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Interactive comment on "Ocean-related global change alters lipid biomarker production in common marine phytoplankton" *by* Rong Bi et al.

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Responses to comments from Reviewer 1

General Comment 1

The authors of this paper investigate experimentally the effects of changes in multiple environmental parameters on the production of phytoplanktonic biomarkers including, PUFAs, sterols, and alkenones in 3 individual species of diatom, cryptophyte, and haptyophyte. These parameters included different temperatures, molar ratios of N:P, and pCO2 concentrations. This manuscript provides valuable insights for the impacts of lipid remodeling on food web dynamics and biogeochemical cycling, particularly in sterol production. The discussion however is lacking in the specific potential effects

of increased FA production and alkenoates on modern biogeochemical dynamics and could be expanded on.

Response:

Fatty acid (FA) composition and contents in phytoplankton have shown significant correlations with zooplankton production and trophic carbon transfer from phytoplankton to zooplankton (Jónasdóttir et al., 2009; Amin et al., 2011; Rossoll et al., 2012; Arndt and Sommer, 2014; Bi and Sommer, 2020). These impacts of FA production remodeling have been discussed in detail in our previous studies (Bi et al., 2014; Bi et al., 2018; Bi and Sommer, 2020). As suggested, we will add some discussion on the potential effects of FA production changes in the revised manuscript.

We think the reviewer referred to the potential effects of "alkenones", instead of "alkenoates" which were not measured in our study. E. huxleyi is mainly grazed by heterotrophic protists in the context of pelagic food webs (Braeckman et al. 2018; Nejstgaard et al. 1997). Moreover, previous studies on copepod feeding clearly showed excretion of alkenones and indicated faecal pellet transport of these compound to sediments. In the present study, we observed non-significant changes in organic carbon-normalized contents of total alkenones in E. huxleyi under variable temperature, N:P ratios and pCO2, demonstrating quantitative application of alkenones as proxies for E. huxleyi biomass in biogeochemical cycles. We will expand the discussion on the potential effects of alkenones in the revised text.

General Comment 2

The authors have a sound experimental design to investigate the effects of multiple environmental drivers however much of the discussion focuses on the impacts of individual variables on the production of each respective biomarker. Greater exploration of the potentially compounding effects of multiple environmental drivers may increase the impact of the manuscript. The interpretations from the PCA (Fig. 3) are hardly mentioned in the discussion and would be worth expanding on as well.

Response:

We accept the reviewer's comment on expanding the interactive effects of multiple environmental drivers. Because non-significant interactive effects were observed on the contents of sterols and alkenones in this study, we will add some discussion on the potential interactions between temperature and N:P supply ratios on carbon-normalized contents of brassicasterol/epi-brassicasterol in E. huxleyi.

We will interpret the PCA results in more detail and highlight specific physiological functions and biosynthetic pathways of three lipid biomarkers. For example, the PCA results interpreted that the responses of TFA carbon-normalized contents to N:P supply ratio were opposite to that of brassicasterol/epi-brassicasterol and alkenones (Fig. 3; Table S5), e.g., strong positive correlations of TFAs with N:P ratios in P. tricornutum and Rhodomonas sp., but negative ones of brassicasterol/epi-brassicasterol in E. huxleyi.

General Comment 3

Greater connection to the interpretation of these the sterols in sediments could be provided by the authors as well. How might these results specifically influence the interpretation of biomarker analysis in the reconstruction of phytoplankton productivity and community composition?

Response:

As suggested, we will provide some interpretations of sterol changes in sediments. Briefly, our study revealed an overall 20% decrease in carbon-normalized contents of brassicasterol/epi-brassicasterol in Rhodomonas sp. and E. huxleyi in ocean-related global change scenarios (warming, N and P deficiency and enhanced pCO2), but not in the diatom P. tricornutum. Because smaller ranges are expected in temperature, N:P supply ratio and pCO2 in individual locations over time, our results provide additional support for the applicability of using sterols in paleoproductivity reconstruction, especially in diatom-dominated areas.

Specific Comment 1

How comparable are carbon or cell normalized concentrations when sterols are often interpreted as biomarkers for productivity and normalized to dry sediment weight? Would this affect paleo interpretations of community structure or productivity?

