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Discussions

Interactive comment on "Effects of ocean acidification on pelagic carbon fluxes in a mesocosm experiment" *by* K. Spilling et al.

K. Spilling et al.

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Response to review

We are grateful for all the constructive comments and suggestions, which have improved the manuscript. Below we have replied to all of the issues raised by the reviewers.

Reviewer #1, Comment #1 The main results show that elevated CO2 conditions increased total particulate carbon and the DOC pool due to a decrease in respiration and bacterial production at elevated CO2 concentrations. I think that this is a very interesting result that needs to be discussed more deeply in the manuscript. I refer the authors to Hopkinson et al. 2010 and Teira et al. 2012 for information about decreases in phyto and bacterial respiration under high CO2 concentrations. Sobrino et

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Discussion paper

al. 2014 can be also used as a reference related to downregulation of phytoplankton metabolism under high CO2, which might be an appropriate topic for the discussion of the manuscript.

Author response: A good point and we will expand the discussion on this topic relating the decrease in respiration to possible downregulation of metabolism.

Reviewer #1, Comment #2 Regarding the data analysis, I like the idea of using estimated instead raw data to make comparisons between variables or when observed values are not available. However the authors should also provide more information to complement or justify the usage of estimated vs. measured data. For example when comparing NPP14C and NPPe the authors only say that results "agree reasonably well" which is a very general contention for this paper. In addition, during Phase III, total respiration was not measured and the authors estimated TR based on the NPPe TR-1 and BP TR-1 ratios during Phase II. Information about their correlations during Phase II would be desirable to justify the estimation carried out during Phase III.

Author response: We will make changes to the estimated variables according to the suggestions of reviewer #2 (see comments below). We will be more specific when comparing different variables and also provide a better justification for the estimates of TR in Phase III. This was done using two methods as to give a range rather than specific number for the TR estimate.

Reviewer #1; Comment #3 Finally, a specific equation for the estimation of bacterial respiration would be nice to see in the Methods.

Author response: This equation will be added

Reviewer #1; Minor comments Minor issues: - Line 234 days - Line 269 correlated to?? - Line 410. Revise sentence " The initial increase in the: : :." - Line 425 during - Fig. 1 filtration - Fig. 2. What about using similar units in the Y axis and legend (i.e. uatm??)

Author response: Appropriate changes will be made

Reviewer #2, Comment #1 Although I am convinced of the scientific relevance of this study, I am not convinced considering this budgeting exercice as a separate manuscript is highly relevant. Spilling et al. under revision in this special issue already reports on decreasing respiration rates at high CO2, causing higher Chla, TPC and DOC concentrations in the high CO2 treatments. The added value of the present manuscript is to estimate plankton rates that have not been directly measured (NPPe, but see later comment on this term; GPPi, but again see later comment; BR; DOC production). I would definitely recommend merging the two Spilling et al. papers to provide a more comprehensive overview of what happened during this experiment. If this suggestion is not followed, this manuscript, in my opinion, needs major revisions in order to improve its clarity and to discuss and criticise more deeply what has been found.

Author response: We do understand this point as having one manuscript was the original idea. During the writing process, however, we decided to present the budgeting exercise on its own in order to keep a more focused paper on respiration and primary production. The present manuscript was submitted as a synthesis paper and additionally presents data from many of the other papers submitted to the special issue, including bacterial production, DOC and a budget for the DIC based on atmospheric exchange. We are confident that following the referees' comments and suggestions will considerably improve our manuscript and justify separate publication.

Reviewer #2, Comment #2 Estimates of DOC, TPC and DIC pools in mol C m-2: I was wondering for quite a while how these initial pools have been calculated and how the authors could provide an error estimate on a single sampling. I saw in the other Spilling et al. that these pools were actually averages of 3 sampling dates at the start of each phase. This must be clarified in the present manuscript. Also, how were integrated pools estimated: it is mentioned (and only for DIC, L136) in the ms that volumetric concentrations in per kg were converted using seawater density. Obviously, they were further multiplied by the considered depth. Please clarify.

Author response: The reviewer is correct, the error estimates where made from consecutive measurements, and this will be mentioned in the materials and methods and table legends (Tables 1-3). We will also add the information that the depth and area of the mesocosms were used to calculate all pools and fluxes in m-2 units.

