

## ***Interactive comment on “Simultaneous shifts in stoichiometric and fatty acid composition of *Emiliania huxleyi* in response to environmental changes” by Rong Bi et al.***

**Rong Bi et al.**

[rongbi@ouc.edu.cn](mailto:rongbi@ouc.edu.cn)

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### Responses to comments from Reviewer 1

Comment 1 Line 30: “PIC” for the first appearance, should be marked it’s the abbreviation of “particulate inorganic carbon”. Also for “POC”.

Response: As suggested, the abbreviation ‘particulate inorganic carbon’ for PIC and ‘particulate organic carbon’ for POC will be added for their first appearance.

Comment 2 Line 31-32: “10:1, 24:1 and 63:1” are the ratios of N:P, the unite “mol mol-1”, not necessarily shown.

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Response: As suggested, we will remove the unit ‘mol mol-1’ and state ‘molar ratios 10:1, 24:1 and 63:1’.

Comment 3 Line 87-92: “E.huxleyi is expanding its range poleward”, why then gave an example of the subtropical area.

Response: The study in the subtropical area was removed. A study in the Bering Sea will be added, in which Harada et al. (2012) found that warming and freshening have promoted *Emiliania huxleyi* blooms in the Bering Sea since the late 1970s.

Comment 4 L149-151: “The target values were chosen to reflect a present and future regime of each factor”, however, the pCO<sub>2</sub> concentrations 560 and 2400  $\mu\text{atm}$  they used, can hardly be considered reasonable. An explanation why a gap in the CO<sub>2</sub> concentrations was so big.

Response: In plankton-rich waters respiration plus atmospheric CO<sub>2</sub>-enrichment can drive regional pCO<sub>2</sub> up to 900  $\mu\text{atm}$  at times today. Considerable seasonal, depth and regional variations of pCO<sub>2</sub> have been observed in the present-day ocean (Joint et al. 2011). For example, up to 900  $\mu\text{atm}$  of pCO<sub>2</sub> was observed in August in the Southern Bight of the North Sea, with a lower pCO<sub>2</sub> (192  $\mu\text{atm}$ ) in April (Schiettecatte et al. 2007). A natural pCO<sub>2</sub> gradient of 292 to 8828  $\mu\text{atm}$  was reported off Culcano Island, Italy (Ziveri et al. 2014). In the future oceans, pCO<sub>2</sub> will increase with rising atmospheric CO<sub>2</sub>, being 851-1370  $\mu\text{atm}$  by 2100 and 1371-2900  $\mu\text{atm}$  by 2150 (RCP8.5 scenario of the IPCC report 2014) (IPCC 2014). In the present study, the chosen values of pCO<sub>2</sub> cover the range of typical levels of pCO<sub>2</sub> in the present-day ocean and future ocean projections. Such a big gap in the value of pCO<sub>2</sub> was used to test the response of *E. huxleyi* to a considerable variation of pCO<sub>2</sub>, which has been observed in the present-day ocean as mentioned above. To clarify the reason of pCO<sub>2</sub> set-up in our study, a detailed explanation will be added in the revised manuscript.

Comment 5 Line 172: Can they write in detail about how “the specific growth rate of 20% of  $\mu\text{max}$  was applied”. I’m curious and puzzled about the reason and methods of

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how the 20% of  $\mu_{max}$  ( $\mu$ ) was realized. Usually, specific growth rate is not expressed by %.

Response: Using % of  $\mu_{max}$  guarantees that the strength of nutrient deficiency is equal through all temperature and pCO<sub>2</sub> treatments. A fixed value of  $\mu$  would mean weak deficiency when  $\mu_{max}$  is low and strong deficiency when it is high. Based on the specific growth rate ( $\mu = 20\% \text{ of } \mu_{max} (\text{day}^{-1})$ ), the equivalent daily renewal rate (D, day<sup>-1</sup>) can be estimated according to the equation  $D = 1 - e^{-\mu t}$ , where t is renewal interval (day) (here t = 1 day). Thus, the volume of the daily renewal incubation water can be calculated by multiplying D with the total volume of incubation water. We will provide the detail about how 20% of  $\mu_{max}$  was realized and applied and the reason of using % of  $\mu_{max}$  in the revised manuscript.

Comment 6 Line175-176: They said that the incubation water was exchanged with fresh seawater, since the culture medium was partially renewed according to the renewal rate D, the N:P ratios might deviate the target supply ratios in the remained medium due to differential consumption of N and P, can they give some information to show that the N:P supply ratios are stable after several rounds of renewal.

