



# Simultaneous shifts in elemental stoichiometry and fatty acids of *Emiliana huxleyi* in response to environmental changes

Rong Bi<sup>1,2,3</sup>, Stefanie M. H. Ismar<sup>3</sup>, Ulrich Sommer<sup>3</sup>, and Meixun Zhao<sup>1,2</sup>

<sup>1</sup>Key Laboratory of Marine Chemistry Theory and Technology (Ocean University of China), Ministry of Education, Qingdao, 266100, China

<sup>2</sup>Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao, 266071, China

<sup>3</sup>Marine Ecology, GEOMAR Helmholtz-Zentrum für Ozeanforschung, Kiel, 24105, Germany

**Correspondence:** Meixun Zhao (maxzhao@ouc.edu.cn)

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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 *Emiliana 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  $p\text{CO}_2$  levels (560 and 2400  $\mu\text{atm}$ ). 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  $p\text{CO}_2$ , both being lower under high  $p\text{CO}_2$  and higher with warming. We observed synergistic interactions between warming and nutrient deficiency (and high  $p\text{CO}_2$ ) on elemental cellular contents and docosahexaenoic acid (DHA) proportion in most cases, indicating the enhanced effect of warming under nutrient deficiency (and high  $p\text{CO}_2$ ). Our results suggest differential sensitivity of elements and FAs to the changes in temperature, nutrient availability and  $p\text{CO}_2$  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.

## 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,  $\text{CO}_2$  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 resulted 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 drawdown of  $\text{CO}_2$  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  $\text{CO}_2$  into the atmosphere) (Rost

and Riebesell, 2004). Owing to the determination of these two processes on ocean–atmosphere exchange of CO<sub>2</sub>, coccolithophores exhibit a complex and significant influence on the global carbon cycle (Rost and Riebesell, 2004). Of all coccolithophores, *Emiliana 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* has been 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<sub>2</sub> (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 the global carbon cycle. However, biogeochemical cycles of the major nutrient elements (nitrogen and phosphorus) and carbon are tightly linked (Hutchins et al., 2009), and thus variability in *E. huxleyi* C : N : P stoichiometry (cellular quotas and ratios of C, N and P) can also be important in ocean biogeochemistry. Moreover, elemental budgets in organisms are primarily determined by the physiology and biochemistry of biochemicals such as proteins and fatty acids (FAs) (Anderson et al., 2004; Sterner and Elser, 2002). Thus, studying simultaneous changes of elements and biochemicals enables the connection between climate change and ecosystem functions such as elemental cycles. However, shifts in resource nutrient content for consumers are often overlooked in climate change ecology (Rosenblatt and Schmitz, 2016). Recently, Bi et al. (2017) investigated responses of C : N : P stoichiometry and FAs to the interactions of three environmental factors in the diatom *Phaeodactylum tricornerutum* and the cryptophyte *Rhodomonas* sp., showing dramatic ef-

fects of warming and nutrient deficiency, and modest effects of increased *p*CO<sub>2</sub>. However, for the key coccolithophore species *E. huxleyi* much less is known about the simultaneous changes in elemental stoichiometry and biochemicals in response to multiple environmental factor changes.

In the present study, we conducted semi-continuous cultures of *E. huxleyi* to disentangle potential effects of temperature, N : P supply ratios and *p*CO<sub>2</sub> on *E. huxleyi* elemental stoichiometry and FAs. The elevated levels of temperature and *p*CO<sub>2</sub> in our study are within the predicted ranges of future ocean scenarios. The interannual average temperature varied between 16 and 22 °C at the Azores (<http://dive.visitazores.com/en/when-dive>), the source 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 *p*CO<sub>2</sub> have been observed in the present-day ocean (Joint et al., 2011). In plankton-rich waters, respiration plus atmospheric CO<sub>2</sub> enrichment can drive high regional *p*CO<sub>2</sub> at times today, e.g., up to 900 µatm in August, with a minimum value of 192 µatm in April, in the Southern Bight of the North Sea (Schiettecatte et al., 2007). In future oceans, *p*CO<sub>2</sub> is projected to increase with rising atmospheric CO<sub>2</sub>, with 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 *p*CO<sub>2</sub>; (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 *p*CO<sub>2</sub> 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 *p*CO<sub>2</sub> 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  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  at a 16 : 8 h light : dark cycle in temperature-controlled rooms. The culture medium was prepared with sterile filtered (0.2  $\mu\text{m}$  pore size, Sartobran® P 300; Sartorius, Göttingen, Germany) North Sea water with a salinity of 37 psu. Macronutrients were added as sodium nitrate ( $\text{NaNO}_3$ ) and potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) to achieve three N : P supply ratios, i.e., 35.2  $\mu\text{mol L}^{-1}$  N and 3.6  $\mu\text{mol L}^{-1}$  P (10 : 1 mol mol<sup>-1</sup>), 88  $\mu\text{mol L}^{-1}$  N and 3.6  $\mu\text{mol L}^{-1}$  P (24 : 1 mol mol<sup>-1</sup>) and 88  $\mu\text{mol L}^{-1}$  N and 1.4  $\mu\text{mol L}^{-1}$  P (63 : 1 mol mol<sup>-1</sup>). Vitamins and trace metals were added based on the modified Provasoli culture medium (Ismar et al., 2008; Provasoli, 1963). Initial  $p\text{CO}_2$  of the culture medium was manipulated by bubbling with air containing the target  $p\text{CO}_2$ . Three replicates were set up for each treatment, resulting in 54 experimental units. Each culture was kept in a sealed cell culture flask with 920 mL culture volume. Culture flasks were carefully rotated twice per day at a set time to minimize sedimentation.

