

Dear Editor,

We thank the referees for their supportive and constructive comments to our manuscript. We have responded to the comments point by point as follows. The revised sentences or contents are underlined.

Responses to comment 1 are following:

General comments:

The manuscript is clearly written and structured. The study is well designed and explained considering how complex experiments tend to get when increasing the number of environmental drivers. Also, the rationale behind using a “environmental clustering” approach is clearly stated and the co-variation of the environmental drivers is well deduced from previous studies and literature. It is interesting to see in this study how much variation in response to nutrient availability and light there is, even though there are clear differences in PIC, POC quota and growth between ambient and future scenarios. These differences are put into context and discussed well in the manuscript. There is however, one critical point that I think is not well discussed and needs to be considered in the discussion. In the beginning of the discussion, the Authors make the comparison between ocean observations of Coccolithophores and try to highlight discrepancies to the lab experiment. While these comparisons are nearly impossible, because laboratory conditions are so different from natural conditions, I feel that this point merits further discussion. Environmental variation due to different geographical regions affects the environmental history an organism has experienced and thus how an organism can and will react to changes in the environment. In addition within species and within functional group variation in plastic responses of growth and other traits is well established (other publications by Zhang et al have already shown this as well) and can affect how “a species” responds to environmental change. Since the Authors want to make a conclusion what their study tells us about how a cosmopolitan and biogeochemically relevant group of phytoplankton react to future ocean scenarios and not only conclude something about the combination and co-variation of environmental drivers, I feel that ecological variability and environmental variability should be taken into account in the discussion.

One way to approach this “gap” of knowledge could be a discussion about what experimental conditions (based on the given study) could now be focused on to further characterize the responses of other coccolithophore strains that i) come from environmentally different regions, ii) are more recently isolated and thus not acclimatized to long times spent in the laboratory.

Response: We agree with the suggestions from the referee, and have revised the discussion and added related analysis on this aspect with further references to extra literatures at lines 543–558: ‘Different *E. huxleyi* strains displayed optimal responses to a broad range of temperature or CO<sub>2</sub> level, and *E. huxleyi* strains isolated from different regions showed local adaptation to temperature or CO<sub>2</sub> level (Zhang et al., 2014; 2018). Strain-specific responses of growth, POC and PIC production rates in *E. huxleyi* isolated from different regions to changing seawater carbonate chemistry have also been documented (Langer et al., 2009). It has been suggested that inter-strain genetic variability has greater potential to induce larger phenotypic differences than the phenotypic plasticity of a single strain cultured under a broad range of variable environmental conditions (Blanco-Ameijeiras et al., 2016). On the other hand, the genetic adaptation to culture experimental conditions over time may no longer accurately represent the cells in the sea, as reflected in a diatom (Guan and Gao, 2008). Phytoplankton species that had been maintained under laboratory conditions might have lost original traits and display different responses to environmental changes (Lakeman et al., 2009). The strain used in this study has been kept in the laboratory for about 30 years, and the data obtained in this work can hardly reflect relation to its biogeographic origin.’

Specific comments:

Line 120: here the Authors imply, that their study will help understand how biogeochemically relevant phytoplankton change in future climate change scenarios but this is not adequately discussed later on (see general comments).

Reponse: We have revised this part as indicated at lines 535–538: ‘We have to admit that results from laboratory experiments can hardly extrapolate to natural conditions. Nevertheless, our data provide mechanistic understanding of the combined effects of ocean climate change drivers, which can be useful in analyzing field observations.’

Line 161: “adding low light” is misleading. Would it be possible to say that light was reduced?

Response: Agree. We have reworded the words, and changed ‘added’ to ‘supplied’ at lines 163

-166.

Line 151 and 161: it would be good to have an idea about nutrient and light concentrations here already. The information following in line 190 comes a bit late and could even be combined as later on the pCO<sub>2</sub> manipulation is in focus.

Response: We added one sentence: 'Initial DIN and DIP concentration were 24  $\mu\text{mol L}^{-1}$  and 1.5  $\mu\text{mol L}^{-1}$ , respectively, and initial light intensity was 60  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ .' in lines 154–155.

Line 175: I do not fully follow the rationale behind adding the nutrient limitation stepwise

Response: we added "Such stepwise reduction of nutrients levels would be useful for us to analyze effects of nitrate and phosphate separately, and be expected to have implications for the cells episodically exposed to different levels of nutrients in the sea." in lines 180–183.

PIC quota Line 434 ff: I stumbled over the way that the effect of future ocean scenarios are increasing PIC quotas followed by the explanation of how PIC is reduced with increasing pCO<sub>2</sub>. It would be helpful if there was one more sentence that relates the different results. In addition it could be helpful to highlight in Fig. 2-6, what parts of all of the results are used for the ambient-future comparison. Then the in-between data that are very interesting could become more clear.

Response: We added one sentence: 'However, the opposite results were found under the elevated CO<sub>2</sub> treatment alone.' in lines 443–444. We added these contents 'The results shown in the black column were used for the ambient-future comparison in figure 2' in figure legends of figures 3–6.

