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Biogeosciences Discussions

Interactive comment on "Build-up and decline of organic matter during PeECE III" *by* K. G. Schulz et al.

Anonymous Referee #1

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General Comments

The manuscript presents the results of carbon and nutrient consumption of a natural phytoplankton community maintained in mesocosm enclosures at initial CO_2 partial pressures of 350, 700 and 1,050 μatm, which correspond to x1, x2, and x3 of present day values. A significant amount of the data in the manuscript has been presented previously (Riebesell et al., Nature, 450, 545-548, 2007). This manuscript details temperature and salinity profiles in more detail as well as discussing observations obtained from sediment traps and the presentation of new ammonium data. I have a number of reservations about this paper that are briefly summarised here and discussed in more detail below. The methods need further clarification and justification. I am particularly concerned about the impact of the aquarium pump on particle dynamics and

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phyto- and zooplankton behaviour in the mesocosm. The sediment trap methods and sampling of the deep-layer need to be more clear. The authors report that there was increased export in the x3 experiments. This is based on indirect observations of lower ammonium in the deep-layers of the x3 experiment. The authors hypothesis is that enhanced organic carbon sedimentation at higher CO_2 levels could have fuelled organic carbon remineralisation by heterotrophic bacteria, leading to stronger oxygen reduction at depth in the x3 than x2 than x1. This is linked to ammonium data by suggesting that ammonium regeneration rates decrease at decreasing oxygen levels. There is no adequate reference provided for the relationship between ammonium regeneration and oxygen to support this tenuous link. Furthermore, the authors have neglected to discuss the process of photorespiration with respect to their ammonium data. In the x3 treatment the CO_2:O_2 of the upper-layer would be highest and thus photorespiration lowest. Given that ammonium is a waste product of photorespiration this could help to explain their results. There are also some other inconsistencies within the data. The authors hypothesise that enhanced export at elevated CO_2 concentrations is linked to increased TEP production at elevated CO_2 concentrations, this would indicate a preferential loss of DOC, which does not seem to be entirely supported by the DOC:DON data presented. The authors also indicate that in the high CO_2 treatments organic nitrogen remineralisation is reduced, however this suggestion is not consistent with the constant sedimentary POC:PON data observed across all the treatments. The authors suggest that enhanced POC remineralisation at high CO_2 could explain these apparent contradictory observations. However, no mechanism is proposed that would cause this effect, furthermore it seems unlikely that these two processes would act to cancel each other out exactly, which is what would be required to explain the constant POC:PON data. Even if these anecdotal observations were correct, the authors offer no views as to what the net effect on export would be under a high CO_2 regime where export is enhanced through TEP formation but presumably reduced through enhanced remineralisation of organic carbon. Detailed comments are presented below.

Specific comments

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Several times throughout the paper you refer to *deep-waters*. Please change this to deep-layer of the mesocosm, in the first instance and deep-layer subsequently. The term deep-water is a bit misleading.

Do the error bars on the figures propagate analytical errors or are they simply plus/minus one standard deviation of the three mesocosm replicates for each CO_2 treatment?

Page 1, line 8 Include some data in the abstract and consider tempering the tone of the conclusions to reflect some of the considerations discussed below.

Page 4, line 11 this sample nomenclature is the opposite way around to that adopted in Riebesell et al., 2007. This may explain the confusion later in the methods; it would be prudent to maintain the same nomenclature between papers.

Page 5 lines 8-12 What were the ambient nutrient concentrations prior to the addition of nitrate and phosphate? It is unclear why some of the nutrients should have been lost to deep-waters during mixing for mesocosms 1-4 only. This suggests that (specifically physical) conditions for all mesocosm bags were not identical prior to the initiation of the experiment. Do you have any suggestions why this should occur for only 4 out of the 9 mesocosm bags? Is it possible that the bags experienced different hydrodynamic forcing externally related to their positions on the array? This could be comparable to trapping efficiencies of different cylindrical tubes on a sediment trap array, where the presence of a structure (tube) influences the flow environment experienced by an adjacent structure (tube). How does this impact the interpretation of your results? Incidentally how were the mesocosm bags arranged spatially? A schematic would be useful. Did you take preliminary nutrient measurements of the deep-water also after the addition of macronutrients? If so do these numbers support your explanation that the nutrients were lost to the deep-water through mixing? Or do you have numbers to plug into your box model to calculate loss as is done later in the paper?

