enhanced pCO_2 on cellular POP content (Table 1; Table S3).
444 An overall synergistic effect was also observed across 171 studies on the responses of
445 marine and coastal systems to multiple stressors (Crain et al., 2008). Furthermore,
446 although a 29% change emerged in cellular POP content with rising pCO_2, we found
447 non-significant single effect of pCO_2 on *E. huxleyi* C:N:P stoichiometry. Previous
448 studies showed that rising pCO_2 seems to change phytoplankton stoichiometry under
449 specific conditions, e.g. at high light intensity (400 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) (Feng et
450 al., 2008) and low nutrient loads (500 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} at N:P supply ratio $\leq$ 15
451 or N:P supply ratio $\geq$ 30) (Leonardos and Geider, 2005a). In our study, we used
452 relatively lower light intensity (100 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) than that in previous
453 studies, and did not investigate irradiance effects. Additional research is required to
454 assess the effects of other environmental factors such as irradiance and their
455 interactions on C:N:P stoichiometry in our *E. huxleyi* strain.

456 Taken together, our results indicate that C:N:P stoichiometry in *E. huxleyi* largely
457 reflected the changes in N:P supply ratios, across different temperatures and pCO_2
458 levels. However, for two algal species from non-calcifying classes (the diatom *P.*
459 *tricornutum* and the cryptophyte *Rhodomonas* sp.) temperature had the most
460 consistent significant effect on stoichiometric ratios in our previous work (Bi et al.,
461 2017). The results above are consistent with the ranking of environmental control
462 factors in Boyd et al. (2010), which showed that temperature, nitrogen and

463 phosphorus were ranked as important factors for major phytoplankton groups.

464 **4.3 Responses of PIC:POC**

465 Both pCO_2 and temperature had highly significant effects on PIC:POC in our study,
466 with enhanced pCO_2 and warming resulting in an overall 49% decrease and a 41%
467 increase in PIC:POC, respectively, while N:P supply ratios showed no significant
468 effect (Table 1; Table 2). This result is in agreement with rankings of the importance
469 of environmental drivers on PIC:POC in a Southern Hemisphere strain of *E. huxleyi*
470 (isolated from the Chatham Rise), showing the order of pCO_2 (negative effect) >
471 temperature (positive effect) and non-significant effect of nitrate or phosphate (Feng
472 et al., 2017b).

473 The negative effect of enhanced pCO_2 on PIC:POC has been widely observed for
474 different strains of *E. huxleyi* (Meyer and Riebesell, 2015 and references therein). The
475 negative response of PIC:POC to rising pCO_2 in our study was driven by the
476 significant decrease in cellular PIC content (calcification), with cellular POC content
477 (photosynthesis) showing non-significant changes (Table 1; Table 2). Previous studies
478 also showed a greater impact of ocean acidification on calcification than on
479 photosynthesis in coccolithophores (De Bodt et al., 2010; Feng et al., 2017a; Meyer
480 and Riebesell, 2015). Feng et al. (2017a) suggested that the decreased calcification in
481 *E. huxleyi* may be caused by the increased requirement of energy to counteract
482 intracellular acidification. The increased activity of carbonic anhydrase (CA) at low
483 pCO_2 may explain the lack of a significant effect of pCO_2 on the photosynthetic or
484 growth rate (Feng et al., 2017a), as up-regulation of CA at low DIC was previously

observed (Bach et al., 2013).