Response:

In paleoceanography, lipid biomarker data have been reported on the basis of both dry sediment weight and total organic carbon (TOC) content (Zimmerman and Canuel, 2002; Zhao et al., 2006; Xing et al., 2016). Dry sediments contain variable organic and inorganic components, thus biomarker contents normalized to TOC can partially eliminate the influence of sedimentation rates, and between-site or temporal changes in the amount of organic matter deposited or preservation conditions (Zimmerman and Canuel, 2002). The application of sediment biomarker contents for paleoproductivity reconstruction is based on two key assumptions: 1) relatively constant ratios of biomarker per cell or per particulate organic carbon (POC) in a specific phytoplankton group, and 2) non-significant changes in biomarker/POC ratios during post production and deposition degradation.

In laboratory culture studies, carbon-normalized and cell-normalized lipid contents provide broadly similar responses to environmental parameter changes in most cases, but there are exceptions (Ding et al., 2019; this study). However, cell-normalization is difficult for sediment reconstruction, as phytoplankton cell counting is often not quantitative (Piepho et al., 2010; Ahmed and Schenk, 2017). In the present study, we have discussed the implications of our findings in ecology and biogeochemistry based on carbon-normalized contents of lipid biomarkers. Because the variations of temperature, N:P ratios and pCO2 in certain locations over time are smaller than that in this study, carbon-normalized contents of lipid biomarkers can to some extent reflect paleoproductivity changes.

We accept the reviewer's comment and will expand our discussion based on the above

interpretation.

Specific Comment 2

In Fig.3, is pCO2 contributing to the distribution of fatty acids in P. triconutum?

Response:

PCA results show that both Dim1 and Dim 2 loadings for pCO2 are very low (0.185 and 0.151, respectively). Thus, the effects of pCO2 on lipid biomarkers were weaker than temperature and N:P supply ratios. Generally, pCO2 showed a stronger correlation with TFAs in E. huxleyi along Dim2, while the contribution of pCO2 to TFAs in P. tricornutum was weak.

We will add more interpretation on the PCA results to clarify the differential roles of temperature, N:P supply ratios and pCO2 on lipid biomarkers.

Specific Comment 3

How does the DIC and pH compare amongst cultures? Are they consistent across the temperature and nutrient trials?

Response:

DIC and pH were monitored during the experiments, and they did not differ substantially across different temperature and N:P ratio treatments in each experimental run in our study. We will clarify this information in the Methods, and add pH values in Table S1 in the revised manuscript.

Specific Comment 4

The prediction of an overall decrease in carbon-normalized contents of sterols and PUFA is based on future open ocean or coastal conditions? The authors suggest that the open ocean may experience more thermal stratification and thus depleted nutrients while the coastal ocean may receive more external inputs of nutrients.

Response:

Surface inorganic N and P concentrations are highly depleted throughout much of the low-latitude oceans (Moore et al., 2013). Nutrient availability in future will be altered by increasing external nutrient inputs, changes in surface ocean chemistry driven by anthropogenic increases in atmospheric CO2, and changes in ocean circulation (Moore et al., 2013). Therefore, inorganic N:P ratios show a strong spatial variation in the oceans, e.g., a low ratio of 6:1 mol mol-1 in the center of the South Pacific Gyre versus a global scenario of an increase driven by the high N:P atmospheric deposition of $\sim$ 370:1 mol mol-1 (Bonnet et al., 2008; Peñuelas et al., 2012).

In our study, N:P supply ratios cover large-scale spatial patterns of nutrient status, and thus our prediction of lipid biomarker production changes is based on both future open ocean and coastal conditions. This information will be clarified in the revised manuscript.

Technical Corrections 1

Missing clear information on the preparation of the fatty acids and their measurement. Please provide details of methodology including quantification. Are only polyunsaturated fatty acids identified and measured or total fatty acids? Please clarify or include citation to previous work in methods section.

Response:

Samples for fatty acids (FAs) were taken on pre-combusted and hydrochloric acid-treated GF/F filters. FAs were measured as fatty acid methyl esters (FAMEs) using a gas chromatograph according to the procedure in Arndt and Sommer (2014). Briefly, 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 μL. Sample aliquots (1 μL) were given into the GC with hydrogen as the carrier gas. Individual FAs were identified with reference to the standards (Supelco 37 component FAME

mixture and Supelco Menhaden fish oil) and integrated using Chromcard software. All FA components, including saturated, monounsaturated and polyunsaturated FAs were identified and measured.

The methods of FA analysis will be clarified in the method section.

Technical Corrections 2

What N:P was used for the pCO2 experiments, 24:1? Please clarify.

Response:

The experiments were designed as shown in the following figure (Fig. 1). It can clearly show that three N:P supply ratios and three temperatures were set for each pCO2 level. If needed, we will add this figure as a supplementary material in the revised manuscript.