Reviewer #2, Comment #3 Estimates of DOC, DIC and TPC rates of change: no information is provided on how these rates have been calculated. I believe these were calculated through linear regressions of each stock evolution during the considered phase. This must be clarified. Looking at Table 1 of the other Spilling et al., there are some discrepancies with rates presented here (e.g. Exp TPC of 7.4 in the first mesocosm compared to 6.6 in this paper, but this is also the case for other rates). Looking at the important errors associated with these rate estimates, it does seem like many slopes are not significantly different from 0. Please comment. In that case, how is it possible to compare these rates between the different mesocosms. Were these differences actually tested?

Author response: This is a good point. It was calculated based on the difference between the start of each period, and using the average of the first two sampling days as the initial value for each period. So they are not slopes per se. There is no statistical testing of the differences in this paper, but we have explained that this was done in the paper where the original data is presented (and here linear regressions were used e.g. Paul et al 2015).

The discrepancy between the table in this paper with the other Spilling et al. paper is that here we did not include the time before the start of the CO2 treatment (this will be changed also in the other Spilling et al paper), i.e. discarding the Exp TPC data from day T-1.

Reviewer #2, Comment #4 Estimates of NPPe and GPPi: Based on observed variations of TPC, DOC and DIC, the authors further calculated biological carbon fluxes. Net primary production measured by the 14C method (over 24h incubations) were compared to, what the authors refer to as NPPe being the missing process closing the organic budget: NPPe = Export + net variation in TPC + net variation in DOC). As the authors correctly mention, NPPe does incorporate total respiration and not only autotrophic respiration, as does the 14C method (this is actually clearly doubtful considering the long incubations that have been performed)). Anyway, this is incorrect to refer to this process as Net Primary production, this is misleading and you really should consider using the proper term: Net Community Production, and as it is based on an organic budget, you should use NCPo. The authors further use an inorganic budget (based on DIC net fluxes, and estimated CO_2 fluxes) to estimate Gross Primary Production. I would strongly recommend for clarity to reconsider this part and to calculate NCPi, being the Net Community Production based on the inorganic budget. This is, I believe, what is shown in Fig. 3 and termed as Biological release or uptake. The authors have thus two estimates of the plankton community metabolism that do provide different outputs. While it seems like the inorganic budget shows that the community was heterotrophic in ambient mesocosms (Biological release of DIC), the organic budget suggests the opposite for all phases. This must be discussed. The paper as it stands is highly confusing with respect to this metabolic aspect. i.e. In the abstract is mentioned that during phase 1, the community under ambient and high CO_2 treatments was autotrophic (i.e. more production than respiration, with capacity to export to the sediment traps and export to the DOC pool). However, it is clearly stated that the community was heterotrophic during the entire experiment under ambient CO_2 conditions. Again, this must be clarified.

Author response: We were a bit back and forth on how to best present the different variables when the manuscript was being written, and we ended up using the estimated net and gross production. The reviewer has a good point suggesting a better distinction between measured primary production and the estimated community production. We will change the NPPe to Net Community Production, organic budget (NCPo) and furthermore add the Net Community Production, inorganic budget (NCPi) as the reviewer suggests.

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Discussion paper

We will carefully go through the suggested points for clarification and discuss more in detail the discrepancy between the organic and inorganic carbon budget.

Reviewer #2, Comment #5 Comparison between the inorganic and organic budget: I already mentioned this, but I would like to insist on the fact that this paper reports on budgets based on both inorganic and organic constituents. Since they do not really agree, this must be deeply discussed. A recommendation on which type of budget is the most relevant and associated with the lowest uncertainties should be further proposed.

Author response: This comment relates to comment #4 above and our reply to that. We will make the distinction between the organic and inorganic carbon budget as suggested and expand on this in the discussion.

Reviewer #2, Comment #6 CO2 effects on estimated rates: I do not see how differences between estimated rates between low and high CO2 treatments have been tested. It is mentioned on L345 that "an effect of the different CO2 treatments was noticeable in the NPPe but not in the NPP14C", how was it tested?

Author response: It was not tested statistically, and the term 'noticeable' refers to visual inspection of the data. We will however make a statistical test to strengthen this conclusion.

Reviewer #2, Comment #7 Comparison between NPP14C and NPPe: as correctly stated by the authors, NPP14C rates should provide equal or higher estimates than NPPe (NCPo, see above). This is not the case and attributed (on top of potential errors in one control mesocosm) to "changed parameterisation during in incubation in small volumes". Based on my experience, we usually observe higher rates in small incubations vs large ones, not really in accordance with the lower rates of NPP14C during phase 1. Alternatively, this offset could be attributed to errors associated with NPPe estimates, since the TPC pool was clearly underestimated. Could you comment on this?