Line 531: Please see the general comment: here the discussion should go further because not only oceanic conditions may be different but ecologically within species and functional groups there are many differences that can affect the results.

Response: We agree with the suggestions of this referee. Please see the response to general comment 1, we have revised the discussion and added related analysis on this aspect with further

references to extra literatures (lines 543–558).

Line 612 ff.: I see how considering TEP as part of POC quota is important. But then the Authors also say that it is negligible. As it is written currently, the two sentences contradict each other a bit. Consider rephrasing.

Response: We have deleted these contents 'However, released organic compounds should be negligible, since they are usually photorespiration-dependent (Beardall, 1989; Obata et al., 2013)' after line 663.

Line 620ff: consider moving this part of the discussion about RNA and protein metabolism to where cell cycle is already discussed in line 580. Could fit better together.

Response: Agree. We have moved the contents of the discussion about RNA and protein metabolism to where cell cycle is discussed in lines 613–626.

Line 643: The conclusion about competitive ability comes a bit "sudden". Consider mentioning the implications of nutrient uptake on competitive ability earlier in the discussion where phosphate and nutrient uptake related changes are discussed.

Response: Agree. We have move the contents 'While substantial evolutionary responses to multiple drivers may help further, our results imply that decreased phosphate availability along with progressive ocean acidification and warming in surface ocean may reduce the competitive capability of *E. huxleyi* in oligotrophic waters.' to where phosphate uptake is discussed in lines 640–643.

Technical/language comments:

Line 234: "taken" should be "took"

Response: Thanks. 'taken' is changed to 'took' in line 239.

Line 571: should say nutrient-replete

Response: Thanks. 'nutrient-replicate' is changed to 'nutrient-replete' in line 598.

Line 598: On the other hand to what? Please rephrase

Response: ‘On the other hand’ is changed to ‘Meanwhile’ in line 643.

Line 620: type: “a” is missing

Response: Thanks. ‘a’ is added in line 613.

Fig. 1: Please indicate in the legend that experimental steps were done in a consecutive manner.

Also this might be helpful to mention again in Fig S1. Visually this implies that the steps are done in parallel, but in the methodological description they are explained as being done one after another.

Response: Thanks. We added these contents ‘Experimental steps were done in a consecutive manner.’ in lines 1003–1004, and in lines 1066–1067.

Responses to comment 2 are following:

1. The authors refer the manipulated conditions as “future conditions” in the discussion. Therefore, it would be better to justify why these environmental conditions represent the future global change scenario. For example, the irradiance levels and the nutrient concentrations set up for the experiment are not within the ranges listed in Table S1. The physiological response of *E. huxleyi* would be different under different levels of environmental conditions (i.e. irradiance and nutrient). How will the results of this study be extrapolated to the future global change scenario?

**Response:** Under the LNLP condition, initial DIN concentration was  $8 \mu\text{mol L}^{-1}$  and initial DIP concentration was about  $0.5 \mu\text{mol L}^{-1}$ . During the incubation, DIN and DIP concentrations reduced to about  $2.7 \mu\text{mol L}^{-1}$  and  $0.1 \mu\text{mol L}^{-1}$ , respectively, at the end of the incubation (Table 2). DIN and DIP were  $0\text{--}4.9 \mu\text{mol L}^{-1}$  and  $0.1\text{--}0.3 \mu\text{mol L}^{-1}$ , respectively, under the future conditions (Table S1). So, nutrient concentrations were within the ranges listed in Table S1 at the end of the incubation where cell concentration, cellular POC and PIC quotas were measured. In addition, high light intensity was  $240 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  during the cultures, and was also within the ranges of irradiance under the future conditions where irradiance was  $156\text{--}455 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (Table S1).

We agree that the physiological response of *E. huxleyi* would be different under different levels of environmental conditions. ‘We have to admit that results from laboratory experiments can hardly extrapolate to natural conditions. Nevertheless, our data provide mechanistic understanding of the combined effects of ocean climate change drivers, which can be useful in analyzing field observations.’ These contents were added in lines 535–538.

2. The coccolithophore *Emiliania huxleyi* is a cosmopolitan species. Previous studies have shown strain-specific responses of *E. huxleyi* to environmental changes (especially ocean acidification). I would suggest the authors to expand the discussion on Table 5 a little further.

**Response:** As mentioned in response to general comment 1, strain-specific responses in growth rate, POC and PIC production rates of *E. huxleyi* to a range of  $\text{CO}_2$  or temperature have been reported by Langer et al. (2009) and Zhang et al. (2014; 2018). In addition, Blanco-Ameijeiras et al. (2016) examined variability in cellular contents of POC and PIC, magnesium (Mg) and strontium (Sr) of 13 *E. huxleyi* strains under identical culture conditions. We added related analysis on this aspect with further references to extra literatures in lines 543–558.

