

Below, please find a list of major changes of our revised manuscript entitled 'High nitrate to phosphorus regime attenuates negative effects of rising pCO₂ on total population carbon accumulation' authored by Birte Matthiessen, Sarah Lena Eggers and Sebastian Krug as well as a point by point response to each of the reviewers' comments.

Major changes:

In order to focus on the main results and streamline the paper we withdrew results on natural variations on pCO₂. Additionally we re-analysed all data and focused only on the important response variables (i.e. POC, PIC, PON, biovolume measured on both cellular and population level, as well as on cell abundance, change in cell size and left-over nitrogen) measured in the stationary phase. Abstract, results and discussion were completely rewritten and streamlined. The discussion now focuses to a large part on hypothetical different mechanisms of phosphorous acquisition triggered by nutrient regimes and its potential consequences for the population response. As in the previous version unfortunately we still cannot provide POP and DOP data which would make the discussion less speculative.

Terminology was changed such that 'net population' response was changed to 'total population' response and 'nutrient ratio' was changed into 'nutrient regime' (as recommended by reviewer #2).

Reviewer #1

Review of the manuscript 'High nitrate to phosphorus ratio attenuates negative effects of rising pCO₂ on net population carbon accumulation' by Krug et al. The authors evaluate interacting effects of ocean acidification and nutrient supply ratios. General comments: -The manuscript is well written and the experimental approach seems appropriate. It contains relevant data and contributes to the on-going controversial discussion on OA effects on calcifying phytoplankton growth.

However, I am not really convinced that the authors fully explored what can be taken from Fig 3 and the corresponding analysis, or better say there seems to be more in when changing the angle a bit. The main conclusion is that no CO₂ effects on μ_{max} are detectable at Redfield nutrient supply, but there is an increase in μ_{max} with increasing CO₂ at high N:P. This tells us that growth at Redfield is non-limited and increasing CO₂ changes something in resource use efficiency, as the authors' state. By the way, any idea what the mechanisms might be? I also agree that this increase in growth rate at high N:P attenuates negative OA effects.

The authors however point several times at the importance of stationary phase phytoplankton growth and system productivity. If we look at the final densities in Fig 3 it seems that there is a decrease in total abundance with increasing CO₂ at Redfield and an increase in total abundance at high N:P. Maybe I just missed some information on that, but I think it is necessary to talk about this result and the possible reasons for contrasting reactions on increasing CO₂.

We very much appreciate this comment regarding the buried results in Fig. 3. We completely reworked the results and discussion section and now show cell abundance in an extra graph (Fig. 2 A) and applied statistical analyses. Cell abundance significantly decreased with increasing pCO₂ in the Redfield whereas it remained constant in the high N:P regime. This in turn is the reason why the negative effect of pCO₂ on the population level (considering how many cells are in the culture) was attenuated in the high N:P regime but not in the Redfield. About the mechanism why growth in the nutrient regime was significantly different we can only speculate. However, we give detailed possible explanations in new discussion of the revised manuscript.

Regarding growth rates we decided to omit them from the manuscript. Indeed growth rates also show different responses to pCO₂ in the two nutrient regimes. However, as the full factorial statistical model with pCO₂, nutrient regime and their interaction (former Table 3) did not explain enough variance to show significant effects on growth rates we cannot justify to render results from separated analyses as significant. This was a mistake in the previous version of the manuscript.

-Aside from the usually discussed negative OA effects on calcifying issues (and the fertilising effects of increased CO₂ as shown by Iglesias-Rodriguez, et al. (2008), which was not confirmed by this study) there is also competition which seems to be influenced by increasing CO₂. *E. hux* is a 'low P specialist' and as shown in this study, is even able to increase its' P allocation efficiency. In terms of competition that means that *E. hux* might benefit from OA if competing species does not respond in the same way. That might be an additional point worth being integrated into the discussion.