Response: 'fresh' seawater here implies freshly made seawater medium with the target N:P supply ratio, but not only fresh seawater. Indeed, nutrient concentrations in semi-continuous culture may deviate from the target values due to consumption. Semi-continuous cultures, as a practical surrogate for fully continuous culture, have been successfully used to study the effect of nutrients on phytoplankton stoichiometry and fatty acid composition (Terry et al. 1985; Lynn et al. 2000; Piepho et al. 2012; Feng et al. 2017). While we did not measure the N and P in the media daily, semi-continuous cultures can be applied to study the effect of N:P supply ratio on *E. huxleyi* stoichiometry and fatty acid composition. We will clarify that the incubation water was exchanged with freshly made seawater medium in the revised manuscript.

Comment 7 Line 178: It seems that the cell concentration was extremely high, the cell

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concentration range should be provided.

Response: In our study, the final cell density at the steady state ranged between  $1.50 \times 10^5 - 17.8 \times 10^5$  cells mL<sup>-1</sup>, with the average value of  $7.95 \times 10^5$  cells mL<sup>-1</sup>. High cell density ( $> 1 \times 10^6$  cells mL<sup>-1</sup>) was observed in six out of 18 treatments. The average value of cell density in our study was consistent with the range of those in previous studies. For example, De Bodt et al. (2010) reported the maximum cell density of  $6.84 \times 10^5$  cells mL<sup>-1</sup> when testing the effects of pCO<sub>2</sub> and temperature on *E. huxleyi* calcification. As suggested, the range of cell densities will be shown in the revised manuscript.

Comment 8 Line 180: What do the authors mean by "the net growth rate (r)", what's the difference between r and  $\mu$ ? Confusing wordings or mis-understood definitions?

Response: In order to clarify the difference between  $\mu$  and r, we will use the term 'gross growth rate' for  $\mu$ , while r (net growth rate) is the difference between the gross growth rate and the loss rate ( $r = \mu - D$ ). The difference between gross growth rate and net growth rate will be clarified in the revised manuscript.

Comment 9 Line 203: Here "was" should be "were".

Response: As suggested, the word 'was' will be replaced by 'were'.

Comment 10 Line 241: Is this theory applicable in all species and in any conditions.

Response: This hypothesis was proposed by Cherif and Loreau (2010), suggesting that realized maximum growth rates (i.e., the observed maximum growth rate in the present study,  $\mu_{max}$ ) should be equal for essential, non-substantial resources for phytoplankton species. This assumption was supported by both theoretical and empirical evidence, 1) lab experiments showed little or no luxury uptake of resources at the highest growth rate; 2) the maximum capacity of the uptake machinery should not be oversized for a given resource based on economical design (Cherif and Loreau 2010). Similar  $\mu_{max}$  in different nutrient conditions has been observed for different phyto-

### C4

plankton species in empirical experiments (e.g., Ahlgren 1985; Baek et al. 2008; Bi et al. 2012). The model presented in Cherif and Loreau (2010) has also been successfully used to study how nutrient gradients influence stoichiometry of autotrophs in natural chemostats (Nifong et al. 2014). In the present study, we had only one value of  $\mu_{max}$  for each nutrient treatment under different temperature and pCO<sub>2</sub> conditions, thus the effect of N:P supply ratio cannot be tested with ANOVA efficiently. In the revised manuscript, we tested the response of  $\mu_{max}$  to temperature, N:P supply ratio and pCO<sub>2</sub> using GLMMs. The results of GLMMs were consistent with those of ANOVA, showing a highly significant effect of temperature on  $\mu_{max}$ . As the chosen best model contained only first order effects, no significant interactions between the three environmental factors were detected. The non-significant response of  $\mu_{max}$  to N:P supply ratio in *E. huxleyi* is consistent with the assumption of Cherif and Loreau (2010). We will revise the Results and Discussion sections according to the new results of GLMMs on  $\mu_{max}$ .

Comment 11 Line1103: Why there is no panel for the pCO<sub>2</sub> effect in Fig.2.

Response: The effect of pCO<sub>2</sub> on stoichiometric C:N:P will be added in Fig. 2.

Comment 12 Line 1112: As I read from the “experimental setup” part, this study investigates the combined effects of temperature, pCO<sub>2</sub> and N:P supply ratios on *E.huxleyi*. Why in Fig 3. the combined effects of N:P supply ratio and pCO<sub>2</sub> are not considered, i.e. pCO<sub>2</sub> is not considered in panel (a), (b), (c), and N:P supply ratio is not considered in panel (d), (e) and (f). The same question for Fig. 4, 5 and 6.?

