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Interactive comment on "Ocean acidification decreases plankton respiration: evidence from a mesocosm experiment" *by* K. Spilling et al.

Anonymous Referee #2

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GENERAL COMMENTS: The manuscript by Spilling et al. reports on the response of the plankton community to a gradient of increasing CO2 concentrations, focusing on the effects that this treatments had on respiration, carbon fixation and carbon export. Authors conclude that respiration decreases under high CO2 concentrations, while primary productivity did not increase as a consequence of such CO2 levels (contrary to the already observed in many studies carried out up to date). The aim of this study was to provide new knowledge of the effects of elevated CO2 in a system such as the Baltic, were no many data sets on this topic are recorded. Therefore the work hereby presented focuses on a relevant and timely topic for scientists working on the effect of global change on aquatic ecosystems.

However, 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

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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 (CO2) 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 CO2 concentrations on the variables would needed (see Tables and Figures in Paul et al. 2015, Crawfurd 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.

The other important issue is that you mention measurement 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 14C, 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 14C 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?

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

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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?

SPECIFIC COMMENTS: Pg. 12.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).

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 CO2 (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.

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

statistically, thus you cannot conclude anything on that.

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 CO2. 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 CO2 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 CO2 there is less electronic demand, pigments should decrease. Indeed Chla sharply decreased from 2 ugL-1 on P1 to 0.8 in PII and III (Paul et al., 2015). However you estate in pg. 22 Ln 6 that CO2 had a positive effect on Chla. Some clarification is needed.

The paragraph on the effect of pH is interesting but maybe worthwhile looking at more updated papers on pH microenvironment in phytoplankton (Flynn et al., 2012. NATURE CLIMATE CHANGE | VOL 2 | JULY 2012 ; Taylor et al., 2012, Trends in Plant Science, November 2012, Vol. 17, No. 11)

General: I think the discussion need more work and a better connexion between sections. Agree with reviewer 1 on addition of a summary paragraph at the end.

TECHNICAL COMMENTS: Substitute "parameter" by "variable". What you are measuring are variables. Parameters are constants that relate variables.

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