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

Anonymous Referee #2

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General comments:

First of all, I want to compliment the authors on their work. There aren't many studies out there that manipulate three climate change related factors in a full-factorial design. The experiment is conducted nicely, the results are presented (mostly) clear and the data behind this study is very valuable in understanding the interaction effects of temperature, CO2 and nutrient conditions on phytoplankton physiology. Furthermore, the results obtained from this study are well imbedded in our current knowledge on these topics in the discussion. 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 CO2 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 CO2 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? 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. 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 CO2 treatments (Fig. 2). 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 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. A discussion on how changes in stoichiometry and fatty acids relate to each other would be a great addition to the discussion section. What would be a great addition to the introduction are hypotheses on how temperature, CO2 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. 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. 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. All in all, I think this is very interesting work that, with adjustments, would be very suitable for publication in Biogeosciences.

Specific comments:

73 Do you mean community structure of phytoplankton? 80-82 'via releasing CO2' is not really clear for me what this means and why coccolithophores are important components of the carbon cycle. 112 What do you mean by a core feature? 'Element' –> 'elemental' 117 Food for which organism? Phytoplankton or zooplankton? 129-136 This comes as a surprise for me here and seems to fit better in the methodological section than in the introduction 138 PIC:POC is already a ratio 147 The manipulated CO2 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' CO2 level (around 400 ppm)? 148 Does the strain have a specific reference number (to make possible comparisons with other studies easier)? 175 does 'fresh' seawater imply that is was taken from sea at that day? 222 Is there a specific reason why you choose GLMM's instead of the more classic ANOVA's? 228 What are link functions? 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? 242 I would not assume mumax 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? 244 Is there a specific reason why you only used w2 for the mumax results and not for other results as for instance figure 7? 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? 274 Did you determine the CO2 effects by post-hoc tests? As there was no overall effect of CO2 on maximum growth rate while you have a significant

interaction effect, wouldn't that mean that the effect of temperature is dependent on the CO2 level, but not vice versa? 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 under N and P deficiency (lines 287-288) relate to that? 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? 292 What is a PIC population yield? 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? 356 These interactions effects don't become clear from table 2, as there you only report the effects of the individual stressors. 369 'strains' instead of 'strain' 370 It would be interesting to link this result with the origin of your strain. Does it fall in excepted patterns? 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? 378 Seems to contrast Table 1 and lines, were you show no effect of CO2 on maximal growth rate. Or is this based on post-hoc comparisons? 387-389 Can you quantify these slopes as they come a bit as a surprise at this point in the manuscript. 393 remove 'and' 394 If it is a conceptual graph you're referring to, I would be interested in the conceptual reasoning behind this response. 403 I would opt for 'C:N:P stoichiometry' instead of biomass ratios. 409 What do you mean by 'prevailed the governing effect'? 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? 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? 461 I'm missing here a coupling to your own experimental set-up, did you not find effects of CO2 on stoichiometry due to light conditions or nutrient loads? Can you compare your set-up with those from the studies you mentioned? 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. 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. 492 Refrain from starting a sentence with 'and' 498 This is vague for me, what other environmental drivers do you mean specifically? 507 'and the present study' should be within brackets? 519 CO2 would not be related to future oceans as the lowest treatment is already elevated. 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? 529 'dynamic' –> 'dynamics' 595 'low trophic levels consumers': do you mean first order consumers? 606 'relationship' –> 'relationships' 612 How does the temperature and CO2 relate to future ocean scenarios? That would be good to add to the introduction. 614 Wouldn't that contradict the argument you made in line 523-524 that these results cannot be scaled up to carbon export?

Comments on figures and tables:

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? 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. Figure 1: I'm missing the results for N:P supply in this figure. Figure 2: I'm missing the results for CO2 in this figure. 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. Fig S2 is missing the (mean +/- SE) from the legend. Or is standard deviation expressed here?

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