Response: In the present study, significant interactions between temperature and N:P supply ratio, and between temperature and pCO<sub>2</sub> were detected for cellular PIC and POC contents, and the proportion of DHA. However, the significant interaction between N:P supply ratio and pCO<sub>2</sub> was only found for the proportion of SFAs. Please see Fig. 7 for a systematic summary. Thus, the interactions between temperature and N:P supply ratio, and between temperature and pCO<sub>2</sub> were shown in figures 3-6, while

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that between N:P supply ratio and pCO<sub>2</sub> was only shown for SFAs in the supporting information Fig. S2.

Responses to comments from Reviewer 2

General comment I My main concerns with the manuscript in its current form are the framing of the experimental manipulations to global patterns and the length of the discussion. There seems to be a mismatch between projected temperature and CO<sub>2</sub> conditions in future oceans and the ones you manipulated. I would like to know how you would translate your results to a future ocean scenario as the CO<sub>2</sub> concentrations in your lowest treatment are higher than currently measured in global oceans (max 440 ppm; Bakker et al. (2016)). On a similar note, how do the three temperature treatments with a difference of 12C relate to future ocean projections?

Response: The chosen levels of pCO<sub>2</sub> and temperature in this study were set based on the reasons below: 1) pCO<sub>2</sub>. Please see our reply to Reviewer 1 Comment 4. In plankton rich waters respiration plus atmospheric CO<sub>2</sub>-enrichment can drive regional pCO<sub>2</sub> up to 900  $\mu$ atm at times today. For example, up to 900  $\mu$ atm of pCO<sub>2</sub> was observed in August in the Southern Bight of the North Sea (Schiettecatte et al. 2007). A much higher pCO<sub>2</sub> (a natural pCO<sub>2</sub> gradient of 292 to 8828  $\mu$ atm) was observed off Culcano Island, Italy (Ziveri et al. 2014). In the future oceans, pCO<sub>2</sub> will increase with rising atmospheric CO<sub>2</sub>, being 851-1370  $\mu$ atm by 2100 and 1371-2900  $\mu$ atm by 2150 (RCP8.5 scenario of the IPCC report 2014) (IPCC 2014). Therefore, the chosen values of pCO<sub>2</sub> in the present study cover the range of typical levels of pCO<sub>2</sub> in the present-day ocean and future ocean projections. 2) Temperature. Water surface temperatures at the Azores vary between ~12 - 29°C (Lafon et al. 2004), with the inter-annual average temperature between 16 - 22°C and peaks usually reaching a maximum of 24 - 25°C (<http://dive.visitazores.com/en/when-dive>; last accessed date: 22.08.2017). 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. Annual mean sea surface temperature

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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). We also chose this setup to compare with our previous results (Bi et al. 2017). 3) The ranges of pCO<sub>2</sub> and temperature in our study is the same with those in our previous work (Bi et al. 2017), which makes the comparison easier between different work. The reason of pCO<sub>2</sub> and temperature setup will be pointed out in Material and Method in the revised manuscript. In the Introduction, we will also explain how the setup of temperature and pCO<sub>2</sub> in our study relates to future ocean scenarios.

General comment II The discussion is quite lengthy and would benefit in my opinion to focus more on the interaction effects observed in the study as these are the core strength of the work and could advance the field. Perhaps you could reduce the amount of wording if you first discuss the solo effects and then go into all interaction effects in one paragraph (for C:N:P stoichiometry, PIC:POC separately). It seems that there is currently a lot of overlap in the things discussed in separate paragraphs.

Response: As suggested, we will first discuss the solo effect and then go into all interaction in the Discussion.

General comment III In addition, I'm missing the inclusion of the PON and POP contents underlying the responses in C:N:P stoichiometry, the results of N:P supply ratio on maximal growth rate and the (though non-significant) results of C:N:P stoichiometry in the different CO<sub>2</sub> treatments (Fig. 2).

Response: As suggested, the results of PON and POP contents, the N:P effect on  $\mu_{max}$  in Fig. 1, and the pCO<sub>2</sub> effect on C:N:P stoichiometry in Fig. 2 will be shown in the revised manuscript.

General comment IV From the introduction it is not clear that PIC and POC production will be discussed. In my opinion, the focus on C:N:P stoichiometry and underlying biochemical composition is the core of your work and introduced very well in the manuscript. I understand the importance of PIC:POC for calcifiers specifically, but I

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would advise to focus less on the PIC and POC contents, production rates and population yields and more on the C:N:P and fatty acids.