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

Significant interactions were observed between temperature and N:P supply ratios, and between temperature and pCO_2 on cellular particulate carbon contents in our study (Table 1). For example, the negative relationship between cellular PIC content and enhanced pCO_2 became weaker at higher temperatures (Fig. 2h). This result is in agreement with the modulating effect of temperature on the CO_2 sensitivity of key metabolic rates in coccolithophores, due to the shift of the optimum CO_2 concentration for key metabolic processes towards higher CO_2 concentrations from intermediate to high temperatures (Sett et al., 2014). Specifically, the interactions between warming and nutrient deficiency (and high pCO_2) synergistically affected both PIC and POC cellular contents in most cases in our study (Table S3), indicating 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 pCO_2 (Fig. 3h; Table 2), 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 directly used to infer population response, as different responses between cellular and population yields of PIC (and POC) (as μg ml^{-1}) to environmental changes were evident in previous work (Matthiessen et al., 2012) and the present study (Table S5, S6; Fig. S3, S4). Thus, scaling our results up to coccolithophores carbon export should consider these uncertainties.

4.4 Responses of fatty acids

Our study provides one of the first experimental demonstrations of the relative importance of temperature, N:P supply ratios and pCO_2 on *E. huxleyi* FA composition. Both temperature and pCO_2 had significant effects on the proportions of MUFAs and PUFAs, with warming causing larger changes in MUFAs and PUFAs than rising pCO_2, while significant effects of N:P supply ratios were only observed for DHA proportion (Table 1; Table 2).

Increasing temperature caused a 20% decline in MUFA proportion and a 13% increase in PUFA proportion in our study (Table 2). This result is consistent with the negative response of MUFA proportion and positive response of PUFA proportion to warming in other haptophytes based on a meta-analysis on 137 FA profiles (Hixson

529 and Arts, 2016), showing an opposite response to general patterns of phytoplankton
530 FAs to warming. Although warming is expected to have a negative effect on the
531 degree of fatty acid unsaturation to maintain cell membrane structural functions
532 (Fuschino et al., 2011; Guschina and Harwood, 2006; Sinensky, 1974), variable FA
533 responses to warming were widely observed in different phytoplankton groups (Bi et
534 al., 2017; Renaud et al., 2002; Thompson et al., 1992). Contradictory findings were
535 even reported in meta-analyses on large FA profiles such as the absence (Galloway
536 and Winder, 2015) or presence (Hixson and Arts, 2016) of the negative correlation
537 between temperature and the proportion of long-chain EFAs in freshwater and marine
538 phytoplankton. While the underling mechanisms of variable FA responses are still
539 unclear, it is known that both phylogeny and environmental conditions determine
540 phytoplankton FA composition (Bi et al., 2014; Dalsgaard et al., 2003; Galloway and
541 Winder, 2015). In our study, we found significant interactions between temperature
542 and pCO_2 (and N:P supply ratios) on the individual FA component DHA, showing that
543 pCO_2 and nutrient availability may alter the effect of warming on *E. huxleyi* FA
544 composition.

545 Enhanced pCO_2 led to an overall 7% increase in MUFAs and a 7% decrease in
546 PUFAs (Table 2), consistent with FA response patterns in the *E. huxleyi* strain PML
547 B92/11 (Riebesell et al., 2000) and the strain AC472 from Western New Zealand,
548 South Pacific (Fiorini et al., 2010). Also in a natural plankton community (Raunefjord,
549 southern Norway), PUFA proportion was reduced at high pCO_2 level in the nano-size
550 fraction, suggesting a reduced Haptophyta (dominated by *E. huxleyi*) biomass and a

551 negative effect of high pCO_2 on PUFA proportion (Bermúdez et al., 2016). To date,
552 several mechanisms have been suggested to explain the reduced PUFAs at high pCO_2
553 in green algae (Pronina et al., 1998; Sato et al., 2003; Thompson, 1996), with much
554 less work conducted in other phytoplankton groups. One possible mechanism was
555 demonstrated in the study on *Chlamydomonas reinhardtii*, showing that the repression
556 of the CO_2-concentrating mechanisms (CCMs) was associated with reduced FA
557 desaturation at high CO_2 concentration (Pronina et al., 1998). Our observed decrease
558 in the proportion and content of PUFAs at higher pCO_2 (Table S6) fits well with the
559 mechanism proposed by Pronina et al. (1998), which may be attributed to the
560 repression of CCMs at high pCO_2 in *E. huxleyi*.