Author response: We do not have a good explanation for the discrepancy between NPPe and NPP14C, but underestimating of NPP14C seems more plausible as this are incubations in small volumes involving more steps than bulk measurements of TPC. Another possible explanation, suggested by the reviewer, is that the discrepancy could be due to an overestimation of NPPe. That would indicate an overestimation of either ΔTPC, ΔDOC or exported TPC (or a combination of these variables). TPC is not likely to be overestimated considering the methodology used, as measuring TPC has a relatively small uncertainty and would miss the <0.7 μm fraction. With this assumption, exported TPC would have been substantially overestimated. ΔTPC or ΔDOC would only be overestimated in the case when there is an underestimate at the start point, an overestimate at the end point or both an underestimate and overestimate increasing the difference between experimental phases in TPC or DOC. The discrepancy between NPPe and NPP14C during Phase I is so consistent for all treatments that we have hard time believing that we would have this consistent overestimation of ΔTPC or ΔDOC in all mesocosm bags.

Concerning the statement that: 'the TPC pool was clearly underestimated', we assume that you refer to the difference between the TPC pool and what was found in the bacterial and virus fraction based on flow cytometry. The small bacterial/virus not caught on the GFF filter did not contribute to the NPPe estimate. Defining the TPC as the >0.7 μm fraction, it is not obvious that TPC is underestimated.

Reviewer #2, Comment #8 Estimates of biological pools: I do not really get what is the added value of calculating and presenting pools of meso- and microzooplankton, micro- and nanophytoplankton, picophytoplankton, bacteria and viruses. I would guess that these informations are already available in other manuscripts from the special issue. Is this not the case? This makes a small paragraph of the Results and Discussion and, apart from showing that measured TPC is much much lower than the cumulated stocks of these biological compartments, I do not see what valuable information it brings.

Author response: There is no paper presenting all of the organism groups together, and this being a synthesis paper we wanted to present these different pools. We agree that this data is not well incorporated into the story and we will expand on this in the discussion, trying to better link the relative contribution of the different groups to the fluxes presented.

Reviewer #2, Comment #9 Estimates of variability: I would recommend the authors to mention the sample size each time SE are provided. Furthermore, I do not really see (as this is not explained) how SEs have been calculated for estimated rates (error propagation). e.g. as NPPe rates are based on DOC net fluxes, therefore the associated errors should be at least equal to the errors associated with DOC net fluxes right? This is not the case, and this must be clarified further.

Author response: We agree that the error estimates needs to be better explained. In the case of NPPe the SE was calculated from the square root of the sum of variance of the three parameters used to calculate the NPPe: DOC TPC and Exported TPC. We will include the sample size as suggested.

Reviewer #2, Comment #10 Minor issues: Abstract: L57: did not transfer, please correct L58: revealed a clear effect of increasing CO2 on carbon production. I don't think this is correct. Carbon production does not seem impacted, while carbon loss is.

Author response: This will be corrected.

Reviewer #2, Comment #11 Materials and Methods: L104: I understood that more mesocosms were initially deployed, I do not see why this is not mentioned here. Everyone must also know how hard such experiment is. L159: Grossart et al. (2006), please correct L185: according to: , please correct L194: Cherny et al. (2013b), please correct. L205: organic carbon pool, please add dissolved + particulate for clarity L207: Direct measurements using : : :, please correct

Author response: We will refer to the Paul et al. (2015) paper where the initial deployments are mentioned and the overall methods are described in more detail and

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Discussion paper

incorporate the suggested corrections.

Reviewer #2, Comment #12 Results and Discussion: L265: While some indication on temporal evolution is provided for the other measured variables, this is not the case for bacterial biomass, please add this information. L280: Spilling et al. 2016), please correct L286: in e.g., please remove e.g. L287: have pointed at, please correct L297: $p <= 0.01$ I believe, please correct. L325: (Paul et al. 2015 (a or b)), please correct and clarify L353: Spilling et al. 2016), please correct

Author response: This will be added and corrections will be made.

Reviewer #2, Comment #13 Figures Fig. 3. As mentioned earlier, for clarity, Biological release or uptake should be referred to as NCPi (based on the inorganic budget). Values are not in mol C m-2 but in mol C m-2 d-1 I think, please correct.

Author response: The reviewer is right and this will be corrected.

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