Response: We will shorten the results and discussion on PIC and POC and focus more on the changes in C:N:P stoichiometry and fatty acids.

General comment V A discussion on how changes in stoichiometry and fatty acids relate to each other would be a great addition to the discussion section.

Response: A discussion on how the changes in stoichiometry relate to those in fatty acids will be added.

General comment VI What would be a great addition to the introduction are hypotheses on how temperature, CO<sub>2</sub> and nutrient supply affect the C:N:P stoichiometry and fatty acid composition. Something similar to Figure 7, but then hypothetical. This would then furthermore help shape the discussion as you could refer back to these hypotheses.

Response: In the last paragraph of the introduction, we will add hypotheses on how temperature, pCO<sub>2</sub> and nutrient supply affect C:N:P stoichiometry and fatty acid composition.

General comment VII A smaller comment, but the use of N:C and P:C ratios instead of C:N and C:P is not very commonly used in literature. The readability and comparison of these ratios to other studies would benefit greatly if they are expressed in C:N and C:P.

Response: We will use POC:PON and POC:POP, instead of N:C and P:C biomass ratios, in the revised manuscript.

General comment VIII Furthermore, the reasoning behind the statistical methods used are not entirely clear for me. Some information in the method section on why this type of statistics are used and what the associated parameters mean would aid the reader in the understanding of the manuscript.

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Response: Generalized linear mixed models (GLMMs) are appropriate for non-normal data such as counts or proportions, while classical statistical procedures such as ANOVA rely on normally distributed data (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., Frère et al. 2010; Jamil et al. 2014; Bracewell et al. 2017), in which data sets are often non-normally distributed. We will explain the reason why to choose GLMMs and what the associated parameters mean in the revised manuscript.

Specific comment 1 73 Do you mean community structure of phytoplankton?

Response: According to Doney et al. (2012), climate change may alter the physiological functioning, behavior, and demographic traits of organisms. These changes cascade from primary producers to upper trophic levels such as fish, seabirds and marine mammals. Therefore, community structure in the sentence in our manuscript means not only for phytoplankton but also for other trophic levels. We hence will clarify this as '— community structure of different trophic levels —'.

Specific comment 2 80-82 'via releasing CO<sub>2</sub>' is not really clear for me what this means and why coccolithophores are important components of the carbon cycle.

Response: This sentence will be revised to clarify that coccolithophores are not only important photosynthetic producers of organic matters (causing a draw-down of CO<sub>2</sub> 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<sub>2</sub> to the atmosphere).

Specific comment 3 112 What do you mean by a core feature? 'Element' → 'elemental'

Response: This sentence will be revised to ' — variability in *Emiliania huxleyi* C:N:P stoichiometry (cellular quotas and ratios of C, N and P) can also be important in ocean biogeochemistry.' 'element' will be changed to 'elemental'.

Specific comment 4 117 Food for which organism? Phytoplankton or zooplankton?

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Response: According to Rosenblatt and Schmitz (2016), shifts in resource nutrient content are generally occur with shifts in consumer physiology and behavior, and they are often overlooked in studies of the responses of food web dynamics to climate change. We thus will clarify this sentence as ' — shifts in resource nutrient content for consumers are often overlooked in climate change ecology —'.

Specific comment 5 129-136 This comes as a surprise for me here and seems to fit better in the methodological section than in the introduction.

Response: As suggested, these two sentences will be moved to the methodological section.

Specific comment 6 138 PIC:POC is already a ratio

Response: It will be revised as 'PIC and POC contents and their ratios' throughout the text.

Specific comment 7 147 The manipulated CO<sub>2</sub> levels came as a surprise to me in the framework of current and future projections. Do you have specific reasons to choose these levels as I would have expected a lower 'ambient' CO<sub>2</sub> level (around 400 ppm)?

Response: Please see our reply to General comment I.

Specific comment 8 148 Does the strain have a specific reference number (to make possible comparisons with other studies easier)?

Response: As suggested, the specific reference number (internal culture collection reference code: A8) will be added.

Specific comment 9 175 does 'fresh' seawater imply that is was taken from sea at that day?

Response: 'fresh' seawater implies that freshly made seawater medium, but the seawater was not taken from the sea on that day. To clarify this, the sentence will be revised as 'The incubation water was exchanged with freshly made seawater medium

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—'.

Specific comment 10 222 Is there a specific reason why you choose GLMM's instead of the more classic ANOVA's?