Page 5 lines 12-18 I think there are some mistakes in this sentence. According to the

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method section, mesocosms 1-3 were aerated at 350 μatm and 4-6 were aerated at 700 μatm. In the sentence starting on line 15 you state that the addition of $NaHCO_3$ and HCl is equivalent to CO_2 aeration, and increased pCO_2 in mesocosms 1-6 to desired values of 700 and 1050 μatm, respectively. Are these not the target values of mesocosms 4-9? Please clear up the confusion.

Page 5 line 18 Please specify what t_0 equates to, is this after nutrient and CO_2 equilibrium was established in all mesocosms (i.e. day 2?) or is it literally day 0 (i.e. 2 days prior to the establishment of chemical equilibrium within the mesocosms?)

Page 5 line 20 The text needs clarifying with regard to the daily sampling procedure. If the tube was lowered into the mesocosm it was presumably open at both ends? Did it have a closing mechanism? If the tube dimensions are 5m long and 6 cm diameter then this would have a volume of: $\pi 3^2$ = 28.27 cm^2 x 500 cm = 14,137 cm^3 or 14.1 litres. Given this where does the 20 L per mesocosm per day number come from?

This section needs to be expanded to include more information on the sediment traps and how deep-water was sampled. How were the sediment traps deployed? Are they moored? Is it one single tube? What preservatives were used? Why did you collect water from the sediment trap tube and not the water around it? There is no description of particle analysis from the sediment traps of any sort. A much clearer description of the methods is required here.

Page 6 Line 4 Please specify how much water was sampled for the various nutrient measurements. Also include how many replicates were measured, and details on the precision and accuracy of your measurements. Include the nominal pore size of GF/F filters (0.7 μm). This paragraph needs to be re-worded, for example you should start a new sentence when discussing the silicate measurements, including information on sample size, replicates, precision etc.

Page 6 line 12 Change to *The supernatant was passed through 0.2μm (polycarbonate?) filters and the filtrate analysed by* Include the type of filter used

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Page 6 line 25 As mentioned above please include information on the number of replicates analysed and the precision of the measurements. Also include information on whether you used an internal standard to constrain the accuracy of the analytical procedure, and which analytical standard was used for the elemental analysis.

Page 6 lines 26-27 At the beginning of section 2.2 you state that all measurements follow standard procedures. Given this statement can you provide a reference for the removal of inorganic carbon with concentrated HCl? Vapour phase methods can fail to remove all traces of carbonate particularly that associated with complicated organic matrices as might be expected in surface samples. Furthermore if there were any calcifying heterotrophs in your sample then this procedure may not have removed all of the carbonate. In my lab I simultaneously fume filters with granular calcium carbonate, and foraminifera to verify the vapour phase is removing all of the carbonate and run these samples as operational blanks. Did you do this? If not please comment on the potential sources of error from fuming over night?

Page 7 line 1-2 Does this mean the particulate measurements were performed from the water in sediment trap tubes? Any sinking particles would have presumably settled to the base of the sediment trap tube. Did you use any preservative? Your methods for sampling the particulate and dissolved phases from the deep-layer are very unclear and need to be explained in much more detail. Also why was the deep-layer only sampled every other day? If this is due to time constraints this needs to be stated.

Page 7 line 11-14 With regards to simulating export production to the deep-layer. The fact that you had an aquarium pump constantly mixing the upper-layer of the mesocosm is very concerning. The processes of particle formation and aggregation are extremely sensitive to small-scale hydrodynamic processes, and I imagine that the pump continually acted disaggregate particles back to suspended material and prevent them from sinking. This has major implications for your export observations. I would question the need to homogenise the mixed layer for dissolved components anyway given the volume of the mesocosm, diffusivity co-efficients of dissolved species, and your sampling

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resolution (once a day at 10:00am). You may have good reason for use of the pump, which incidentally requires justifying, but this severely compromises any accurate reproduction of particle dynamics in your experiments. You need to discuss these issues both in response to this review, but more importantly in your manuscript. In addition, you should mention what the effect of this pump is on phytoplankton. Dinoflagellates, for example, exhibit very acute responses to shear stress and will form cysts that will sink to your deep-layer. Please comment on how this pump may have affected the community composition of the phytoplankton assemblage and what impact this has on your results.