Technical Corrections 3

Consider switching the order of sections 4.2 and 4.3.

Response:

In the manuscript, we consistently present the findings of sterols followed by alkenones in all sections including abstract, introduction, methods, results, discussion and conclusion.

We thank the reviewer for this comment. While the order of sections 4.2 and 4.3 could be switched, we prefer to keep the consistent order in all sections. We hope the reviewer will agree with this.

Responses to comments from Reviewer 2

General comment

The manuscript by Bi et al. describes the response of three species of phytoplankton to a combination of three parameters relevant for the adaptation of algae to future

climate change. The highlights of the manuscript are the multifactorial nature of the experiments and their meticulous execution, including a full account of carbonate system parameters. That being said, the manuscript is a little lean on the methods section such as details of the cultivation. I have only minor comments on the manuscript and believe that all conclusions are sound, but I have the feeling that major opportunities to derive novel and far-reaching insights were missed. For instance, the discussion could be more specific on the geological implications of changing alkenone ratios and sterol compositions and abundance. Similarly, the impacts on the food web (past and present) could be discussed in more detail. I was also expecting an analysis of the caloric content or of energy storage molecules (e.g. triacylglycerols), which could be a great addition to the study.

Response:

We thank the reviewer for the positive comments on our paper and suggestions for improvements.

1) Previous studies showed that C37/C38 alkenone ratios not only varied with temperature and physiological stages (such as growth stage), but also differed between alkenone-producing species (Conte et al., 1998; Pan and Sun, 2011; Nakamura et al., 2014). We observed that C37/C38 alkenone ratios varied significantly with the changes in temperature, N:P supply ratios and pCO2, indicating that besides temperature, other environmental factors such as nutrient availability and pCO2 can also significantly influence C37/C38 alkenone ratios. In contrast, C38 Et/Me ratios in our study responded significantly only to temperature changes. Our results suggest the importance to consider the effects of multiple environmental factors on C37/C38 alkenone ratios, and further strengthen the importance of temperature in regulating C38 Et/Me ratios in geological application of alkenone ratios.

Considering the implications of sterol contents, we will add discussion based on the differential responses of diatoms with other two species, i.e., non-significant changes in

carbon-normalized contents of brassicasterol/epi-brassicasterol in the diatom P. tricornutum, but significant decreases in brassicasterol/epi-brassicasterol in Rhodomonas sp. and E. huxleyi under ocean-related global change scenarios (warming, N and P deficiency and enhanced pCO2). Because smaller ranges are expected in temperature, N:P supply ratio and pCO2 in individual locations over time, our results imply applicability of sterols in paleoproductivity reconstruction, especially in diatom-dominated areas.

Based on these important applications of alkenone ratios and sterols, we will expand the discussion on geological implications of these lipid biomarkers.

2) We accept the review's comment and will add discussion on the impacts of changing lipid biomarker contents on food webs. Both FA and sterols are important for zooplankton production. For example, we can speculate that an increase in PUFA contents in the diatoms in cold period may have positive effects on the production of higher trophic levels; in contrast, a decrease in PUFA contents in E. huxleyi at enhanced pCO2 may reduce trophic transfer efficiency in food webs.

3) We thank the reviewer for this important comment. We agree that analysis of caloric content or of energy storage molecules would be a great addition for our study, but such analysis is beyond the scope of the present work and the methods. We will revise the conclusion and suggest future studies to consider the influence of these and other environmental changes on other energy storage molecules such as triacylglycerols.

Line comment 1:

Line 65-70: It would be useful to describe the directionalities of these changes. Were these changes significant so that they prompted the present study?

Response:

Yes, some of previous studies presented significant changes in phytoplankton sterol contents. We will describe the directionalities of these changes.

Line comment 2:

Line 102-139: Were the culture axenic? If not, how was bacterial growth controlled?

Response:

The algal cultures were non-axenic. We did not control bacterial growth. Because the biomass of bacterial was very low, the bacterial influence on phytoplankton was negligible.

Line comment 3:

Line 103: Could more information be supplied for the identity of the Rhodomonas strain such as a strain number and/or statements on deposition in a culture collection?

Response:

This strain was isolated from the Kiel Bight and identified by the working group at GEOMAR. But it has not been deposited in a culture collection, thus no a strain number. However, it is maintained and still available from GEOMAR for laboratory culture.

Line comment 4:

Line 117-132: It is not clear how target pCO2 was maintained in the semi-continuous cultures.

Response:

As suggested, detail information on the maintenance of target pCO2 in semi-continuous cultures will be added. Briefly, renewal of the cultures was carried out at the same hour every day using fresh filtered seawater preacclimated to target pCO2, and CO2-enriched water.