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

572 In summary, our study showed stronger effects of pCO_2 and temperature, and a

573 weaker effect of N:P supply ratios on the proportions of unsaturated FAs in *E. huxleyi*.
574 It should be noted that using different units to quantify FA composition may cause
575 contradictory results, e.g., an increase in PUFA proportion (% of TFAs) but
576 non-significant changes in PUFA contents per biomass (μg mg C^{-1}) with increasing
577 temperature in our study (Table S5, S6). Moreover, PUFA contents per biomass in two
578 species of non-calcifying classes (*P. tricornutum* and *Rhodomonas* sp.) showed a
579 different response pattern from that observed in *E. huxleyi* in our study, i.e., a
580 significant negative effect of enhanced pCO_2 on PUFA contents in *E. huxleyi* (Table
581 S6), but a non-significant effect of pCO_2 on PUFA contents in *P. tricornutum* and
582 *Rhodomonas* sp. (Bi et al., 2017). This different response between phytoplankton
583 groups is in agreement with findings in mesocosm studies (Bermúdez et al., 2016;
584 Leu et al., 2013), suggesting that changes in taxonomic composition can cause
585 different relationships between PUFAs and pCO_2 in natural phytoplankton
586 community.

587 **4.5 Implications for marine biogeochemistry and ecology**

588 We observed an overall increase in POC:PON (with warming and N deficiency)
589 and POC:POP (with N and P deficiency) in *E. huxleyi*, while enhanced pCO_2 showed
590 no significant effects (Table 2). This result indicates that nitrogen and phosphorus
591 requirements in *E. huxleyi* are likely to reduce under projected future changes in
592 temperature and nutrient availability, and show minor changes in response to higher
593 pCO_2. Likewise, Hutchins et al. (2009) suggested negligible or minor effects of
594 projected future changes in pCO_2 on most phytoplankton phosphorus requirements.

595 Moreover, the overall low PIC:POC under future ocean scenarios (warming and
596 enhanced pCO_2) indicates that carbon production by the strain *E. huxleyi* in our study
597 acts as a carbon sink. This argument is consistent with the findings of the decreased
598 calcification with increasing pCO_2 in most coccolithophores (Beaufort et al., 2011;
599 Hutchins and Fu, 2017), which may reduce vertical exported fluxes of sinking
600 calcium carbonate and minimize calcification as a carbon source term, ultimately
601 downsizing the ocean's biological carbon cycle (Hutchins and Fu, 2017).

602 Besides the overall increase in POC:PON and POC:POP, we found an overall
603 increase in the proportions of PUFAs (with warming and enhanced pCO_2) and DHA
604 (with warming, N and P deficiency and enhanced pCO_2) in *E. huxleyi* (Table 2), but a
605 decrease in PUFA and DHA contents per biomass with enhanced pCO_2 (Table S6).
606 The relationship between changes in stoichiometry and FA composition in
607 phytoplankton varies in a complex way with environmental conditions and algal
608 taxonomy (Bi et al., 2014; Pedro Cañavate et al., 2017; Sterner and Schulz, 1998). For
609 example, the correlation between PON:POC and PUFA contents per biomass was
610 negative in *Rhodomonas* sp. and positive in *P. tricornutum* under N deficiency (Bi et
611 al., 2014). Our findings thus indicate that elemental composition responses may be
612 coupled with responses in essential FA composition in the strain of *E. huxleyi* studied
613 under certain configurations of environmental drivers. Such a linkage between
614 stoichiometric and FA composition is important in studies of food web dynamics, as
615 the C:N and C:P stoichiometry and PUFAs both have been used as indicators of
616 nutritional quality of phytoplankton, with high POC:PON (and POC:POP) and low

