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Interactive comment on "Environmental controls on the elemental composition of a Southern Hemisphere strain of the coccolithophore *Emiliania huxleyi*" *by* Yuanyuan Feng et al.

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Dear reviewer and editors,

The authors would like to thank the reviewer for the helpful comments provided in order to improve the manuscript. The manuscript has been carefully checked and revised based on the reviewer's comments. The responses to the detailed comments are listed below.

"MAJOR COMMENTS: Results: I am concerned about the "cellular content response (POC, PON, POP) to environmental drivers". Organic matter quotas are strongly determined by the cell cycle. POC/cell, for example, will be much lower directly after cell division than right before. Thus, you can only compare cell quotas among treatments, when you are sure that all treatments were in the same cell cycle stage. The Authors do not indicate if samples were taken at the same time. This information would be a step forward because it could then at least be assumed that cell division was synchronized during night. However, even if sampling times were identical, it remains questionable if this assumption is valid for every treatment. Growth rates are not reported here but I assume that they are below 0.69, at least in some treatments (e.g. the low temperature treatment). If the cells divide less than once per day and only divide during night, it means that some cells of the population are packed with POC while others are depleted. Since you only sample once at an unknown cell cycle stage, it may become difficult (if not impossible) to disentangle the cell cycle-specific response from the actual treatment response. I therefore think that the results on cell quotas presented here (but also elsewhere in the literature) could potentially be misleading. My suggestion would be to show production rates (μ x cell quota) instead of cell quotas. These also have theoretical issues but should more robust. " The authors agree that the cellular elemental quotas depend on the cell cycles. The samples in our study are taken during the same time window so that most of the cells were in the same stage during sampling. The information was also added in the results section as "Samples were collected for cell counts, Chl-a biomass, and elemental components, including particulate organic carbon (POC), particulate inorganic carbon (PIC), particulate organic nitrogen (PON), and particulate organic phosphorus (POP), starting 2 hours after the beginning of the light incubation phase and finishing within 2 hours for all the experimental treatments". In addition, the cells were examined under the microscope; there were no significantly enlarged cells in division observed, even at the lowest temperature. Therefore, the results of the elemental compositions presented in our study are comparable among different treatments.

ADDITIONAL COMMENTS: "Page 2 Line 1: the "each" could probably be removed." The word "each" has been removed.

“Page 2 Line 1: perhaps remove “cellular” because PIC is extracellular.” The word “cellular” has been removed.

“Page 2 Line 2: “implications for coccolithophore biogeochemistry”. This is a rather vague formulation. What is coccolithophore biogeochemistry? Do you mean the influence coccolithophores have on biogeochemical cycles? I think a bit more precision would improve the final sentence? Do you mean their influence on the nutrient cycle? Carbon export?” The text has been revised to “. . .with wide-reaching implications for coccolithophore related marine biogeochemical cycles...”.

“Section 2.1 provides a thorough description of the culturing methodology. One crucial information should be added, however. Were all samples taken at the same time (within an appropriate time window, e.g. ∼2 hours)? This is important because cell quotas change over the day and these can only be compared when all treatments were in the same cell cycle state when sampled (see also MAJOR COMMENT).” All the samples from each manipulation experiments were collected in a similar and appropriate time window. This description has been added in the results section as stated above.

“Page 6 Line 5: Agreed but a reference for this statement would probably be useful.” A reference has been added.

“Page 12 Line 10: “with lowest nitrate and phosphate concentrations of 3.6 and 0.4 μM, respectively”. In this case, your results may not really be comparable to Paasche’s and others. Your nutrient concentrations were not leading to zero growth whereas those of Paasche et al were.” The authors agree that the lowest nutrient concentrations were not as low (leading to zero) as those in the cited references. However, here we made the comparisons only to point out the difference of the results we observed in our study and those under nutrient depleted conditions. It is stated in the manuscript that “the present study used a semi-continuous incubation method with higher and relatively steady nutrient concentrations (with lowest nitrate and phosphate concentrations of 3.6 and 0.4 μM, respectively) and the cells were grown and sampled at a healthy exponential growth phase". And thus "further studies at extremely low nutrient concentrations (<0.1 μM) in a steady-state growth phase are still needed to understand the potential connection between carbon production and extreme nutrient limitation".

"Page 13 Line 26: check spelling of 'cell'." The original typo has been corrected.