Response: Please see our reply to General comment VIII.

Specific comment 11 228 What are link functions?

Response: 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](https://www.ibm.com/support/knowledgecenter/SSLVMB_21.0.0/com.ibm.spss.statistics.help) 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. We will explain in the text what the link function is.

Specific comment 12 222-239 This part of the statistics is quite difficult for me to follow. Could you explain a bit more about the different procedures and what they do?

Response: We will explain more about the different procedures in GLMMs.

Specific comment 13 242 I would not assume  $\mu_{max}$  to be the same between nutrient treatments as that was the case in another study. Did you test this and was this the case in your study?

Response: We tested the changes of  $\mu_{max}$  between different nutrient treatments. Because there was only one data of  $\mu_{max}$  in each nutrient treatment under different temperature and pCO<sub>2</sub> conditions, the effect of N:P supply ratio cannot be tested with ANOVA efficiently. In the revised manuscript, we will show the results of GLMMs on the response of  $\mu_{max}$  to temperature, N:P supply ratio and pCO<sub>2</sub> using GLMMs, which are consistent with those of ANOVA, showing a highly significant effect of temperature and non-significant effect of N:P supply ratio and pCO<sub>2</sub>. As the chosen best model contained only first order effects, no significant interactions between the three environmental factors were detected. In the literature, there are limited data on the response of

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$\mu_{max}$  in *E. huxleyi* to nutrient availability, while several studies reported the response of specific growth rate. According to Cherif and Loreau (2010), realized maximum growth rates (i.e., the observed maximum growth rate in the present study,  $\mu_{max}$ ) should be equal for essential, non-substantial resources for phytoplankton species. This assumption was supported by both theoretical and empirical evidence, 1) lab experiments showed little or no luxury uptake of resources at the highest growth rate; 2) the maximum capacity of the uptake machinery should not be oversized for a given resource based on economical design (Cherif and Loreau 2010). For *E. huxleyi*, luxury consumptions for phosphate and nitrate are lower than other phytoplankton taxa (Rost and Riebesell 2004). Thus, the non-significant response of  $\mu_{max}$  to N:P supply ratio in *E. huxleyi* in our study is consistent with the assumption of Cherif and Loreau (2010). Future work is suggested to study the response of  $\mu_{max}$  in *E. huxleyi* under a wider range of nutrient conditions. We will revise the Results and Discussion sections according to the new results of GLMMs on  $\mu_{max}$ .

Specific comment 14 244 Is there a specific reason why you only used w2 for the  $\mu_{max}$  results and not for other results as for instance figure 7?

Response: It would be better to show w2 for all responses; however, error mean square cannot be obtained from GLMMs and thus w2 cannot be calculated for response variables tested with GLMMs. In the revised manuscript, we will show the response of  $\mu_{max}$  using GLMMs. Thus, the results of w2 will be removed and percent changes of  $\mu_{max}$  will be calculated.

Specific comment 15 248 Why would you use nested models when you have a full factorial design? In other words, what is the added value of these statistical tests? Can you relate your chosen temperatures to acclimatization of *E. hux* in your lab or the original population that was sampled? How are average annual water temperatures at the Azoren?

Response: It is possible to use a nested model in a full-factorial design setting. The

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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. The difference to e.g. a test including straight-forward interaction effects is that interaction terms describe systematic variation of one factor's effects over a gradient of the other, whereas a nested model can highlight if for example pCO<sub>2</sub> plays a role for fatty acid content only at intermediate temperature. Please see our reply to General comment 1 regarding average water surface temperatures. The chosen temperature setup in our study is within the range of sea surface temperature at the Azores.

Specific comment 16 274 Did you determine the CO<sub>2</sub> effects by post-hoc tests? As there was no overall effect of CO<sub>2</sub> on maximum growth rate while you have a significant interaction effect, wouldn't that mean that the effect of temperature is dependent on the CO<sub>2</sub> level, but not vice versa?

Response: In our study, a post hoc test was applied only if there were significant effects. We thus did not determine the effect of pCO<sub>2</sub> by the post hoc test, as the effect of pCO<sub>2</sub> was not significant according to ANOVA. We agree with the reviewer that the effect of temperature is dependent on the CO<sub>2</sub> level. In the revised manuscript, we will use GLMMs to test the response of  $\mu_{max}$  (Please see our response to Specific comment 13). The results showed no significant interactions between temperature and pCO<sub>2</sub>, while there was still a different trend of  $\mu_{max}$  to increase with increasing temperature between the two pCO<sub>2</sub> treatments. We will revise the Results and Discussion sections accordingly.