First, batch culture experiments were performed to obtain an estimate of the observed maximal growth rate ( $\mu_{\text{max}}$ ,  $\text{d}^{-1}$ ) under three temperatures, three N : P supply ratios and two  $p\text{CO}_2$  levels.  $\mu_{\text{max}}$  was calculated based on the changes of population cell density within exponential phase (Bi et al., 2012). Once batch cultures reached the early stationary phase, semi-continuous cultures were started with the algae from batch cultures. The gross growth rate ( $\mu$  ( $\text{d}^{-1}$ ), resulting from the process of reproduction alone due to negligible mortality in cultures lacking predators; Lampert and Sommer, 2007) was applied as 20 % of  $\mu_{\text{max}}$ . Using percentage of  $\mu_{\text{max}}$  guarantees that the strength of nutrient deficiency is equal through all temperature and  $p\text{CO}_2$  treatments. A fixed value of  $\mu$  would mean weak deficiency when  $\mu_{\text{max}}$  is low, and strong deficiency when it is high. Based on  $\mu$ , the equivalent daily renewal rate ( $D$ ,  $\text{d}^{-1}$ ) can be calculated according to the equation  $D = 1 - e^{-\mu \cdot t}$ , where  $t$  is renewal interval (here  $t = 1$  day). The volume of the daily renewal incubation water can be calculated by multiplying  $D$  by the total volume of incubation water (920 mL). The incubation water was exchanged with freshly made seawater medium with the target N : P supply ratios, as well as pre-acclimated to the desired  $p\text{CO}_2$  level. To counterbalance the biological  $\text{CO}_2$  drawdown, the required amount of  $\text{CO}_2$ -saturated seawater was also added. Renewal of the cultures was carried out at the same hour every day. The steady state in semi-continuous cultures was assessed based on the net growth rate ( $r$  ( $\text{d}^{-1}$ ), the difference between the gross growth rate and the loss rate

( $r = \mu - D$ )). When  $r$  was zero (at steady state),  $\mu$  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  $1.50 \times 10^5$  and  $17.8 \times 10^5$  cells  $\text{mL}^{-1}$ , with an average value of  $7.95 \times 10^5$  cells  $\text{mL}^{-1}$ ). pH measurements were conducted daily in semi-continuous cultures (Fig. S1 in the Supplement), 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  $\mu\text{m}$ , Minisart RC25; Sartorius, Göttingen, 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  $p\text{CO}_2$  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 in the Supplement).

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 12 h. Before analysis, filters were dried at 60 °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  $\mu$  by cellular PIC and POC content, respectively. As the physiological (i.e., cellular) PIC and POC variations cannot directly be upscaled to total population response (Matthiessen et al., 2012), PIC and POC contents in our study were shown both on the cellular (as  $\mu\text{g cell}^{-1}$ ) and the population (as  $\mu\text{g mL}^{-1}$ ) levels.

Fatty acid samples were taken on pre-combusted and hydrochloric acid-treated GF/F filters (Whatman GmbH, Dassel, Germany) and stored at  $-80$  °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  $\mu\text{L}$ . Sample aliquots (1  $\mu\text{L}$ ) 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 ( $\mu\text{g mg C}^{-1}$ ), 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  $\mu_{\text{max}}$ , 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  $\mu_{\text{max}}$ , elemental stoichiometry (elemental cellular contents (as  $\text{pg cell}^{-1}$ ) and their molar ratios), POC and PIC population yield (as  $\mu\text{g mL}^{-1}$ ) and production (as  $\text{pg cell}^{-1} \text{d}^{-1}$ ), FA proportion (as % of TFAs) and contents (as  $\mu\text{g mg C}^{-1}$ ), with temperature, N:P supply ratios and  $p\text{CO}_2$  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/>). 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 corrected Akaike information criterion (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). If 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 if significant second-order interactions were detected in GLMMs. The question a nested model addresses is 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 analyzed according to Christensen et al. (2006). The observed combined effect of two factors was compared with their expected net additive effect (e.g., (factor<sub>1</sub> – control) + (factor<sub>2</sub> – control)), which was based on the sum of their individual effects. If the observed combined effect exceeded their expected additive effect, the interaction was defined as synergism. In contrast, if the observed combined effect was less than the additive effect, the interaction was defined as antagonism.

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

## 3 Results

### 3.1 Maximal growth rate ( $\mu_{\text{max}}$ )

We observed a highly significant effect of temperature (bold letters in Table 1) and non-significant effects of N:P supply ratios and  $p\text{CO}_2$  on  $\mu_{\text{max}}$  in *E. huxleyi*. Increasing temperature stimulated  $\mu_{\text{max}}$ , causing  $\mu_{\text{max}}$  to be 2 to 3 times higher at the highest temperature than those at the lowest temperature (Fig. 1).