contents in certain PUFAs often constraining zooplankton production by reducing trophic carbon transfer from phytoplankton to zooplankton (Hessen, 2008; Jónasdóttir et al., 2009; Müller-Navarra et al., 2000; Malzahn et al., 2016). In addition, other factors such as the cell size of phytoplankton and nutritional requirements of consumers can also influence trophic transfer efficiency (Anderson and Pond, 2000; Sommer et al., 2016). Nevertheless, studies on plant-herbivore interactions reported that changes in elemental and biochemical composition in phytoplankton can translate to higher trophic levels (Kamya et al., 2017; Malzahn et al., 2010; Rossoll et al., 2012) and refer to direct effects of environmental changes on low trophic level consumers, which can be modified by indirect bottom-up driven impacts through the primary producers (Garzke et al., 2016; Garzke et al., 2017).

5 Conclusions

Our study shows that N:P supply ratios had the strongest effect on C:N:P stoichiometry, while temperature and pCO_2 played more influential roles on PIC:POC and PUFA proportions in *E. huxleyi*. The specific response patterns of elemental ratios and FAs have important implications for understanding biogeochemical and ecological functioning of *E. huxleyi*. The observations presented here suggest differential responses of elements and FAs to rising temperature, nutrient deficiency and enhanced pCO_2 in *E. huxleyi*, being to some extent unique compared to algal species from non-calcifying classes. Thus, the role of multiple environmental drivers under the biodiversity context should be considered to truly estimate the future functioning of phytoplankton in the changing marine environments.

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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Fig. 1 Responses of the observed maximal growth rate (μ_{max}; mean ± SE) to temperature, N:P supply ratios and pCO_2 in *Emiliania huxleyi*. The selected model contains only the first order effects of the three environmental factors, with the results of AICc shown in Table S2.

Fig. 2 Responses of cellular contents of (a, e) particulate organic carbon (POC), (b, f) particulate organic nitrogen (PON), (c, g) particulate organic phosphorus (POP) and (d, h) particulate inorganic carbon (PIC) (mean ± SE) to temperature, N:P supply ratios and pCO_2 in *Emiliania huxleyi*. The selected models contain the first order effects, and second order interactions of the three environmental factors for the four response variables, with the results of AICc shown in Table S2.

Fig. 3 The ratios of (a, e) particulate organic carbon vs. particulate organic nitrogen (POC:PON), (b, f) POC vs. particulate organic phosphorus (POC:POP), (c, g) PON vs. POP (PON:POP) and (d, h) particulate inorganic carbon vs. POC (PIC:POC) (mean ± SE) in response to temperature, N:P supply ratios and pCO_2 in *Emiliania huxleyi*. The selected models contain only the first order effects of the three environmental factors for the four response variables, with the results of AICc shown in Table S2.

Fig. 4 Responses of the proportions of (a, c) monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), and (b, d) docosahexaenoic acid (DHA) (mean ± SE) to temperature, N:P supply ratios and pCO_2 in *Emiliania huxleyi*. For MUFA and PUFA proportions, the selected models contain only the first order effects of the three environmental factors, and that for DHA proportion contains also second order interactions, with the results of AICc shown in Table S2.

Table 1. Results of the selected GLMMs testing for the effects of temperature, N:P supply ratios and pCO_2 on the observed maximal growth rate (μ_{max}), elemental stoichiometry and fatty acid proportions in *Emiliania huxleyi*. Significant *p* values are shown in bold; T: temperature; N:P: N:P supply ratios; TFA: total fatty acid; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; DHA: docosahexaenoic acid. Results of AICc are shown in Table S2.