Page 9: The following text has been integrated in the discussion:

*Moreover, in case that other phytoplankton species, especially other coccolithophores, are not able to increase their phosphorous allocation as *E. huxleyi* could even mean that *E. huxleyi* might have a competitive advantage in a future more acidified ocean. Whereas Riegman et al. (2000) were able to show that *E. huxleyi* is superior in terms of phosphorous allocation compared to a number of not closely related phytoplankton species this question remains to be answered for other coccolithophores.*

-One thing I do not really understand is that the authors did not analyse phosphorus. The authors state that P is important, they manipulate N and P but did only investigate N (along with C). I can see no reason other than that N comes for free with the C analysis which justifies the decision to present N but no P data (especially when studying a low P specialist). Having this lack of data in mind one can even argue that the ms might benefit from just focussing on C and excluding the whole N dataset (which would not change the main conclusion of the ms and prevent questions regarding the biological meaning of the decreasing C:N later on in the specific comments).

We absolutely agree that the missing data about POP (and DOP) is the weakest point of this manuscript. The reason for this data gap is that we lost them during analyses. However, we decided to keep data on N in the manuscript as they are an essential part for the new though highly speculative discussion on potential different mechanism of P acquisition triggered through nutrient regimes (i.e. induction of alkaline phosphatases (APase) in high N:P regime). N is required for protein biosynthesis and thus reinforce our otherwise hypothetical discussion on the mechanism behind our results.

Specific comments: -Abstract The abstract is rather long and does not read smooth.

There are lots of words which can be deleted (e.g. L17 Thereby cell size and total cell abundance was taken into account can be deleted and cell size and total abundance could be mentioned in the sentence before together with PIC and POC).

Further: L18 Corresponding to literature can be deleted. Additionally, same sentence, the results did not show any response, it was the algae.

We applied all specific comments on the abstract and rewrote it in order to make it shorter and smoother.

L11ff Rewrite -Introduction Mention somewhere that loss of DIC by primary production cannot be immediately balanced by atmospheric exchange. This would help understand why you did not supply CO₂ in the corresponding partial pressure continuously.

Page 3 (and of introduction): The following sentence was inserted:

Initial CO₂ concentrations were manipulated instead of continuously supplying the corresponding pCO₂ to match natural conditions where loss of DIC by primary production cannot be immediately balanced by atmospheric exchange.

Results: L166ff I got lost several times while reading this paragraph and had to start again. Please read it carefully and check if it can be improved.

Results were completely reanalyzed and rewritten. The text should read smoothly and much clearer now.

Fig 2B Is there a biological explanation for the decrease in C:N?

Looking at Fi 1 there is a very slight decrease in POC and a very slight increase in N, which results in the decreasing C:N. Does it mean anything?

The biological meaning of declining C:N ratio is now incorporated in the discussion about responses in the Redfield regime on page 8.

Conclusions:

Technical corrections: Throughout the text: Decide for either ':' or '/' to express ratios.

You use both right now.

In the revised version of this manuscript nutrient regimes are defined as high N:P and Redfield regime without any quotation marks.

L184 Fig 2A is Fig 2B Fig 2 Why circles and squares?

All figures are new with consistent open and closed diamonds for high N:P and Redfield regime, respectively.

-Check the reference list. The Deep Sea Research references are unclear. Deep Sea Res I or II, or even Part A?

Further give species names in italics. The words in the titles of several references are in caps. And so on. All typical errors based on downloading from different databases. Check your EndNote/ReferenceManager or whatever database.

All species names are in italics (except for the reference list according to BGS author instructions). References were corrected. However, the correct abbreviation for Deep Sea Research Part A is Deep Sea Res.

Anonymous Referee #2

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The manuscript by Krug et al. represents an interesting piece of original research, which is moreover timely and of great value for the growing ocean acidification community.

The experiments on which the manuscript is based were well done and clearly presented in terms of tables and figures. My criticism concerns the interpretation of the data and the conclusions drawn. Although I have the feeling that the latter are not essentially wrong, they are phrased in a way that makes them appear wrong. In the following I'll explain in detail what I mean.

Page 6834, lines 15-17: What do you mean by "cellular nutrient accumulation"? The reason for the higher cell densities is simply that there was initially more phosphate.