### 3.2 Elemental stoichiometry

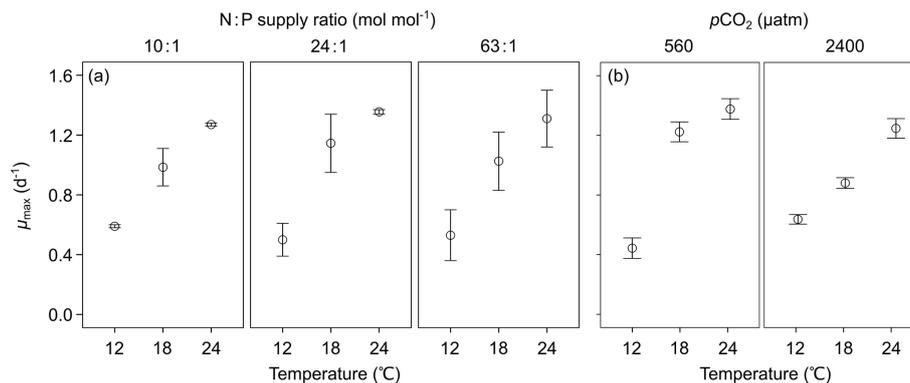
GLMM results showed that cellular contents of POC, PON, POP and PIC responded significantly to temperature and the interactions between temperature and N:P supply ratios (bold letters in Table 1). Moreover, there were significant effects of  $p\text{CO}_2$  on cellular PIC content, and significant interactions between temperature and  $p\text{CO}_2$  on cellular POP and PIC contents. For cellular contents of POC, PON and POP, increasing temperature and nutrient deficiency showed synergistic interactions (Table S3), resulting in lower values at higher temperatures under N deficiency (N:P supply ratio = 10 : 1 mol mol<sup>-1</sup>) and increasing values with increasing temperature under P deficiency (N:P supply ratio = 63 : 1 mol mol<sup>-1</sup>) (Fig. 2a–c; nested model,  $p < 0.001$ ). Synergistic interactions were also observed between increasing temperature and enhanced  $p\text{CO}_2$  on cellular POP content

**Table 1.** Results of the selected GLMMs testing for the effects of temperature, N : P supply ratios and  $p\text{CO}_2$  on the observed maximal growth rate ( $\mu_{\text{max}}$ ), elemental stoichiometry and fatty acid proportions in *Emiliana huxleyi*. Significant  $p$  values are shown in bold;  $T$ : temperature; N : P: N : P supply ratios; TFAs: total fatty acids; 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	Coefficient $\pm$ SE	$t$	$p$
$\mu_{\text{max}}$ ( $\text{d}^{-1}$ )	Intercept	$-1.368 \pm 0.225$	-6.075	< <b>0.001</b>
	$T$	$0.074 \pm 0.010$	7.082	< <b>0.001</b>
	$p\text{CO}_2$	$< 0.001 \pm < 0.001$	-0.472	0.644
	N : P	$< 0.001 \pm 0.002$	-0.162	0.873
POC cellular content ( $\text{pg cell}^{-1}$ )	Intercept	$3.683 \pm 0.377$	9.779	< <b>0.001</b>
	$T$	$-0.089 \pm 0.020$	-4.577	< <b>0.001</b>
	$p\text{CO}_2$	$< 0.001 \pm < 0.001$	-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
	$T \times \text{N : P}$	$0.001 \pm < 0.001$	3.477	<b>0.001</b>
	$p\text{CO}_2 \times \text{N : P}$	$< 0.001 \pm < 0.001$	-0.359	0.721
PON cellular content ( $\text{pg cell}^{-1}$ )	Intercept	$1.208 \pm 0.491$	2.458	<b>0.018</b>
	$T$	$-0.083 \pm 0.026$	-3.259	<b>0.002</b>
	$p\text{CO}_2$	$< 0.001 \pm < 0.001$	-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
	$T \times \text{N : P}$	$0.001 \pm 0.001$	2.802	<b>0.007</b>
	$p\text{CO}_2 \times \text{N : P}$	$< 0.001 \pm < 0.001$	0.165	0.870
POP cellular content ( $\text{pg cell}^{-1}$ )	Intercept	$-0.564 \pm 0.468$	-1.206	0.234
	$T$	$-0.091 \pm 0.024$	-3.751	< <b>0.001</b>
	$p\text{CO}_2$	$< 0.001 \pm < 0.001$	-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	<b>0.021</b>
	$T \times \text{N : P}$	$0.001 \pm < 0.001$	2.410	<b>0.020</b>
	$p\text{CO}_2 \times \text{N : P}$	$< 0.001 \pm < 0.001$	0.572	0.570
PIC cellular content ( $\text{pg cell}^{-1}$ )	Intercept	$3.293 \pm 0.406$	8.122	< <b>0.001</b>
	$T$	$-0.067 \pm 0.021$	-3.193	<b>0.003</b>
	$p\text{CO}_2$	$-0.001 \pm < 0.001$	-5.519	< <b>0.001</b>
	N : P	$-0.003 \pm 0.009$	-0.292	0.772
	$T \times p\text{CO}_2$	$< 0.001 \pm < 0.001$	4.584	< <b>0.001</b>
	$T \times \text{N : P}$	$0.001 \pm < 0.001$	2.340	<b>0.024</b>
	$p\text{CO}_2 \times \text{N : P}$	$< 0.001 \pm < 0.001$	0.111	0.912
POC : PON ( $\text{mol mol}^{-1}$ )	Intercept	$2.741 \pm 0.081$	33.823	< <b>0.001</b>
	$T$	$-0.008 \pm 0.004$	-2.169	<b>0.035</b>
	$p\text{CO}_2$	$< 0.001 \pm < 0.001$	0.153	0.879
	N : P	$-0.004 \pm 0.001$	-5.430	< <b>0.001</b>
POC : POP ( $\text{mol mol}^{-1}$ )	Intercept	$5.423 \pm 0.128$	42.300	< <b>0.001</b>
	$T$	$-0.007 \pm 0.006$	-1.242	0.220
	$p\text{CO}_2$	$< 0.001 \pm < 0.001$	0.069	0.945
	N : P	$0.012 \pm 0.001$	9.617	< <b>0.001</b>
PON : POP ( $\text{mol mol}^{-1}$ )	Intercept	$2.702 \pm 0.145$	18.590	< <b>0.001</b>
	$T$	$0.001 \pm 0.007$	0.157	0.876
	$p\text{CO}_2$	$< 0.001 \pm < 0.001$	-0.169	0.866
	N : P	$0.016 \pm 0.001$	11.200	< <b>0.001</b>