Variable	Factor	Coefficienct ± SE	*t*	*p*
μ_{max} (d^{-1})	Intercept	-1.368 ± 0.225	-6.075	**<0.001**
	T	0.074 ± 0.010	7.082	**<0.001**
	pCO_2	<0.001 ± <0.001	-0.472	0.644
	N:P	<0.001 ± 0.002	-0.162	0.873
POC cellular content (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
	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
PON cellular content (pg $cell^{-1}$)	Intercept	1.208 ± 0.491	2.458	**0.018**
	T	-0.083 ± 0.026	-3.259	**0.002**
	pCO_2	<0.001 ± <0.001	-0.873	0.387
	N:P	-0.008 ± 0.011	-0.709	0.482
	T × pCO_2	<0.001 ± <0.001	1.549	0.128
	T × N:P	0.001 ± 0.001	2.802	**0.007**
	pCO_2 × N:P	<0.001 ± <0.001	0.165	0.870
POP cellular content (pg $cell^{-1}$)	Intercept	-0.564 ± 0.468	-1.206	0.234
	T	-0.091 ± 0.024	-3.751	**<0.001**
	pCO_2	<0.001 ± <0.001	-1.656	0.104
	N:P	-0.018 ± 0.010	-1.840	0.072
	T × pCO_2	<0.001 ± <0.001	2.396	**0.021**
	T × N:P	0.001 ± <0.001	2.410	**0.020**
	pCO_2 × N:P	<0.001 ± <0.001	0.572	0.570
PIC cellular content (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
POC:PON (mol mol^{-1})	Intercept	2.741 ± 0.081	33.823	**<0.001**
	T	-0.008 ± 0.004	-2.169	**0.035**
	pCO_2	<0.001 ± <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
	pCO_2	<0.001 ± <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
	pCO_2	<0.001 ± <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**
	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

Table 2. The changes in cellular elemental contents (as pg cell^{-1}), elemental molar ratios and the proportions of major fatty acid groups and docosahexaenoic acid (DHA) (as % of total fatty acids) in response to warming, N and P deficiency and enhanced pCO_2 in *Emiliania huxleyi*. Here, only significant changes are shown based on GLMM results in Table 1. Red and blue arrows indicate a mean percent increase and decrease in a given response, respectively.

Response	Effect				
	Warming	-N	-P	Enhanced pCO_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

↑↓ Changes ≥ 25% ↑↓ Changes < 25% – No significant change

Fig. 1

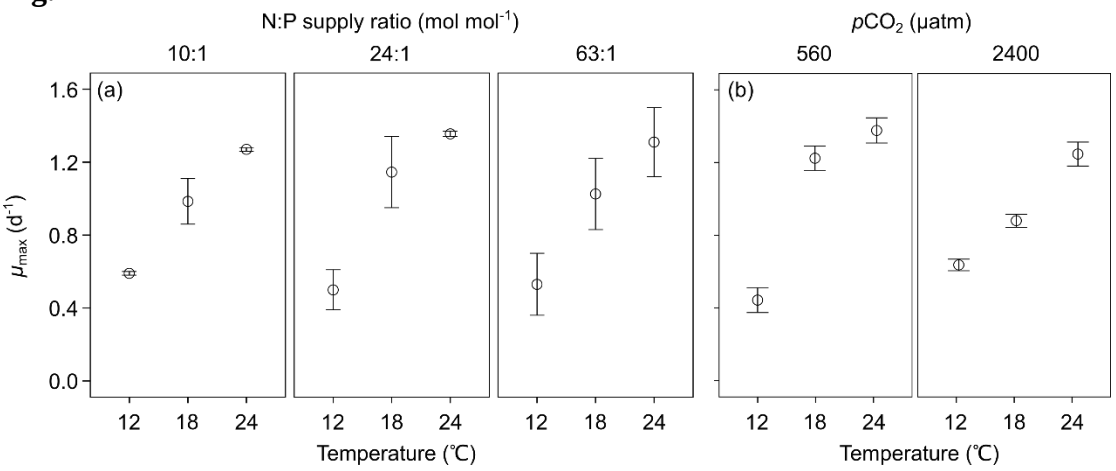

N:P supply ratio (mol mol-1)
10:1
24:1
63:1
pCO2 (µatm)
560
2400
(a)
(b)
µmax (d-1)
1.6
1.2
0.8
0.4
0.0
12
18
24
Temperature (℃)