Some other specific comments:

Lines 163: “low nitrogen was added: : :” I don’t think this is a correct expression of introducing low nitrate concentration. Could the authors also specify how the nitrate concentration was reduced? The same for line 164, “low phosphate was added..”.

**Response:** Agree. We changed ‘added’ to ‘supplied’ in lines 163–166.

Line 269: The cell diameter was measured for the whole coccospHERE, with coccoliths attached. However, both PIC quota and PIC/POC ratio was changed by different experimental manipulations, especially by alteration of pCO<sub>2</sub>. This would have also resulted in changes in coccolith thickness. I was wondering if the authors have considered this when calculating the cell-volume normalized particulate organic elemental quotas.

Response: We agree with the suggestions of this referee. We have calculated the cell-volume normalized POC and PIC quotas in figures S6 and S7. POC (or PIC) quota and the cell-volume normalized POC (or PIC) quota showed similar trends in response to different environmental conditions (Figures 4; 5; S6; S7). We added 'and the cell-volume normalized POC quotas' in line 650.

Lines 527-531: This sentence is too long, please split to two.

Response: This sentence was reduced to 'Our results from laboratory experiments with multiple drivers experiment instead predicted a different trend with progressive ocean climate changes.' in lines 533–535.

Line 556: "low-pH inhibited growth.." Here the authors indicate it was mainly the effects of pH instead of changing pCO<sub>2</sub>, please add some explanations on this.

Response: We added these contents 'In ocean acidification condition, the negative effect of low pH on growth rate of the same *E. huxleyi* strain PML B92/11 was larger than the positive effect of high CO<sub>2</sub> concentration (Bach et al., 2011). Our data further showed that low-pH inhibited growth to lesser extent under the high light than under low light (Fig. 3e; Table 2).' in lines 579–583.

Bach, L. T., Riebesell, U., and Schulz, K. G.: Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliania huxleyi*, Limnol. Oceanogr., 56, 2040–2050, doi: 10.4319/lo.2011.56.6.2040, 2011.

Line 559: Please add a reference after the sentence "photosynthesis under the high light regime could generate more energy-conserving compounds".

Response: Fernández et al. (1996) reported that high light intensity facilitates carbohydrate accumulation and low light intensity reduces cellular carbohydrate content. So we cited this reference in line 585.

Fernández, E., Fritz, J. J., and Balch, W. M.: Chemical composition of the coccolithophorid *Emiliania huxleyi* under light-limited steady state growth. J. Exp. Mar. Biol. Ecol., 207, 149–160. doi: 10.1016/S0022-0981(96)02657-3, 1996.

Line 561: Please specify the strain of the *E. huxleyi* examined in Jin et al., as well as in line 569.

Response: Jin et al. (2017) examine responses of growth rate, POC and PIC quotas of *E. huxleyi* strains PML B92/11 and CCMP 2090 under different levels of incident solar radiation. '*E. huxleyi*' was replaced by '*E. huxleyi* strains PML B92/11 and CCMP 2090' in line 587, and by '*E. huxleyi* strain PML B92/11' in line 596.

Line 575: Why was PIC quota increased under high light? Please add some explanations.

Response: One explanation could be that high light intensity makes cells to remove H<sup>+</sup> faster and then reduce the negative effects of low pH on calcification of *E. huxleyi* (Jin et al., 2017). These contents 'increased light levels can partially counteract the negative effects of OA on calcification' were changed to 'high light intensity could make cells to remove H<sup>+</sup> faster and then reduce the negative effects of low pH on calcification of *E. huxleyi* (Jin et al., 2017)' in lines 603–604.

Line 617: The sentence "released organic compounds should be negligible: : :" contradicts to the previous expression of "over-synthesis of cellular organic carbon might be released as dissolved organic carbon.." in lines 612-613.

Response: Thanks. We have deleted these contents 'However, released organic compounds should be negligible, since they are usually photorespiration-dependent (Beardall, 1989; Obata et al., 2013)' after line 663.

Fig. 1 Please label the symbols in the graph for a better understanding of the treatments.

Response: Thanks. We have done.

Fig. S1 I think it would be better to move this figure to the main manuscript, instead of being in the supplementary materials, in order to make a better understanding of the step-wise experimental design.

Response: We try to move the figure S1 to the main manuscript, whereas we find that figure S1 and figure 1 seem to repeat in terms of the experiment setup. So we would like to keep the figure S1 in the supplemental information. If the referee persists in, we will do it.

Fig. S11 How was the RNA concentration measured? This is not presented in the methods section.

Response: The sentence: 'In this study, RNA content per cell was verified by a SYBR Green method (Berdalet et al., 2005).' is added in line 614–615.

Berdalet, E., Roldán, C., Olivar, M. P., and Lysnes, K.: Quantifying RNA and DNA in planktonic organisms with SYBR Green II and nucleases. Part A. Optimisation of the assay, Sci. Mar., 69, 1–16, doi: 10.3989/scimar.2005.69n11, 2005.