Table 1. Continued.

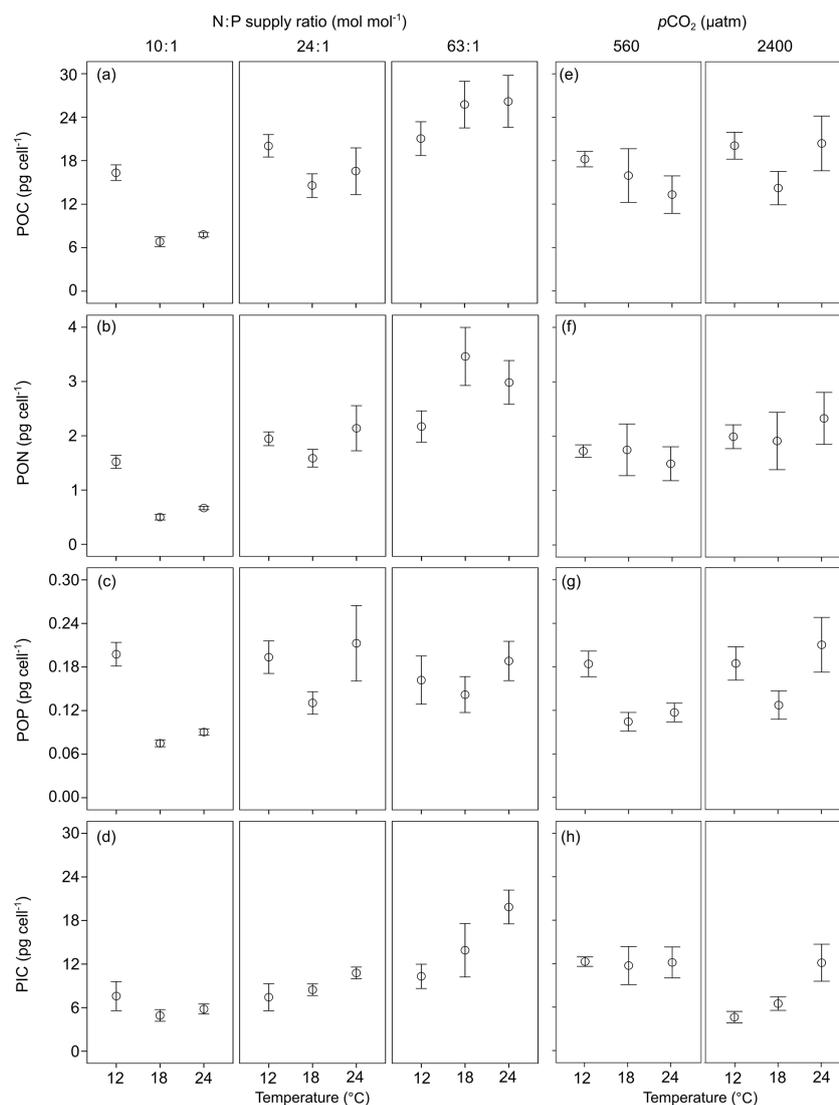
Variable	Factor	Coefficient $\pm$ SE	<i>t</i>	<i>p</i>
PIC : POC	Intercept	0.460 $\pm$ 0.066	7.010	< <b>0.001</b>
	<i>T</i>	0.025 $\pm$ 0.003	8.184	< <b>0.001</b>
	<i>pCO</i> <sub>2</sub>	< 0.001 $\pm$ < 0.001	−12.837	< <b>0.001</b>
	N : P	< 0.001 $\pm$ 0.001	−0.166	0.869
SFA proportion (% of TFAs)	Intercept	3.506 $\pm$ 0.145	24.178	< <b>0.001</b>
	<i>T</i>	−0.012 $\pm$ 0.008	−1.538	0.131
	<i>pCO</i> <sub>2</sub>	< 0.001 $\pm$ < 0.001	−0.238	0.813
	N : P	−0.004 $\pm$ 0.003	−1.248	0.218
	<i>T</i> $\times$ <i>pCO</i> <sub>2</sub>	< 0.001 $\pm$ < 0.001	1.816	0.076
	<i>T</i> $\times$ N : P	< 0.001 $\pm$ < 0.001	1.657	0.104
	<i>pCO</i> <sub>2</sub> $\times$ N : P	< 0.001 $\pm$ < 0.001	−2.487	<b>0.016</b>
MUFA proportion (% of TFAs)	Intercept	30.259 $\pm$ 1.344	22.518	< <b>0.001</b>
	<i>T</i>	−0.579 $\pm$ 0.063	−9.240	< <b>0.001</b>
	<i>pCO</i> <sub>2</sub>	0.001 $\pm$ < 0.001	2.269	<b>0.028</b>
	N : P	−0.014 $\pm$ 0.014	−1.050	0.299
PUFA proportion (% of TFAs)	Intercept	32.264 $\pm$ 2.300	14.028	< <b>0.001</b>
	<i>T</i>	0.638 $\pm$ 0.107	5.949	< <b>0.001</b>
	<i>pCO</i> <sub>2</sub>	−0.002 $\pm$ 0.001	−2.769	<b>0.008</b>
	N : P	0.034 $\pm$ 0.023	1.453	0.152
DHA proportion (% of TFAs)	Intercept	2.204 $\pm$ 0.185	11.887	< <b>0.001</b>
	<i>T</i>	0.054 $\pm$ 0.010	5.611	< <b>0.001</b>
	<i>pCO</i> <sub>2</sub>	< 0.001 $\pm$ < 0.001	1.874	0.067
	N : P	0.010 $\pm$ 0.004	2.735	<b>0.009</b>
	<i>T</i> $\times$ <i>pCO</i> <sub>2</sub>	< 0.001 $\pm$ < 0.001	−2.946	<b>0.005</b>
	<i>T</i> $\times$ N : P	−0.001 $\pm$ < 0.001	−2.898	<b>0.006</b>
	<i>pCO</i> <sub>2</sub> $\times$ N : P	< 0.001 $\pm$ < 0.001	1.249	0.218