Fig. 2

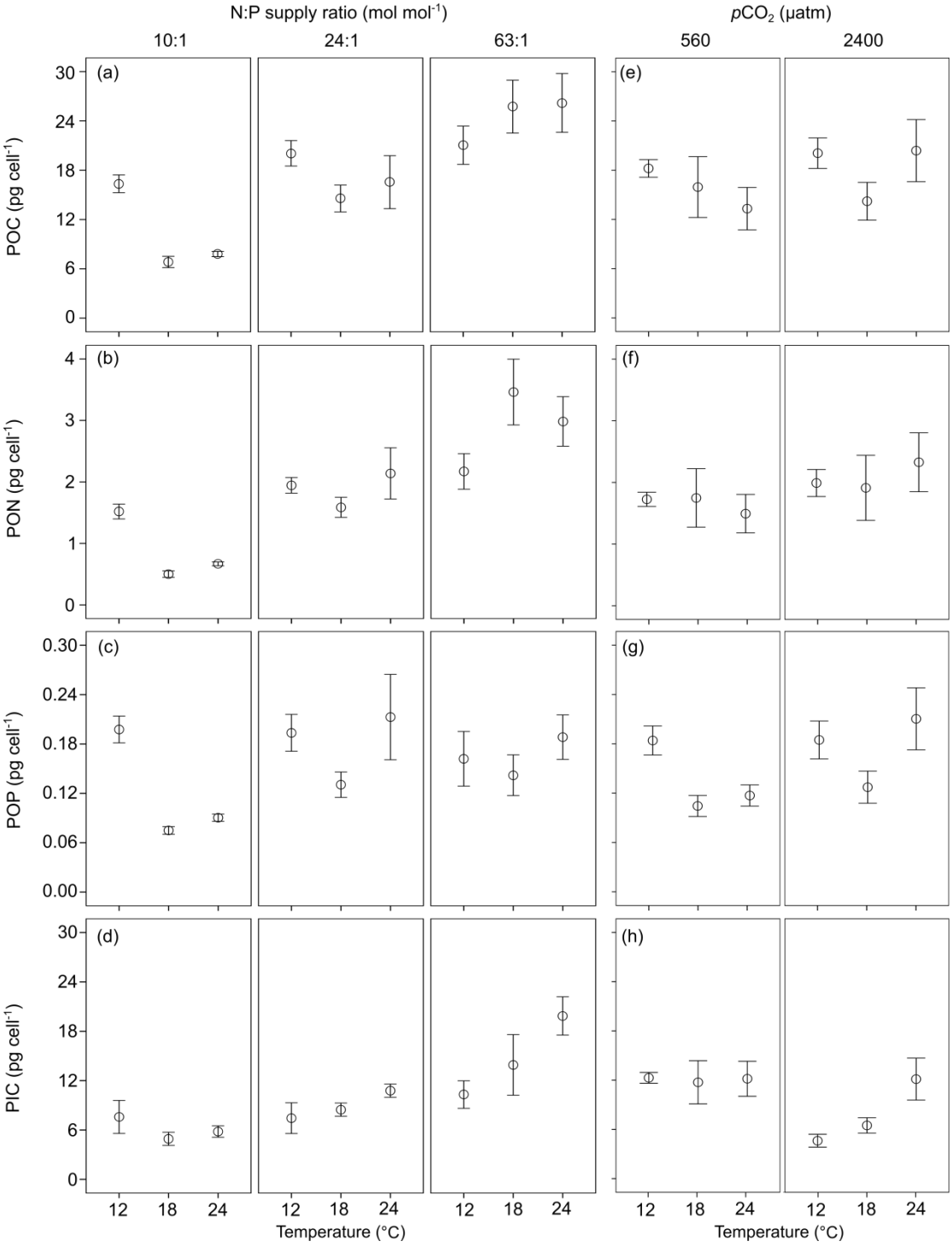

N:P supply ratio (mol mol-1)
10:1
24:1
63:1
pCO2 (µatm)
560
2400
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
POC (pg cell-1)
PON (pg cell-1)
POP (pg cell-1)
PIC (pg cell-1)
Temperature (°C)
Temperature (°C)

