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Comment

Interactive comment on “Primary production during nutrient-induced blooms at elevated CO₂ concentrations” by J. K. Egge et al.

J. K. Egge et al.

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Both Referee #1 and Referee #3 suggest that we merged the data set of the present MS (Egge et al.) with other related dataset as osmotrophs abundance (Paulino et al.), bacteria production (Allgeier et al.) and description of the set up of the experiment (Schulz et al). There already exists such combined papers from the previous PeECE experiment, especially the one from 2001. The reason why the PeECE group decided to go for a special issue this time, instead of larger overview papers, was that we here should have the possibility to go more into details on different topics. As the other papers are found in the same special issue, it should not be too difficult to find the other datasets. We feel that merging more data into this manuscript would tend to “drown” the messages without really providing much more insight into what happened in the mesocosms. With joint publication in a special issue, we feel the

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disadvantages to outweigh the advantages of merging the data into one manuscript, and have chosen not to follow this advice.

Answer to the 3 referees -

Referee #1 The referee was concerned about the authors' use of terminology with respect to gross and net production, and the potential mismatch of measurement time-scales. He/she was also concerned about the level of replication of certain measurements, and conclusions reached without robust statistical support.

The planktonic community in an enclosed water mass like a mesocosm will not be independent of the development in the previous days. If one of the mesocosm has highest biomass on one day, it will also have the highest biomass the next day if the growth rate is similar in all mesocosms. This dependency in day to day measured data limits our possibility to statistical analysis, and we have therefore only used simple statistics, ANOVA t-Test: Paired Two Sample for Means, to identify statistical (significant) differences between treatment in cumulative production and toward the end of the experiment. This gives a statistically significant higher production in 3x compared to 1x as will be included in the revised version of the manuscript.

In terms of measurement time-scales, the bulk 14C experiments appeared to have been made in both years using 4 hour incubations. Presumably, these measurements would approximate something close to gross primary production. Why weren't the size fractionated measurements made on the same time-scale?

14C was incubated both for 4h and 24h in 2005. There were 2 reasons for doing the 24h incubation in addition to 4h (routine). The first reason was that we then had the same incubation period for O₂ and 14C. The second was because we wanted to fractionate the incubated volume, and therefore had to make sure activity of 14C was measurable in the subsample of 25 ml. If only 4h incubations we would have had to use stronger activity and or larger sample volume, resulting in a higher use of 14C. We could also have increased the volume, but would then have got into problems with a

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very long filtration time.

In terms of terminology, the authors seem to equate their measured 24 h O₂ changes with gross production. Yet, given the length of these incubations, and the possibility for significant autotrophic respiration, I suggest that these measurements really reflect net primary production. Had the authors used the H₂¹⁸O labeling technique, they would have measured true gross production. Similarly, the term net community production is normally taken to include all community respiration including zooplankton. Typically, this measurement cannot be properly assessed using bottles, but would instead be inferred from net O₂ saturation in the mixed layer. Given these caveats, I'm not sure what the authors are really discussing. No explicit statement is made of how the authors calculated community respiration. Is this from bottle incubation data? If so, would it include the contribution of large zooplankton?

Our use of these terms is, consistent with standard terminology for this technique (e.g. Aristegui and Harrison 2002 *Aquatic Microbial Ecology* 29:199-209). There are several aspects of these calculations, such as e.g. the assumption that respiration in light and dark are equal, quite thoroughly discussed in the literature (e.g. P.J.L. Williams et al. (2004) *Deep Sea Res I* 51:1563-1578). Repeating this discussion without new data to shed light on the problems seems to us to diverge the discussion from the focus of the potential CO₂-effects. Our response is therefore the inclusion of a more explicit statement of the calculations in Materials Methods. The 125 ml bottles used for O₂ measurement will not include respiration from large zooplankton. Since the lack of large zooplankton would give the same effect in light and dark bottles, this exclusion should not affect the calculations of gross primary production. The contribution to respiration from large zooplankton is usually assumed to be minor compared to the microbial part. As a rough calculation, the maximum copepod biomass in the PEECE experiment was ca 20 mgC/m³ (Nejstgaard pers comm.). Using a specific respiration of ca 1% per day (Nishibe and Ikeda 2008, *Mar. Biol.* 153:397-404), this amounts to a total respiration around 0.02 μmolO₂l⁻¹d⁻¹, below detection limit for our method. We

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can well include this estimate, but feel again that it is not contributing to the focus of this paper.

Given that size fractionated ^{14}C uptake samples were only collected from a single mesocosm per CO_2 treatment, there would appear to be no reliable way to quantify the true biological variability in the CO_2 dependent response. The variability presented in the graphs (standard deviations) reflects the precision of the ^{14}C method rather than the true variability in the CO_2 response. As such, it would not seem possible to gauge the biological significance of the observed effects, and I feel that the results presented in this section cannot be considered robust. For the oxygen measurements, I was unclear as to whether samples were collected for each mesocosm.

We did only measure fractionated ^{14}C production in one of each treatment, although we realize we should have measured in all 9 but that was not possible due to the time schedule. For the O_2 , all 9 mesocosms were measured, 3 light and 3 dark bottles from each mesocosm. Our community respiration is based on dark bottle measurement. This will be included in the Material and methods

Basic nutrient and phytoplankton biomass data should be included. The majority of papers in this special issue are from the same mesocosm experiment, PeECE III, 2005. In order not to repeat the description of the experimental set up and the development of basic parameters like nutrient concentration and phytoplankton biomass (Chl a) the decision was that this information goes into one paper, (Schulz et al. submitted) and all other BGD papers refer to this one.