Specific comment 17 280 any particular reason to use N:C ratios as opposed to C:N ratios? The latter is used more often in literature and makes the comparison with the Redfield Ratio easier. For instance, a hump-shaped curve to temperature (or U-shaped curve, line 286) is also observed for a marine cyanobacterium (Fu et al. 2014). By having the ratios in N:C instead of C:N, comparison with other studies like these can get confusing. Furthermore, you did not report interaction effects of temperature and N:P supply ratio on N:P ratios. So how does the difference in temperature response

C13

under N and P deficiency (lines 287-288) relate to that?

Response: We will present the results of POC:PON and POC:POP in the revised manuscript to make the comparison with the Redfield Ratio and the results in the literature easier. We found that, similar to the results in Fu et al. (2014), a hump-shaped curve to temperature was also observed for POC:PON in response to increasing temperature under N deficiency in our study. Indeed, there was non-significant interaction between temperature and N:P supply ratio on PON:POP according to GLMMs. However, POC:PON responded significantly to temperature, showing a different trend of changes to increasing temperature under different N:P supply ratio. We thus presented this nutrient-dependent response, as it need not be universal to constitute 'significant discovery'.

Specific comment 18 283 instead of biomass ratios, would it make sense to use PON:POP or POC:PON as that would already imply that it is biomass related. Related to that question, is the C:N ratio composed of TPC:PON or POC:PON? Furthermore, what is underlying the changes in stoichiometry? You have the results for POC content in Figure 4, but how do PON and POP change?

Response: As suggested, we will use POC:PON, POC:POP and PON:POP in the revised manuscript. The C:N biomass ratio is composed of POC:PON. To explore what is underlying the changes in C:N:P stoichiometry, we analyzed the responses of cellular PON and POP contents. For example, a U-shaped curve was observed for the responses of cellular POC and PON contents to increasing temperature under N deficiency, which can explain the observed hump-shaped curve for the response of POC:PON. The detail results will be included and discussed in the revised manuscript.

Specific comment 19 292 What is a PIC population yield?

Response: A population yield of PIC is the PIC content per ml ( $\mu\text{g ml}^{-1}$ ). This will be clarified in the revised manuscript.

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Specific comment 20 347 Technically, C:N:P is not a ratio but is composed of C:N and C:P ratios. Additionally, why did you chose to only highlight the N:P results?

Response: As suggested, C:N:P biomass ratio will be changed to C:N:P stoichiometry throughout the text. The response of N:P biomass ratio was highlighted here because it had the highest percent changes among the three stoichiometric ratios. To clarify this, a 'e.g.' will be added in this sentence.

Specific comment 21 356 These interactions effects don't become clear from table 2, as there you only report the effects of the individual stressors.

Response: Indeed, we did not observe significant effects of the three stressors on all response parameters, with significant interactive effects only observed for cellular POC content, and SFA and DHA proportion. We will clarify this information in the first paragraph in the Discussion.

Specific comment 22 369 'strains' instead of 'strain'

Response: As suggested, the word will be corrected to 'strains'.

Specific comment 23 370 It would be interesting to link this result with the origin of your strain. Does it fall in excepted patterns?

Response: In our study,  $\mu$ max of *E. huxleyi* (from the Azores,  $\sim 38^\circ\text{N}$ ) was two to three times higher at the highest temperature than that at the lowest temperature, showing a similar change pattern with that in *E. huxleyi* (1.6 times higher at the higher temperature) from the Sargasso Sea ( $\sim 20\text{--}35^\circ\text{N}$ ). We will add the comparison between our results and the results in the literature in the revised manuscript.

Specific comment 24 375 I could also argue it the other way, that the biogeographic origin of an *E. huxleyi* strain is important for their response to temperature. Like mentioned before, could you elaborate on this more?

Response: We agree that the results show the importance of the biogeographic origin

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of an *E. huxleyi* strain for their response to temperature. We will revise this sentence according to this comment and Specific comment 23.

Specific comment 25 378 Seems to contrast Table 1 and lines, were you show no effect of CO<sub>2</sub> on maximal growth rate. Or is this based on post-hoc comparisons?

Response: Please see our response to Specific comment 16.

Specific comment 26 387-389 Can you quantify these slopes as they come a bit as a surprise at this point in the manuscript.

Response: We will quantify these slopes both in the Result and Discussion.

Specific comment 27 393 remove 'and'

Response: 'and' will be removed.