Page 7 line 10-15 You are wise to abandon any flux measurements obtained from the sediment traps. I would also be sceptical about the elemental ratios obtained from the flux data for two reasons: i) Over-and under trapping is caused by the hydrodynamic flow regime at the top of the sediment trap, it is widely acknowledged (see review by Buesseler et al., Journal of Marine Research, 65, 345-416, 2007) that this leads to some form of particle sorting based on type. Combing these trap observations with those of inter-specific plasticity in nutrient uptake stoichiometry may influence the measured elemental ratios. ii) If particles have been re-suspended from the bottom of the mesocosm bag then it is difficult to resolve the measurements temporally. The particles may have undergone continued remineralisation in the deep-layer before being sampled thus skewing the measured elemental ratios. Please comment on these issues with respect to the presentation of your sediment trap results.

You should be able to present export measurements by adopting a budgeting approach using all of your other observations. This has presumably been done, at least in part, to arrive at your conclusion of over-trapping. I would like to see these numbers for the individual mesocosm experiments. Did you take into account the standing stocks of particulate and dissolved material in the upper-layer, as well as the removal of inorganic nutrients when assessing the sediment trap data? If not consideration of this data would accentuate the problem of over-trapping.

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Page 8 line 26-27 Presumably you are talking about ammonium concentrations here, please state this. Change deep-water to deep-layer. The end of the sentence is clumsily worded. Present the values for each treatment as you do in the previous sentence for the surface layer.

Page 9 line 29 The metazooplankton biomass will increase in part due to the exclusion of natural predators from the mesocosm experiments, please mention this. As mentioned earlier, please address the affect of the aquarium pump on the behaviour of the zooplankton community?

Page 11 line 16 I agree that sedimentation processes are difficult to evaluate within this set-up. Export numbers could have been constrained with an elemental budgeting approach with correct sampling of both layers. This approach would have been slightly compromised by remineralisation of particles in the deep-layer. Why was this not considered, at least as a complimentary approach to the deployment of traps in the deep-layer? This could still be carried out and included in the paper. If the relevant measurements of both water masses were made this should be included. If the measurements were not made this needs to be identified as an experimental short-coming and recommendations for future sampling protocols discussed. The necessary corrections arising from mixing of the two layers could also be applied as these numbers are presented elsewhere (e.g. fig 2).

Page 11, line 24-25 Please avoid phrases like *seemingly* and *appeared* when discussing data. You have the data, and enough replicates to state within a valid statistical framework whether or not the chlorophyll/POC was higher under different treatments. A quick scan of figure 7 for example suggests that they are probably not significantly higher when you consider the error bars.

Page 12, line 1 By using the phrase *on the other hand* you are implying that this observation is different to the one presented in the previous sentence. You state here that the increase in DOC, DON, and DOP are not significantly different between treatments. Do

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you mean statistically they are not significantly different? You did not demonstrate that the POC, PON, POP data were statistically significantly different between treatments. Please tidy these discussion points up with presentation of appropriate statistics either in this section or in the results section.

Page 12, line 21-23 Is there any evidence in the literature that ammonium regeneration is oxygen-dependent in the water column? I do not think it is appropriate to provide a sedimentary reference here.

Page 12, line 23 You have not presented oxygen concentrations for the deep-layer, so how can you ask this question? You are assuming that the oxygen is low based on the ammonium data. You have not provided a reference in the water-column for the relationship between ammonia and oxygen. Even if the sedimentary references are valid here, which they may not be, and then what is the relationship between ammonium regeneration and oxygen like over your ammonium concentrations, linear? exponential? You are making a big assumption about oxygen based on your ammonium data. Why did you not measure oxygen in the deep-layer?

You have also ignored the process of photorespiration to explain your ammonium data. In the x3 treatment the CO_2:O_2 ratio in the surface waters will be higher than the x2 and x1 treatments. This will mean that photorespiration is lowest in the x3 treatment and highest in the x1 treatment. Ammonia is a waste product of photorespiration and will thus be lowest in the x3 treatment and highest in the x1 treatment. Do you think this mechanism can help explain your ammonium results? This obviously affects your conclusions regarding enhanced carbon export based on ammonium data. The text needs to be modified to reflect this.