We agree that 'cellular nutrient accumulation' is confusing. What was meant is nutrient content. In the revised version the term 'nutrient accumulation' was used only when referring to total population nutrient content.

Moreover, I cannot follow the argument. It is actually the other way round; the Redfield treatment features the weaker cellular response.

We agree that wording was confusing. The abstract in the revised version has been fully rewritten and should read smoothly now.

Page 6834, lines 18-21: I don't understand that conclusion. See comments

on chapter 4.2

Please see response on comments on chapter 4.2

Page 6834, lines 24-26: How is that possible? I understand that you showed that this is impossible.

Abstract: We changed the sentence as following:

It is therefore necessary to consider both effects of nutrient limitation on cell physiology and their consequences for population size when predicting the influence of coccolithophores on atmospheric pCO₂ feedback and their function in carbon export mechanisms.

Page 6843, line 6: POP was not measured. How do you know about POP then?

As unfortunately all POP and DOP measurements from our experiment were lost, in the revised version we make clear that we make only assumptions about POP content based on what we see from PON. The discussion part was completely rewritten and now makes clear distinctions between what we see and what we assume.

Page 6843, lines 7-10: The final N-conc in the Redfield is close to the detection limit. So there's the possibility of N-limitation here. The latter is supported by Fig 2b showing higher C/N ratio in the Redfield.

We apologize for the confusion we raised. However, in this part of the text we discussed results from high N:P but not Redfield treatments. As the discussion part now is completely rewritten this issue should be clarified.

Page 6843, line 11: The "limiting resource" can only be named with confidence for the "high N/P" (see comment on lines 7-10).

See response to comment above

The "reason for higher cell abundance" is the higher initial conc of phosphate in the Redfield. Page 6843, line 19: You might as well cite Hoppe et al. (2011, JEMBE) here for a comprehensive analysis.

Though we do not exactly understand to which part of the text the reviewer refers to we think that the confusion arose from our unprecise wording. Here we did not refer to the absolute higher cell number which, as the reviewer correctly states, is higher in the Redfield regime due to higher P concentration. What we meant was that within the high N:P regime (in contrast to Redfield) cell abundance remained constant (i.e. incorrectly phrased as higher) with increasing pCO₂. These confusing sentences were clarified throughout the whole manuscript.

Page 6843, line 20: How do you know about the "cellular response to P-limitation"? You haven't got a control, have you?

Plus there are no references for the "previous studies". If you look at the literature you see that there is no uniform response. So what does it coincide with?

'Previous studies' on cellular responses to P-limitation referred to responses of cell size (i.e. increase of cells) shown by Riegman et al., 2000; Müller et al., 2008. For clarification on page 9 we moved citations of Müller and Riegman up to the sentence from which the confusion arose.

Page 6843, line 24: This is a contradiction to lines 7-10. Page 6843, lines 25/26: That's rather a more severe P-limitation than an "over-supply of N" (cells tend to get fatter under N-limitation as well).

We agree. As the discussion was completely rewritten the contradiction should be clarified.

Page 6843, lines 26-28: What does that sentence mean?

Good question!!

The text has been clarified

Page 6844, lines 7-12: It is not necessarily the nutrient ratio, because there's an N/P co-limitation in the Redfield only and the limitation is much stronger in the "high N/P". I suggest calling it "nutrient regime" instead of "nutrient ratio".

We appreciate this comment. Nutrient Ratio was changed into nutrient regime throughout the text.

Chapter 4.2: page 6844, line 24: what is a "TPC ratio"? On the chapter in general: I'm unable to follow the argument, because the consumption of DIC does tell you nothing about the shift in the system, ie pH. The latter is determined by the ratio of DIC and TA consumption which in turn is determined by the PIC/POC ratio.

We agree that this explanation was confusing. To clarify and focus on the important results, findings on natural variations in pCO₂ were omitted from the manuscript

On the whole, I believe by clarifying the above issues it should be possible to make the interpretation as good as the data.

We hope that this major revision of the manuscript improved the clarity and possible explanations of our findings.