

## ***Interactive comment on “Ocean acidification decreases plankton respiration: evidence from a mesocosm experiment” by 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.

Dr. Neale Reviewer #1, comment #1. While the results do demonstrate decreased respiration for samples from the higher CO<sub>2</sub> enrichments, I do have some concern about how representative these rates are of processes in the mesocosms. A depth integrated water sample was taken and incubated at “ambient” temperature. But it can be seen from Paul et al (2015) that there was a strong temperature gradient over the mesocosm’s depth range, at times as much as 10\_C, so it is not clear what was

C1

“ambient” temperature. Moreover, mixing waters of differing temperatures may bias the respiration measurement at a fixed temperature vs. the “real” average, i.e. combining warm, lower particle concentration surface water with cooler, high particle (or nutrient) concentration bottom water could stimulate respiration versus the average of the two.

Author response: It is true that temperature stratification varied. We kept the incubation temperature at the surface temperature, and we will add this information to the Materials and Methods chapter. Dr. Neale makes a valid point that there might be a bias due to mixing water of different temperature rather than averaging measurements taken at different temperature. Logistical constraints prevented us from making respiration incubations at several temperatures. We will take up this potential bias in the Discussion chapter.

Reviewer #1, comment #2. The authors also indicate that respired carbon was about 10x greater than net production (pg. 17 line 7). Some more explanation is needed for why such comparison is made since a determination of whether the system is net heterotrophic or autotrophic would require comparison of gross primary production with total community respiration, as stated on page 21 line 9. The statement on page 21 line 26 implies that the authors have some idea of gross primary production, could this be compared to respiration rate?

Author response: We did try to estimate the gross primary production and after the submission of this paper we made a carbon budget for the whole experiment, which is presented in a synthesis paper (Spilling et al 2016). We will remove these statements from the present paper and place a reference to the budget paper in order to have a more clear focus.

Reviewer #1, comment #3. The authors also speculate that the net primary productivity method may not have been sensitive enough to detect difference between treatments, so that enhanced production at increased CO<sub>2</sub> was not detected. Small incubation volumes are suggested to contribute to uncertainty but the authors give no indication

C2

of what was that measurement uncertainty. Nevertheless, they state that the measurements were comparable to previous ones in the same regions using similar methods (Kivi et al. 1993) which would argue against any substantial bias. One other factor to consider as to whether the NPP assay would detect an enhancement effect was that the incubations were conducted outside the bags. According to Riebesell et al. (2013), the mesocosm material (thermoplastic polyurethane) removes all UV whereas glass scintillation vials used for the NPP incubation transmit UV-A and most UV-B so rates in the vials could have been substantially more inhibited in the near surface samples than phytoplankton in the mesocosms that were protected from UV. Moreover, some studies have shown that phytoplankton grown under CO<sub>2</sub> enhanced conditions are more sensitive to UV. It is possible that NPP was higher in the mesocosms with CO<sub>2</sub> enrichment but the effect was dampened in incubations outside the bag due to a counterbalancing increase in sensitivity to UV (see, e.g., Sobrino et al. 2008, 2009). Also, as the lead author knows (since he was co-author on the paper), Sobrino et al. (2014) observed lower rates of DOC release during short term PPR incubations by phytoplankton acclimated to CO<sub>2</sub> enhanced conditions but this effect was much less when incubations included UV. This DOC would be quite labile and rapidly respired so might not affect the bulk DOC pool but a reduction in DOC release could decrease bacterial respiration.

Author response: A very good point that we will take up in the Discussion chapter. The DOC concentration in the Baltic Sea is very high compared with most other oceans and coastal seas (like the Mediterranean that is referred to). Most of this is refractory DOC, which effectively absorbs in the UV region, and typically the depth at which 1% of UVB remains is <50 cm (e.g. Piazena and Häder 1994). UVA penetrates a little deeper and may have affected slightly the incubation platform moored at 2 m depth. We do not believe, however, that UV light have caused major inhibition of our primary production measurements (or affected labile DOC production), but we will point this out with the reasoning described above.