"Page 13 Line 26: It is unclear in this sentence whether you measured cell size or you refer to earlier results. Please clarify." The new supplemental figure (Fig. S1) has been added in order to provide the cell size information from the temperature experiments.

"Page 15 Line 1: This final speculation in the temperature section is a bit too extreme. It became clearer during the last couple of years that extrapolations from the (monoclonal) bottle to the global ocean should be avoided since way too many factors (e.g. ecology) are neglected." The last sentence has been revised to "Similarly, Toseland et al. (2013) suggested that future warming might accentuate nitrate limitation in the oceans" to avoid over extrapolations from our bottle incubation experiments.

"Page 15 Line 9: 'In general, cell growth of E. huxleyi is less limited by low CO2 concentrations than in other phytoplankton groups (Clark and Flynn, 2000; Paasche et al., 1996; Riebesell et al., 2000a).' This statement implies that E. huxleyi would have a particularly efficient CCM but is this supported by the evidence provided in the cited references? I suggest to check the MIMS-based papers by for example Björn Rost's group because these provide K1/2 values for carbon uptake and they have investigated quite a number of different species that can be compared with E. huxleyi." The reference of Rost et al. (2003) that examined the K1/2 values for carbon uptake of several phytoplankton species is now cited in the revised manuscript.

"Page 15 Line 27: I do not understand where the 'both' is referring to." The word "both" refers to the two parameters 1. cellular PIC:POC ratio in the present manuscript and 2. the ratio of calcification rate vs. photosynthesis rate in Feng et al. (2017) being commonly used in research papers to indicate the relative change of PIC vs. POC production in coccolithophores, and thus they have implications for the marine rain ratio.

“Page 15 Line 27: ‘ecological implications’? Do you mean ‘biogeochemical implications’?” The word “ecological” has been revised to “biogeochemical”.

“Page 16 Line 2: Confusion: The 14C measurement is not referring to your study, or is it? 14C measurements have not been described in the methods or did I miss something?” The paper Feng et al. (2017) is now referred to in the text.

“Page 16 Line 5: Reference missing in the reference list. (Please check the entire list since there some others missing as well).” The missing references are thoroughly checked and added in the reference list.

“Page 16 Line 25: Semicolon” The semicolon has been changed to comma.

“Page 17 Line 16: ‘. . .future research on a full environmental matrix is still necessary.’ It would be valuable to add that the goal of such a matrix should not be to simply combine different factors and then use the outcome to extrapolate it to the future. The goal of culture studies should be to understand the underlying mechanisms of synergistic effects. For example: ‘How does the light intensity modify the temperature response and why?’ ” The goal of these research on full environmental matrix has been added in the revised manuscript as: “These experiments will not only help to further explore the potential interactions (i.e. synergistic or agnostic effects) between environmental drivers, but also provide a better understanding of the underlying mechanisms of these interactive effects”.

“Page 18 Lines 14-17: I am not so sure about the final conclusion and the concomitant suggestion. If we design experiments to mimic anticipated physico-chemical conditions of the future as close as possible than the results can in most cases only be used to project findings from a culture experiment to the global ocean in a one to one manner. This, however, is questionable since many factors in that can significantly modify the outcomes are neglected in the experiment. Perhaps it may be more sustainable to

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suggest that experimentalists should design experiments in such a way that underlying mechanisms for synergistic effects can be understood." The authors agree that there are many factors in the oceanic environments neglected in our experiment. However, it is a general limitation of laboratory manipulation experiments. Our manipulation experiments focused on the single driver effects, which provide some helpful diagnostic information for further explaining the interactive effects of multiple drivers. As such, the final conclusions have been further extended as: "For future multi-factorial manipulation experimental designs, our results suggest that the magnitudes of change in each environmental driver need to be determined/decided cautiously and should have environmental relevance in order to make more accurate predictions, and the understanding of interactive effects of multiple environmental drivers and the underlying mechanisms should be further explored.".

"Figure 7: Perhaps rather call it conceptual figure. Furthermore, were abbreviations "Q" defined in the text?" The figure legend has been changed to "conceptual figure", and the abbreviation of "Q" has also been defined as cellular quota.

We look forward to hearing back from you again. Thank you very much.

Sincerely, Yuanyuan Feng and the coauthors

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2017-332/bg-2017-332-AC2-supplement.pdf

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-332, 2017.

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