Specific comment 28 394 If it is a conceptual graph you're referring to, I would be interested in the conceptual reasoning behind this response.

Response: The conceptual reasoning behind is still unclear. The authors who proposed the conceptual graph suggested that one possible explanation is that increasing temperature may modulate the balance between a fertilizing effect of ocean carbonation and a metabolic repression by ocean acidification (Sett et al. 2014). This explanation will be added at the end of this paragraph.

Specific comment 29 403 I would opt for 'C:N:P stoichiometry' instead of biomass ratios.

Response: 'C:N:P biomass ratios' was revised to 'C:N:P stoichiometry' throughout the manuscript.

Specific comment 30 409 What do you mean by 'prevailed the governing effect'?

Response: Skau (2015) tested the effects of temperature and phosphorus on stoichiometry in three haptophytes, showing that phosphorus treatments had a stronger

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effect on C:P ratios in *E. huxleyi* compared to temperature. We will revise this sentence as '— nutrient availability had a stronger effect on stoichiometric ratios in *E. huxleyi* compared to temperature'.

Specific comment 31 415-417 I really like Figure 7 as it gives a nice overview about your results. But what I'm missing there is the change in cellular N and P content. These results could help you in making conclusions about the changes in PON:POP, whether that is mainly due to N or P deficiency. Furthermore, should it be a table instead of a figure?

Response: We will add the changes in cellular N and P contents. Yes, Fig. 7 should be a table, which will be changed in the revised manuscript.

Specific comment 32 445-447 But given your result that the changes in N:C (or C:N) are stronger than those of P:C, what would be the mechanism behind that? Is there any current literature on that respect? Furthermore, if you bring in the argument of less P rich ribosomes with warming, wouldn't you have expected an decrease in P:C instead of the increase you observed?

Response: In the literature, variable changes of PON:POC and POP:POC to warming were observed in *E. huxleyi*, showing positive (Feng et al. 2008; Matson et al. 2016), negative (Borchard and Engel 2012) and U-shaped responses (Rosas-Navarro et al. 2016). Similar to our study, Borchard and Engel (2012) also found a stronger change of PON:POC than that of POP:POC at higher P condition, while both biomass ratios decreased with increasing temperature. The mechanism behind the different responses of PON:POC and POP:POC 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). We will revise this part of discussion to clarify the mechanism behind the changes of C:N:P stoichiometry in response to warming.

Specific comment 33 461 I'm missing here a coupling to your own experimental set-

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up, did you not find effects of CO<sub>2</sub> on stoichiometry due to light conditions or nutrient loads? Can you compare your set-up with those from the studies you mentioned?

Response: We will add comparison of experimental set-up between our study and previous work. For example, Feng et al. (2008) reported that rising pCO<sub>2</sub> caused the increase in POC:PON only at the high light condition (400  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>). The light intensity in our study (100  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>) was lower than that in Feng et al. (2008). In our study, the effect of light intensity was not tested, while the effect of pCO<sub>2</sub> was not found under different N:P supply ratios. Thus, future work is suggested to address the effect of light intensity on the *E. huxleyi* strain used in our study.

Specific comment 34 462 This is a rather fast transition for me from stoichiometry to cellular biomass. Perhaps this part fits better with the discussion paragraph on growth rates.

Response: In this sentence, we still discuss the responses of C:N:P stoichiometry and not cellular biomass. We will revise this sentence as 'Taken together, our results indicate that the changes in N:P supply ratios are reflected in C:N:P stoichiometry in *E. huxleyi*, —'.

Specific comment 35 472 But you haven't looked at taxonomic composition as you study one species. As there is already such variability between strains and experiments with *E. hux*, I would shorten this paragraph and focus more on the drivers of variation in responses.

Response: As suggested, the discussion in this paragraph will be revised to focus more on the drivers of variation in stoichiometric responses: 'Taken together, our results indicate that the changes in N:P supply ratios are reflected in C:N:P stoichiometry in *E. huxleyi*, across different temperatures and pCO<sub>2</sub> levels, showing the absence of significant interactions between the three environmental factors. However, for two algal species from non-calcifying classes (the diatom *P. tricornutum* and the cryptophyte *Rhodomonas* sp.) temperature had the most consistent significant effect on N:C

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and P:C biomass ratios and showed significant interactions with N:P supply ratios and pCO<sub>2</sub> in our previous work (Bi et al. 2017). The results above are consistent with previous studies, which showed that both temperature and N:P supply ratios were ranked as important factors in regulating phytoplankton stoichiometry in previous studies (Boyd et al. 2010; Feng 2015).’.