We will make appropriate changes to all the specific comments.

C3

#### Reviewer #2

Reviewer #2, comment #1. I have several concerns that (in my opinion) warrant further attention from the authors. I found surprising the lack of real independent mesocosms replicates. Only the controls do replicate (M1 and M5). Under these circumstances an appropriate statistical analysis cannot be performed, compromising the significance of the results. In its place, regression coefficient significance tests have been done to analyse the significance of the results. Although valid, these tests compare the mesocosms between them, but the behaviour, obviously implying variability within each specific treatment cannot then be ruled out, because without replicates is not possible to discern if the response is due to the controlled factor (CO<sub>2</sub>) or to any other uncontrolled factor, and or their interaction. At least, significance differences obtained from the R comparisons tests should be mentioned in the text adding the p values (in results section) and marked in the Figures as an asterisk or letter to indeed demonstrate that there are some differences. A table including the results of all linear regression analyses indicating the significant effects of the different CO<sub>2</sub> concentrations on the variables would be needed (see Tables and Figures in Paul et al. 2015, Crawford et al., 2015, Bermudez et al., 2015-this special issue-as examples of what I am referring to). In my opinion, in this manner the Ms would benefit of a better understanding of the results.

Author response: The mesocosm bags are relatively large scale operations, 55 m<sup>3</sup> in each enclosure, and this puts some constraints on how many units can be used. Lack of replication does not, however, prevent proper statistical analysis of results: for example gradient experiments of a single variable or factorial design experiments with multiple variables, both provide data that can be statistically tested for treatment effects (see e.g. the discussion by Oksanen 2001 and Hurlbert 2004). In our case, a gradient of different CO<sub>2</sub> additions was used. The statistical test was in the figure legend, and we will include it also in the results section as suggested by the reviewer.

Reviewer #2, comment #2. The other important issue is that you mention measure-

C4

ment uncertainties at some points. I do not understand how or why can be a measurement uncertainty working with small volumes, can you specify? How this affect reliability? Regarding the incubation time with  $^{14}\text{C}$ , I think it is widely demonstrated that this method is quite sensitive. I agree that it may be more estimative of NPP, but, in incubations long as 24h, the same  $^{14}\text{C}$  molecule can be fixed and respired several times (the eternal discussion). Do you think you could be getting an underestimate of your measurement? Said this, I think the point raised by reviewer 1 regarding the effect of UV on C fixation during incubations would be much more relevant in terms of affecting PP (not commenting on UVR as I totally agree and support reviewer 1 comments). Also said by reviewer 1, if you think there are uncertainties, how your data compare to former published studies?

Author response: There will always be measurements uncertainties (depending on the methodology, instrument etc) and this would be independent of the volume, and we are not quite sure that we got your point. Perhaps you refer to the primary production measurements. In that case the incubation volume was relatively small, and we did not remove the grazers, which could have introduced a bias with respect to grazing pressure, i.e. the number of grazers could have been quite variable depending on how many by chance got into the relatively small incubation volume. With respect to the UV point, please see our response above to reviewer #1.

Reviewer #2, comment #3. It is not clear to me whether you also mention measurement uncertainties on the TPC data, it seems so. In this regards, if there exist such an uncertainty in TPC, how this translates into figures 4 and 5 that are normalised by TPC? The -under or -sub estimations would then be included in your calculations on the cumulative PP and TR and vertical C flux?

Author response: There are of course also measurements uncertainties in the TPC, and yes these would be included in the data presented in Figs 4 and 5. However, it does not affect the main conclusion of the paper.