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

(Table S3), showing the lowest value at low  $p\text{CO}_2$  level and the highest one at enhanced  $p\text{CO}_2$  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  $p\text{CO}_2$  affected cellular PIC content synergistically (Table S3), with the negative response of cellular PIC content to enhanced  $p\text{CO}_2$  being significantly weaker as temperature increased (Fig. 2h; nested model,  $p < 0.001$ ).



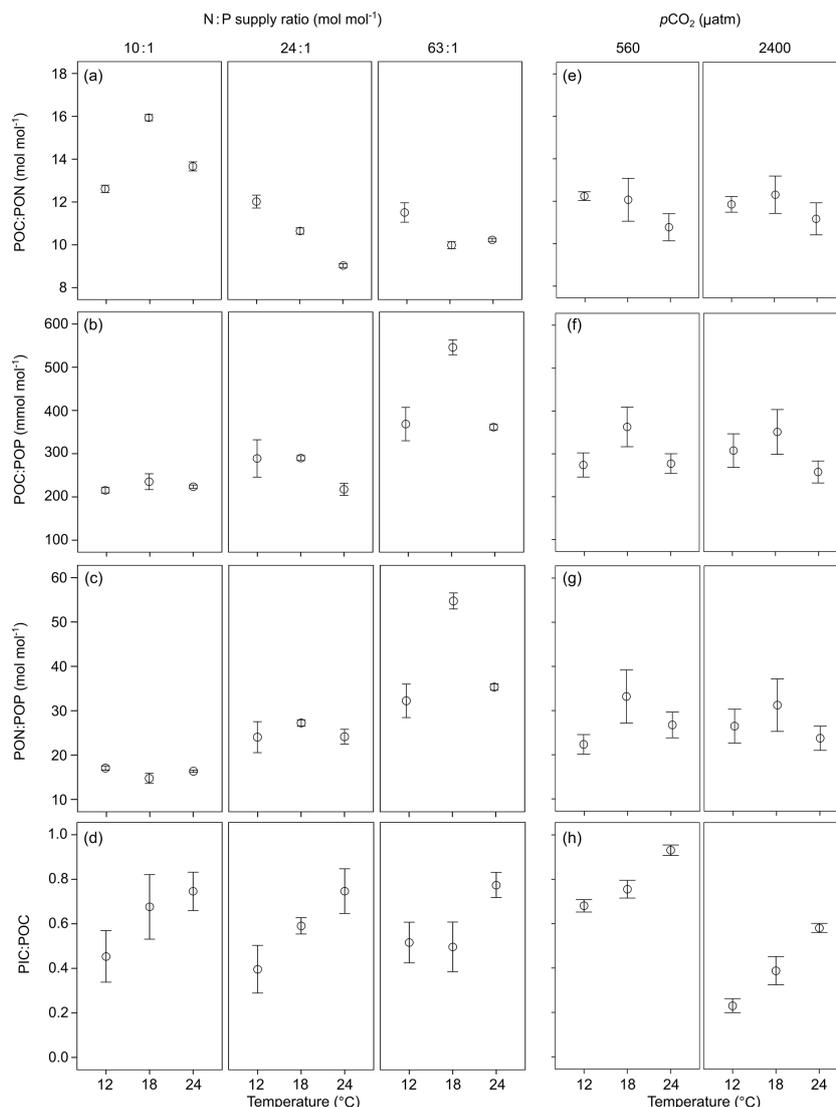
**Figure 2.** Responses of cellular contents of (a, e) particulate organic carbon (POC), (b, f) particulate organic nitrogen (PON), (c, g) particulate organic phosphorus (POP) and (d, h) particulate inorganic carbon (PIC) (mean  $\pm$  SE) to temperature, N:P supply ratios and  $p\text{CO}_2$  in *Emiliania 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.