Line comment 5:

Line 134: From the description it is not quite clear why these cultures are considered semi-continuous. Was a large volume of sample withdrawn directly from the reactor?

How much volume was taken and what was the sampling interval? After what interval (turnovers?) was the culture considered to be back in steady state?

Response:

The renewal volumes were about 10% of the total culture volume (100 – 200 mL out of 920 mL). Renewal interval was 1 d. Steady state was reached at ca. 10 d with slight difference between the individual cultures. More details will be added to clarify the setup of semi-continuous cultures.

Line comment 6:

Line 141: How were cells counted/fixed

Response:

Algal cells were counted using an improved Neubauer hemacytometer under microscope. We will describe this information clearly in the revised manuscript.

Line comment 7:

Line 153-154: What was the volume of the samples?

Response:

The filtration volume of sterol and alkenone samples was 100 – 200 mL. Only 10% of the extractions were used for GC quantification, with the rest stored for later use.

Line comment 8:

Line 278: POC is mentioned here for the first time but it was not described in the methods how it was determined.

Response:

POC and PON were measured using an elemental analyzer. We will add this information in the methods.

Line comment 9:

Line 310-313: Would this comparison change if the maximum growth rates of the batch cultures would be used for comparison? Can a stationary batch culture be reasonably compared to a continuous culture in terms of energy availability for remodeling?

Response:

Because growth rate strongly affects sterol contents according to previous studies, the comparison on lines 310-313 would change if the maximum growth rates of batch cultures were used for comparison. In a continuous culture, phytoplankton has a constant growth rate under a certain dilution rate, while the growth rate at the stationary phase of batch culture is zero. Thus, energy availability for sterol remodeling differ between the two culture systems. We will revise the paper to address this suggestion.

Line comment 10:

Line 410-412: Could this be expanded on by considering expression of lipid biosynthesis genes and/or energy storage?

Response:

Indeed, the genes related to sterol biosynthesis have been sequenced in several algal species including diatoms and cyanobacteria (Kaneko et al., 2001; Brumfield et al., 2010; Gallo et al., 2020). We will expand the discussion here by considering expression of lipid biosynthesis genes and its links to transcriptomic and metabolomic responses.

Line comment 11:

Line 417: Unclear if the “strong increase” in PUFAs is significant.

Response:

The increase in PUFA contents were significant ($p = 0.003$). We will clarify this response in the revised manuscript.

Line comment 12:

Line 810: Spell out Dims.

Response:

The word “Dims” refers to “dimensions”. We will clarify this in the legend of Fig. 3.

Line comment 13:

Figures 1-3: It would be great to add information on error bars (standard error? triplicates?) to all figure and table captions.

Response:

Error bars in Fig. 1-2 refer to standard errors, and triplicates were set for each treatment. As suggested, we will make the information clearly in all figure and table captions.

Please also note the supplement to this comment:
https://bg.copernicus.org/preprints/bg-2020-183/bg-2020-183-AC1-supplement.pdf

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2020-183, 2020.

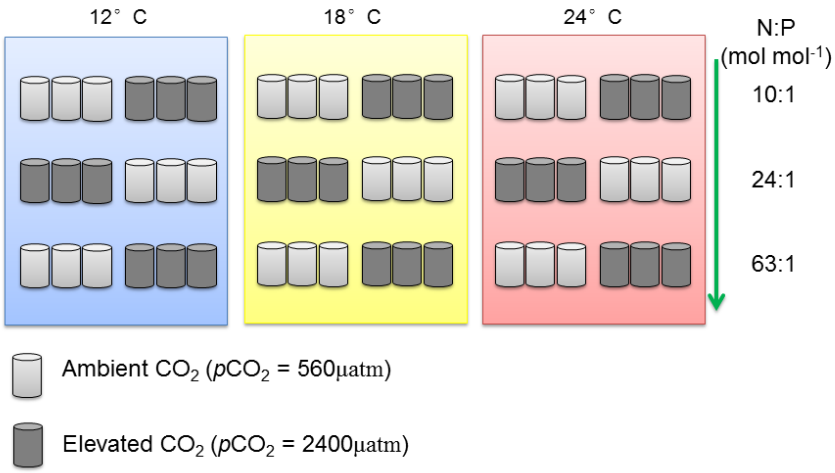

Fig. 1. Experimental setup. The three phytoplankton species were grown semi-continuously under a full-factorial combination of three temperatures, three N:P supply ratios and two pCO2 levels