Fig. 3

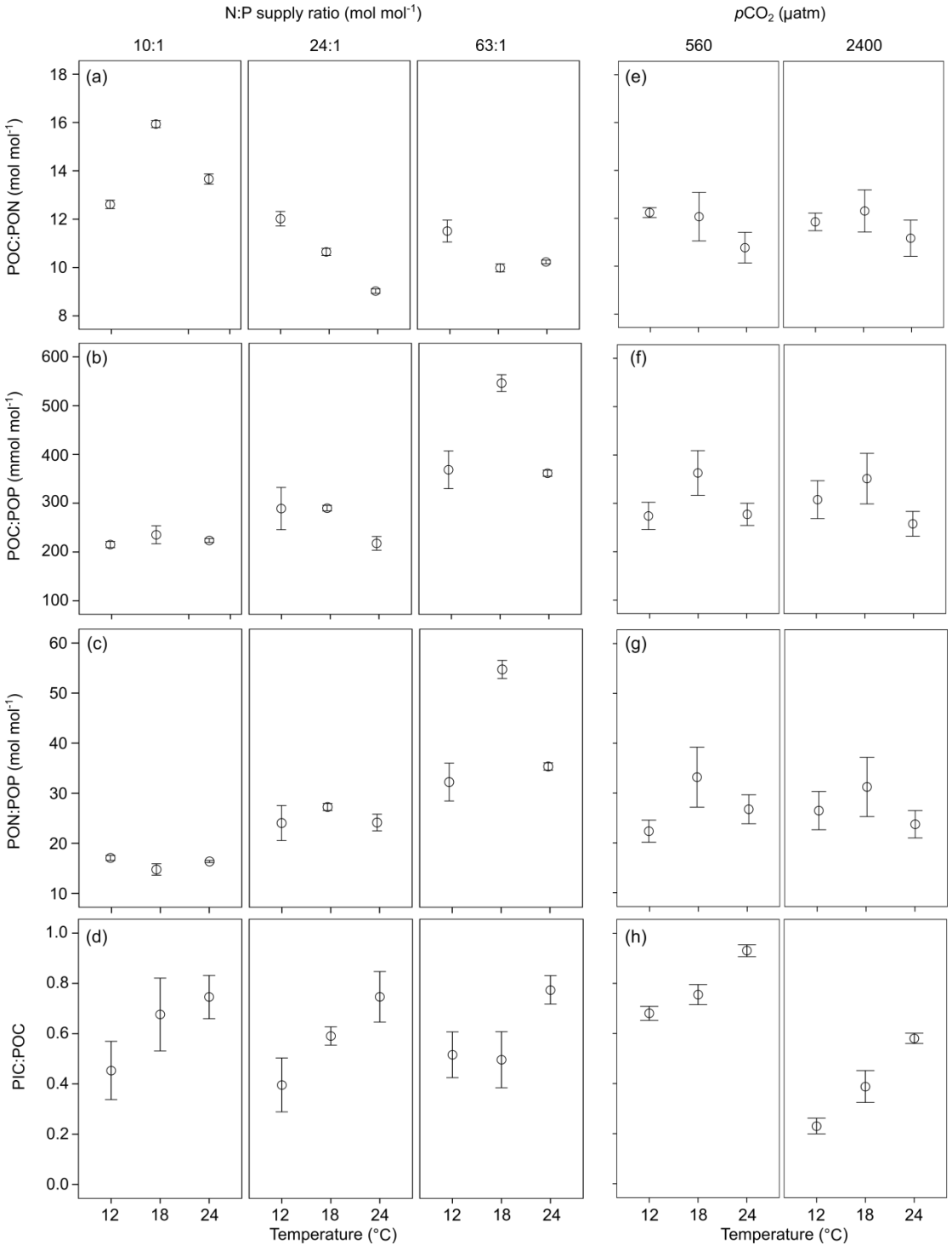
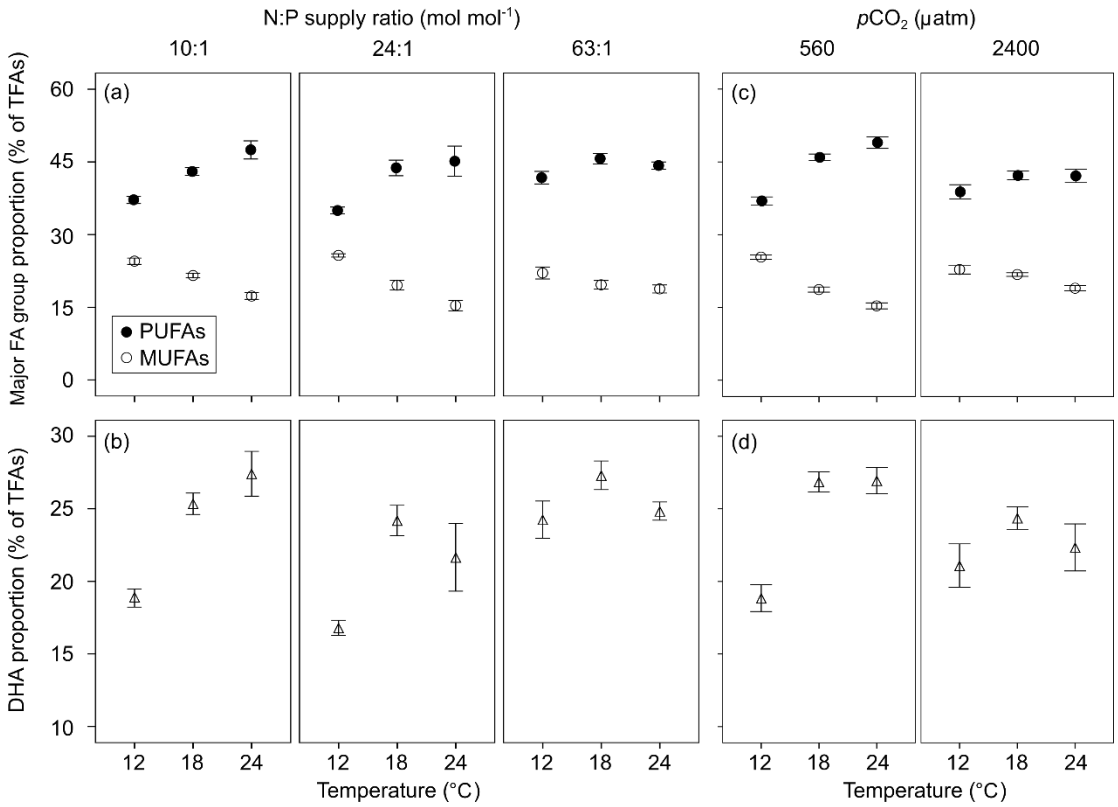

N:P supply ratio (mol mol-1)
10:1
24:1
63:1
pCO2 (µatm)
560
2400
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
POC:PON (mol mol-1)
POC:POP (mmol mol-1)
PON:POP (mol mol-1)
PIC:POC
Temperature (°C)
Temperature (°C)

Fig. 4


N:P supply ratio (mol mol-1)
10:1
24:1
63:1
pCO2 (µatm)
560
2400
(a)
(b)
(c)
(d)
Major FA group proportion (% of TFAs)
DHA proportion (% of TFAs)
PUFAs
MUFAs
Temperature (°C)
Temperature (°C)