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 a non-significant effect of  $p\text{CO}_2$  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  $p\text{CO}_2$ , with a non-significant effect of N:P supply ratios detected (Table 1).

PIC : POC increased with increasing temperature and decreased with enhanced  $p\text{CO}_2$  (Fig. 3d 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  $p\text{CO}_2$  (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 were mainly represented by 14 : 0 (13–23 %) and 16 : 0 (5–11 %). The major individual MUFA was 18 : 1n-9 (8–21 %).

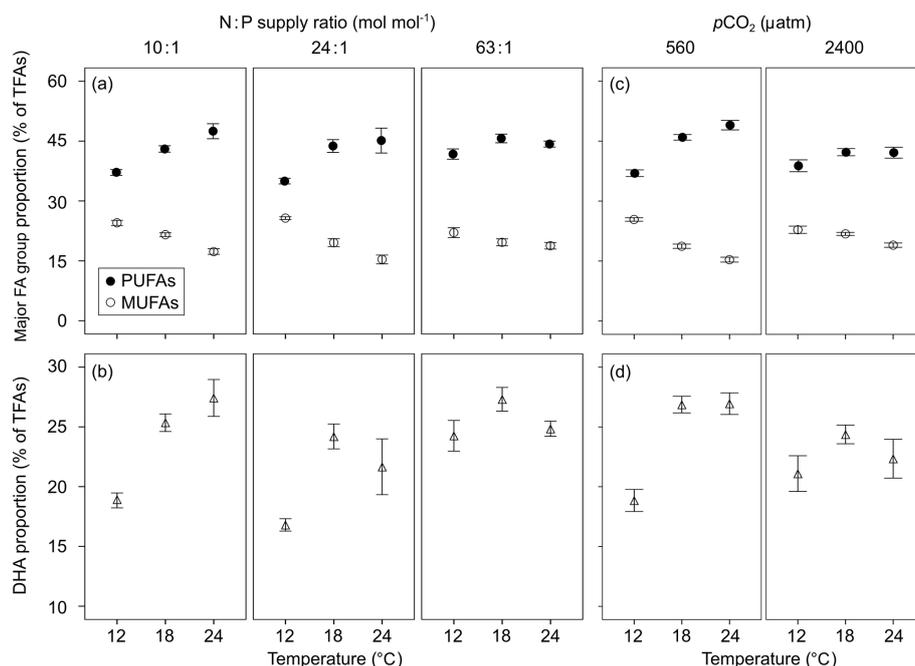


**Figure 3.** The ratios of (a, e) particulate organic carbon vs. particulate organic nitrogen (POC : PON), (b, f) POC vs. particulate organic phosphorus (POC : POP), (c, g) PON vs. POP (PON : POP) and (d, h) particulate inorganic carbon vs. POC (PIC : POC) (mean  $\pm$  SE) in response to temperature, N : P supply ratios and  $p\text{CO}_2$  in *Emiliana 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.

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

The proportion of major individual PUFA (DHA) showed significant responses to temperature and N : P supply ratios,

and the interactions between temperature and N : P supply ratios (and  $p\text{CO}_2$ ) (bold letters in Table 1). Increasing temperature and N : P supply ratios caused an overall increase in DHA (Fig. 4b). The interactions between increasing temperature and nutrient deficiency (and enhanced  $p\text{CO}_2$ ) affected DHA synergistically (Table S3), and the positive effect of temperature became more pronounced at lower N : P supply ratios (nested model,  $p < 0.001$ ) and at low  $p\text{CO}_2$  (nested model,  $p < 0.001$ ) (Fig. 4b and d).



**Figure 4.** Responses of the proportions of (a, c) monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), and (b, d) docosahexaenoic acid (DHA) (mean  $\pm$  SE) to temperature, N:P supply ratios and  $p\text{CO}_2$  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 also contains second-order interactions, with the results of AICc shown in Table S2.