The referee asks for the reason for the unbalanced emphasis on the 2005 data relative to the 2003 data. We try to explain in the text that although CO_2 effects were observed on the phytoplankton community in 2003, no differences in the production was observed. In 2003 only 2 out of 3 parallel mesocosms were sampled. The 2005 data set is more extensive (3 parallels pre treatment) and also includes the fractionated production. We therefore decided to present 2003 just as average production in a table. In

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addition, not many papers are being published from the experiment in 2003 compared to 2005, and it is therefore difficult to discuss those data. To avoid confusions we will only refer to the 2003 data as unpublished data in the revised version.

Specific comment like more information needed in the Material and method section, and moving text has been carried out. We have also improved the text in paragraphs where we can see the text is not clear.

What is a osmothroic organism?? An organism that absorbs nutrients from solution, as opposed to ingesting particulate matter. We still believe this is rather well known, and we did therefore not include a definition in the paper.

8217. Referee asks if not the possible effect from heterotrophic bacteria (bacteria production) in 0.2-1 μ m fraction, would this be corrected for by subtracting dark production. Answer: Not really. 14C will not be incorporated in algae due to photosynthesis in the dark bottles, and DO14C will therefore not leak from the cells. As the DOC taken up by bacteria in the dark bottles will not be labelled, it will not be corrected for by subtracting the dark values from the dark bottles. The phrase: "in the light bottles"; is included in 4394 | 5 to make this clearer.

Referee #2The referee believes we have contradictory results by concluding in abstract that there is a trend towards higher cumulative primary production, while in the results we say there are no statistical differences between treatments.

Towards the end of the experiment there was a statistically significant higher cumulative 14C production in 3x compared to 1x production. This has now been included in the manuscript. In the BGD version we say both in the abstract and in the results that there was a trend of increasing production with increasing CO2 in cumulative 14C production. A similar trend could not be seen neither the in vitro rates of gross nor net oxygen as we write in the results. We do not refer to O2 production, but only 14C in the abstract, and it refers to the experiment in 2005 (PeECE III). Table 2, however, referred to in the results from the PeECE II in 2003, where we had a less intensive measurement program (only

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2 out of 3 parallels) and where we did not see any effect of CO₂ treatment. We can see there might be some confusion about which of the experiments we refer to, and will change that in the abstract where we only refer to the results from 2005. The reason we include data from 2003 is as we wrote (4391, l 26-27, and top of 4392) because there was a change in phytoplankton community based on CO₂ treatment which could not be observed in the primary production measurements. We therefore wanted to go one step further in 2005 by fractionate the ¹⁴C production. To avoid confusions we will only refer to the 2003 data as unpublished data in the revised version.

The general conclusion of the data would according to the referee be that there is no effect of CO₂. For the first 10 days of the experiment, when Si and P were available and the phytoplankton biomass was high, we completely agree with the referee. We can however not hide the fact that we see a different picture towards the end of the experiment. In the second part of the experiment we had a more regenerating system, we had no diatoms and other species dominated. Cumulative ¹⁴C production shows statistically higher production in the 3x compared to 1x CO₂ towards the end of the experiment.

The referee is right in all the detailed comments and this has now been corrected in the paper

Referee #3 Referee wanted more information on the primary production method which will be given in the Materials and Methods, and we did of course use the daily measured inorganic carbon concentration in the different mesocosms over time to calculate primary production. The incubation of ¹⁴C and O₂ bottles were done in the sea outside the mesocosms, and at the same location and depth. This is included in the M & M

Can a loss of up to 75 % of DOC be explained ? We calculated, as we say in the paper, a very low ratio (1:4) between mol C (¹⁴C) and gross production (O₂), while Gazeau et al. (2007) measured a ratio of nearly 1:1. Gazeau et al. (2007) used GF/F filters in their ¹⁴C measurements which Karl et al. (1998) found to overestimate PO₁₄C

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because up to 30 % DO14C was absorbed in the filter compared to polycarbonate filters, as we used. Karl et al. (1998) also found DO14C production to be as high as 50 % of PO14C production. Taken DOC into account will lower the ratio, and the most important factor was probably, however, the fact that we incubated the flasks for 24 h, and not from dawn to dusk as was the case in the 2 aforementioned investigations. Leakage of DO14C will continue also when irradiance is too low for photosynthesis, e.g. particulate production accumulation. Karl et al. 1998 observed higher DO14C during darkness than during the light period in natural samples. These factor may not fully account for the difference between the ratios, but surely shows that the ratio between mol C (14C) and gross production (O₂) can be lower than 1:1 depending on the method used. We have tried to improve this part of the discussion.

Referee found it hard to see how this paper added to previous one (Riebesell et al. 2007) and in some places there appeared to be some contradictions between the results The referee does not say where he/she sees a contradiction between the results, so it is difficult to answer this comment.

Table 2, Why two lines of each treatment? The table will be removed as the data from 2003 is only referred to as unpublished data in the revised version.

Figure 4. Missing error bars. The figure shows average production over the whole experimental period in the different fractions. For example, the $> 10\mu\text{m}$ fraction varies from close to $10\ \mu\text{mol C L}^{-1}\ \text{d}^{-1}$ when the bloom peaked to less than 1 when the experiment finished, meaning the error bars would have been big. But Fig 4 is based on the data in Fig 3, where error bars are given, and sums up the different fraction.

The authors wish to thank the three referees for valuable comments to our Discussion Paper. Their suggestions and comments will be included and improve the revised version of our paper

Interactive comment on Biogeosciences Discuss., 4, 4385, 2007.