Page 13, lines 1-3 According to table 1 TEP measurements were made on the samples. Why is this data not presented here? Does the TEP data support the hypothesis and work of Engel (Journal of Plankton Research, 24, 49-53, 2002) that TEP formation was enhanced under elevated CO_2 conditions?

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Page 13, lines 1-3 As mentioned previously, do you think that the conditions in the mesocosm bags and the presence of aquarium pumps are representative of particle dynamics? The transfer of dissolved and colloidal exopolymers to particles (TEP) is mediated by spontaneous assembly and shear (Passow, Marine Ecology Progress Series, 113, 185-198, 2000). Given the presence of the pump, if the export measurements were mediated by enhanced TEP formation then the experiment is of limited use in considering how these mechanisms might operate in a natural environment. Please comment.

Page 13, lines 1-3 I thought the conclusion of Engel 2002 was that a further increase in atmospheric CO_2 (as simulated in your experiments) would not lead to a higher rate of DIC to TEP conversion, since the rate of exopolymer carbohydrate production seems to be already at its maximum under the ambient CO_2 concentrations. Please comment on how this affects your explanation of the data and how it compares with your direct TEP measurements at different CO_2 levels.

Page 13, line 11 As discussed above, ammonium in your mesocosms is probably mediated by photorespiration, at least in part. Please bring this in here.

Page 13, lines 24-27 If this explanation is correct you would expect that the x3 treatment showed the largest deviation away from Redfield values. According to Figure 10d on day 22 the DOC/DON numbers for the x3 experiment were the highest and closest to Redfield. Please comment how this can be given your explanation of enhanced TEP formation, preferential DOC export, and lower DOC:DON ratios in the x3 treatment. On days 10-12 the DOC:DON seems to be lower in the x3, but the same as in the x2 which is at least in the right direction but still not consistent with progressively enhanced TEP production with increasing CO_2. Please comment.

It is thought that polysaccharide exudation and TEP production are the result of cellular carbon overflow, when ever nutrient acquisition limits biomass production but not photosynthesis. Consequently the TEP production should occur primarily after the nu-

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trients become exhausted on day 10. Do you have DOC:DON measurements prior to day 9? If so are they close to Redfield? Why are they not included in Figure 10d?

Page 14, lines 9-11 It seems rather unlikely that the proposed mechanisms of reduced organic nitrogen remineralisation and increased organic carbon remineralisation at elevated CO_2 would act to cancel each other out and result in constant sedimentary POC:PON values. The PON:POP changes are similar to the POC:POP changes and are probably related to POP rather than PON. If this reasoning is kept you need to justify it much better. For example do you have any ideas about a systematic mechanism under which elevated CO_2 would enhance carbon remineralisation? If so what is the scale of this process, would it act to cancel out the supposed increase in export at elevated CO_2? You seem to be contradicting yourselves by saying, that at increased CO_2 more carbon is exported but it is remineralised more effectively. Therefore what is the net affect of elevated CO_2 on export efficiency given these opposing mechanisms?

Page 15 lines 1-2 I would question points 2 and 3 based on some of the comments made above.

Technical Corrections

Page 1, line 4 Delete subsequent decreasing and replace with consequent decrease in

Page 1, line 8 Change mixed surface waters to upper layer of mesocosm.

Page 2, line 17 Change 21 to 21st

Page 2, line 18 Change climate relevant to climatically active

Page 2, line 26 Change to 21st

Page 3, line 12 Change to 21st

Page 5 line 10 Change to deep-layer

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Page 6 line 9 Insert immediately between which and were

Page 6 line 11 8211; Try to avoid starting sentences with then

Page 6 line 18 Delete prevent and replace with minimise the effect of

Page 6 line 26 Delete Before and replace with Prior to

Page 7 line 11 Insert layer after deep

Page 12, line 8 8211; Delete could be caused by and replace with indicates that

Page 12, line 9 Delete which. At the end of the sentence add over the range examined in this study

Page 12, line 17 Change to deep-layer

Page 13 line 24 This should be section 4.2

Interactive comment on Biogeosciences Discuss., 4, 4539, 2007.