## 4 Discussion

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

### 4.1 Responses of maximal growth rate

Increasing temperature significantly accelerated  $\mu_{\text{max}}$  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 ( $\sim 42^\circ\text{N}$ ) was 1.2 times higher at 26 °C than that at 16 °C, while growth rate of *E. huxleyi* from the Sargasso Sea ( $\sim 20\text{--}35^\circ\text{N}$ ) was 1.6 times higher at the higher temperature (Paasche, 2002). In our study,  $\mu_{\text{max}}$  of *E. huxleyi* (from the Azores,  $\sim 38^\circ\text{N}$ ) was 2 to 3 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

**Table 2.** The changes in cellular elemental contents (as  $\mu\text{g cell}^{-1}$ ), 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  $p\text{CO}_2$  in *Emiliana 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.  $T$ : temperature; N : P : N : P supply ratios.

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

↑ ↓ Changes  $\geq 25\%$     ↑ ↓ Changes  $< 25\%$     - No significant change

under P deficiency) in *E. huxleyi* in our study, with a weaker effect of warming (a 6 % decrease in POC : PON) and a non-significant effect of  $p\text{CO}_2$  observed (Tables 1, 2). Similarly, previous lab experiments have also reported that nutrient availability played a more important role than temperature and  $p\text{CO}_2$  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 the capacity of *E. huxleyi* to thrive under a wide range of environmental conditions. This capacity was largely revealed by a pan-genome assessment, which distributed genetic traits variably between strains and showed a suit of core genes for the uptake of inorganic nitrogen and N-rich compounds such as urea (Read et al., 2013). In spite of strain diversity within *E. huxleyi*, a recent study suggested that the global physiological response of this species to nutrient environments is highly conserved across strains and may underpin its success under a variety of marine environments (Alexander, 2016).

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

The single effects of nutrient availability and temperature described above can be modulated by their interactions. We observed synergistic interactions between warming and nutrient deficiency on cellular contents of POC, PON and POP, and between warming and enhanced  $p\text{CO}_2$  on cellular POP content (Tables 1, S3). An overall synergistic effect was also observed across 171 studies on the responses of marine and coastal systems to multiple stressors (Crain et al., 2008). Furthermore, although a 29 % change emerged in cellular POP content with rising  $p\text{CO}_2$ , we found a non-significant single effect of  $p\text{CO}_2$  on *E. huxleyi* C : N : P stoichiometry. Previous studies showed that rising  $p\text{CO}_2$  seems to change phytoplankton stoichiometry under specific conditions, e.g. at high light intensity ( $400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ )

(Feng et al., 2008) and low nutrient loads ( $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  at N:P supply ratio  $\leq 15$  or N:P supply ratio  $\geq 30$ ) (Leonardos and Geider, 2005a). In our study, we used relatively lower light intensity ( $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) than that in previous studies, and did not investigate irradiance effects. Additional research is required to assess the effects of other environmental factors such as irradiance and their interactions on C:N:P stoichiometry in our *E. huxleyi* strain.

Taken together, our results indicate that C:N:P stoichiometry in *E. huxleyi* largely reflected the changes in N:P supply ratios, across different temperatures and  $p\text{CO}_2$  levels. However, for two algal species from non-calcifying classes (the diatom *P. tricornutum* and the cryptophyte *Rhodomonas* sp.) temperature had the most consistent significant effect on stoichiometric ratios in our previous work (Bi et al., 2017). The results above are consistent with the ranking of environmental control factors in Boyd et al. (2010), which showed that temperature, nitrogen and phosphorus were ranked as important factors for major phytoplankton groups.

### 4.3 Responses of PIC:POC

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

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

peratures was driven by a markedly increased cellular PIC content (28%) and a decreased cellular POC content (−8%) (Tables 1, 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  $p\text{CO}_2$  on cellular particulate carbon contents in our study (Table 1). For example, the negative relationship between cellular PIC content and enhanced  $p\text{CO}_2$  became weaker at higher temperatures (Fig. 2h). This result is in agreement with the modulating effect of temperature on the  $\text{CO}_2$  sensitivity of key metabolic rates in coccolithophores, due to the shift of the optimum  $\text{CO}_2$  concentration for key metabolic processes towards higher  $\text{CO}_2$  concentrations from intermediate to high temperatures (Sett et al., 2014). Specifically, the interactions between warming and nutrient deficiency (and high  $p\text{CO}_2$ ) synergistically affected both PIC and POC cellular contents in most cases in our study (Table S3), indicating that nutrient deficiency and high  $p\text{CO}_2$  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  $p\text{CO}_2$  (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  $\mu\text{g mL}^{-1}$ ) to environmental changes were evident in previous work (Matthiessen et al., 2012) and the present study (Tables S5, S6; Figs. 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  $p\text{CO}_2$  on *E. huxleyi* FA composition. Both temperature and  $p\text{CO}_2$  had significant effects on the proportions of MUFAs and PUFAs, with warming causing larger changes in MUFAs and PUFAs than rising  $p\text{CO}_2$ , while significant effects of N:P supply ratios were only observed for DHA proportion (Tables 1, 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 re-