Specific comment 36 492 Refrain from starting a sentence with ‘and’

Response: ‘and’ will be removed from the beginning of the sentence.

Specific comment 37 498 This is vague for me, what other environmental drivers do you mean specifically?

Response: According to previous studies, the interaction of pCO<sub>2</sub> with other environmental factors such as irradiance and temperature may be potential drivers on the changes in PIC:POC (Feng et al. 2008; De Bodt et al. 2010). We will revise this sentence to specify what other environmental drivers are.

Specific comment 38 507 ‘and the present study’ should be within the brackets?

Response: ‘the present study’ will be added within brackets.

Specific comment 39 519 CO<sub>2</sub> would not be related to future oceans as the lowest treatment is already elevated.

Response: Please see our reply to General comment I.

Specific comment 40 524 This argument is not clear to me and does not follow logically from your work. Yes, you have changes in PIC and POC yields with environmental changes, but why would that not scale up to carbon export?

Response: We will revise this sentence as ‘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) to environmental changes were evident in previous work (Matthiessen

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et al. 2012) and the present study (Fig. 7). Thus scaling our results up to cocolithophores carbon export should consider these uncertainties.’.

Specific comment 41 529 ‘dynamic’ → ‘dynamics’

Response: ‘dynamic’ will be revised to ‘dynamics’.

Specific comment 42 595 ‘low trophic levels consumers’: do you mean first order consumers?

Response: Here we would prefer ‘low trophic levels consumers’, which includes not only first order consumers but also second order consumers. Dietary preferences of zooplankton may change with environmental conditions such as temperature (Boersma et al. 2016). For example, the copepod *Temora longicornis* preferred the cryptophyte *Rhodomonas salina* at higher temperatures, while it preferred the heterotrophic dinoflagellate *Oxyrrhis marina* at lower temperatures (Boersma et al. 2016). In the studies we cited (Garzke et al. 2016; Garzke et al. 2017), the influences of warming and ocean acidification were studied in a community of calanoid copepods, which showed feeding preferences between phytoplankton and microzooplankton. Thus, it is more precise to use the term ‘low trophic levels consumers’ here.

Specific comment 43 606 ‘relationship’ → ‘relationships’

Response: ‘relationship’ will be revised to ‘relationships’.

Specific comment 44 612 How does the temperature and CO<sub>2</sub> relate to future ocean scenarios? That would be good to add to the introduction.

Response: Please see our reply to General comment I.

Specific comment 45 614 Wouldn’t that contradict the argument you made in line 523-524 that these results cannot be scaled up to carbon export?

Response: The argument about carbon export will be revised. Please see our reply to Specific comment 40.

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Specific comment 46 Table S2: the meaning of the column effect builder is not clear to me. What does main, two way and three way mean and how do these model outputs relate to the ones in table 2?

Response: In Table S2, 'main', 'two way' and 'three way' mean models containing first order effects of the three factors, second order interactions of all factors, and third order interactions of all factors, respectively. The selected models in Table 2 are shown in bold in Table S2. We will clarify the meaning of the column effect builder and the relationship between Table S2 and Table 2 in the revised manuscript.

Specific comment 47 Table 2: It is not clear to me what a significant intercept in these models mean? Furthermore, I'm missing interaction terms for some of the variables. I would change PIC (ug/ml) to PIC population yield (ug/ml) to make it easier to connect with the text.

Response: A significant intercept means that the regression curve (or in case of linear correlations: regression line) does not pass through the origin. Table 2 only shows the results of selected models. For some variables such as PON:POC, the model with only first order effects of the three factors was selected, because it can best predict targets. Thus, there were no interaction terms for the variable PON:POC. PIC (and POC) population yield will be used to easily connect with the text.

Specific comment 48 Figure 1: I'm missing the results for N:P supply in this figure.

Response: We will add the results for N:P supply ratio in Fig. 1.

Specific comment 49 Figure 2: I'm missing the results for CO2 in this figure.

Response: We will add the results for pCO2 in Fig. 2.

Specific comment 50 Table S4 seems to be the only results in which standard deviations instead of standard errors are reported. For consistency reasons I would opt for standard errors here.

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Response: We will show standard errors in Table S4.

Specific comment 51 Fig S2 is missing the (mean +/- SE) from the legend. Or is standard deviation expressed here?

Response: Data in Fig. S2 are expressed as mean  $\pm$  SE. As suggested, this information will be clarified in the revised manuscript.

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