C5

Reviewer #2, comment #4. Phytoplankton community composition. As data are presented it is not clearly seen that there is dominance of some groups over others. Only Euglenophytes seem to be absent in t0. Dinoflagellates, cyanobacteria, diatoms and chlorophytes look like having similar proportions in t0 and t17 (p values needed), while "other" increase at 1333 ppm. What organisms does "other" comprise? Stacked area plots would give a much better idea of the temporal evolution and trend followed by the community and so significances could be better appreciated. Thus I suggest to re-plot figures 1 and 2 including all days and treatments in stacked area charts. How your data compare to Bermudez et al. this issue-seems that taxonomy differs a little in between the two studies (for instance Euglenophyta).

Author response: We wanted to present a general overview of the plankton community composition, and a more in-depth analysis and presentation of all the dates are provided in Bermudez et al 2016 and Lischka et al 2015. The presented phytoplankton data is the same as Bermudez et al 2016, but here we have additionally included counts of phytoplankton  $>20\mu\text{m}$ , affecting the biomass of e.g. Euglenophyta.

Reviewer #2, comment #5. Considering that your study deals with the plankton food web, bacterial production, or at least abundances have not been analysed. Although probably low in volume and biomass contribution as compared to phyto and zooplankton groups, they are important too since they have been reported to react positively to increased  $\text{CO}_2$  (a number of papers published on this topic by Grossart, Schulz and Riebesell). I see bacterial contribution is further discussed in pg. 20 based on former reports. How about bacterial production/abundances in this very mesocosms experiment? Neither you say anything about viruses affecting C losses, which is important for C cycling and definitively affect C export. These two (bacteria and viruses) in my opinion shall be at least being discussed (succinctly if you wish) within the framework of the whole mesocosm experiment.

Author response: A very good point and we will incorporate this into the discussion. Bacterial production was measured (Hornick et al 2016), but this was not out yet at the

C6

time of review. Heterotrophic prokaryotes were enumerated and this data is presented in Crawford et al (2016).

Reviewer #2, comment #6. Pg. 18. Ln 20. "The larger-scale mesocosms . . . interacting effects between different components of the food web are included". Pg. 19 Ln 21. Subheading "Interacting effects and community composition". Also in pg. 20 Ln 10 interactive effects are mentioned. I find this an overstatement since you have not analysed interactive effects

Author response: This section is under the discussion of advantages of mesocosm experiments on a general level. We will change this to "possible interacting effects. . ." to make it more clear.

Reviewer #2, comment #7. Pg. 18 Ln 22-pg. 19 Ln 6. Instead of discussing higher plants which do not deal with carbonic /carbonate equilibrium and the systems are different, I think it would make much more sense to focus on explaining the mechanisms why respiration might be reduced in aquatic organisms such as phytoplankton at high CO<sub>2</sub>. Can the decreased TR be related to CCMs? Both photosynthesis and respiration generate energy that can be used for CCMs since they are mechanisms highly-energy-demanding. Under increased CO<sub>2</sub> it is well known that CCMs are downregulated. If there are no active CCMs, then respiration and photosynthesis might also be downregulated, and the energy consumed by them is "available" for other purposes. On the other hand, such energy could also be directed to growth (i.e. PP) that is what you are describing. This would mean that respiration could be downregulated but not PP. Such uncoupling is what is important to discuss in depth. Also, how is this related to pigment concentration? Since under high CO<sub>2</sub> there is less electronic demand, pigments should decrease. Indeed Chl a sharply decreased from 2 ugL<sup>-1</sup> on P1 to 0.8 in PII and III (Paul et al., 2015). However you state in pg. 22 Ln 6 that CO<sub>2</sub> had a positive effect on Chl a. Some clarification is needed.

Author response: A very good point and a more thorough discussion around CCMs will

C7

be incorporated into the Discussion chapter. As for the Chl a, the major change was driven by change in total phytoplankton biomass, e.g. the overall decrease from P1 to PII and PIII, and the higher Chl a in the high CO<sub>2</sub>.

We will make appropriate changes to all the specific comments and technical suggestions raised by the reviewer.

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C8

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