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

Enhanced  $p\text{CO}_2$  led to an overall 7 % increase in MUFAs and a 7 % decrease in PUFAs (Table 2), consistent with FA response patterns in the *E. huxleyi* strain PML B92/11 (Riebesell et al., 2000) and the strain AC472 from western New Zealand, South Pacific (Fiorini et al., 2010). Also in a natural plankton community (Raunefjord, southern Norway), PUFA proportion was reduced at high  $p\text{CO}_2$  level in the nano-size fraction, suggesting a reduced Haptophyta (dominated by *E. huxleyi*) biomass and a negative effect of high  $p\text{CO}_2$  on PUFA proportion (Bermúdez et al., 2016). To date, several mechanisms have been suggested to explain the reduced PUFAs at high  $p\text{CO}_2$  in green algae (Pronina et al., 1998; Sato et al., 2003; Thompson, 1996), with much less work conducted in other phytoplankton groups. One possible mechanism was demonstrated in a study on *Chlamydomonas reinhardtii*, showing that the repression of the  $\text{CO}_2$ -concentrating mechanisms (CCMs) was associated with reduced FA desaturation at high  $\text{CO}_2$  concentration (Pronina et al., 1998). Our observed decrease in the proportion and content of PUFAs at higher  $p\text{CO}_2$  (Table S6) fits well with the mechanism proposed by Pronina et al. (1998), which may be attributed to the repression of CCMs at high  $p\text{CO}_2$  in *E. huxleyi*.

N and P deficiency caused no significant changes in the proportions of MUFAs and PUFAs, while a 14 to 22 % increase in DHA proportion was observed (Table 2). While nutrients often play a major role on phytoplankton lipid composition (Fields et al., 2014; Hu et al., 2008), the less pronounced effects of nutrient deficiency in our study indicate a unique lipid biosynthesis in *E. huxleyi*. Indeed, Van Mooy et al. (2009) suggested that *E. huxleyi* used non-phosphorus

betaine lipids as substitutes for phospholipids in response to P scarcity. Genes are also present in the core genome of *E. huxleyi* for the synthesis of betaine lipids and unusual lipids used as nutritional/feedstock supplements (Read et al., 2013). Therefore, the lack of significant nutrient effects on most FA groups in *E. huxleyi* in our study may be caused by the functioning of certain lipid substitutions under nutrient deficiency.

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

#### 4.5 Implications for marine biogeochemistry and ecology

We observed an overall increase in POC : PON (with warming and N deficiency) and POC : POP (with N and P deficiency) in *E. huxleyi*, while enhanced  $p\text{CO}_2$  showed no significant effect (Table 2). This result indicates that nitrogen and phosphorus requirements in *E. huxleyi* are likely to reduce under projected future changes in temperature and nutrient deficiency, and will respond less strongly to changes in  $p\text{CO}_2$  than to temperature or nutrient regime shifts. Likewise, Hutchins et al. (2009) suggested negligible or minor effects of projected future changes in  $p\text{CO}_2$  on most phytoplankton phosphorus requirements. Moreover, the overall low PIC : POC under future ocean scenarios (warming and enhanced  $p\text{CO}_2$ ) indicates that carbon production by the strain *E. huxleyi* in our study acts as a carbon sink. This argument is consistent with the findings of the decreased calcification with increasing  $p\text{CO}_2$  in most coccolithophores (Beaufort et al., 2011; Hutchins and Fu, 2017), which may reduce vertical exported fluxes of sinking calcium carbonate and minimize calcification as a carbon source term, ultimately downsizing the ocean's biological carbon cycle (Hutchins and Fu, 2017).

Besides the overall increase in POC : PON and POC : POP, we found an overall increase in the proportions of PUFAs

(with warming and enhanced  $p\text{CO}_2$ ) and DHA (with warming, N and P deficiency and enhanced  $p\text{CO}_2$ ) in *E. huxleyi* (Table 2), but a decrease in PUFA and DHA contents per biomass with enhanced  $p\text{CO}_2$  (Table S6). The relationship between changes in stoichiometry and FA composition in phytoplankton varies in a complex way with environmental conditions and algal taxonomy (Bi et al., 2014; Pedro Cañavate et al., 2017; Sterner and Schulz, 1998). For example, the correlation between PON : POC and PUFA contents per biomass was negative in *Rhodomonas* sp. and positive in *P. tricornutum* under N deficiency (Bi et al., 2014). Our findings thus indicate that elemental composition responses may be coupled with responses in essential FA composition in the strain of *E. huxleyi* studied under certain configurations of environmental drivers. Such a linkage between stoichiometric and FA composition is important in studies of food web dynamics, as the C : N and C : P stoichiometry and PUFAs both have been used as indicators of 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, 2017).

## 5 Conclusions

Our study shows that N : P supply ratios had the strongest effect on C : N : P stoichiometry, while temperature and  $p\text{CO}_2$  played more influential roles in 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  $p\text{CO}_2$  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 changing marine environments.

**Data availability.** Data sets are available upon request by contacting Meixun Zhao (maxzhao@ouc.edu.cn, maxzhao04@yahoo.com).

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**Competing interests.** The authors declare that they have no conflict of interest.

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