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Biogeosciences Discussions

Interactive comment on "Combined effects of inorganic carbon and light on *Phaeocystis globosa* Scherffel (Prymnesiophyceae)" *by* A. Hoogstraten et al.

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Response of the authors to the comments of the reviewers on the manuscript entitled: Combined effects of inorganic carbon and light on Phaeocystis globosa.

Dear editor and reviewers,

First we would like to thank both reviewers for their helpful and guiding comments concerning our manuscript. Hereby we submit our response to these comments. We have

read the concerns of the reviewers and answered their questions in the text below the specific comments (marked by bullet points). We agree with the reviewers that several things need to be clarified, such as the experimental setup, which we have clarified in our response, and we have implemented this in a revised version of the manuscript. Furthermore, as both reviewers suggest, we have rewritten the introduction and the discussion, in order to allow for a more elaborate comparison with the results of Wang et al 2010(a&b) and Chen and Gao (2011). As suggested by the second referee, D. Hutchins, we have compared our results with those of studies on other Phaeocystis species in the discussion. Therefore, we maintained the first paragraph in the introduction, describing the three Phaeocystis species. A more elaborate description of the species P. globosa has been added to the introduction. However, we did not want to repeat all current knowledge on this species, which has been published in thorough reviews (e.g. Rousseau et al., 2007 and Schoeman et al., 2005). Therefore we have kept this section relatively short. As both reviewers suggest, it would have been great to have data available on DMS/P, however we did not take samples for this. We decided to have a well-defined carbonate system in our cultures, which is very important in carbon perturbation experiments. Since this is time-consuming and not easy to do, most studies focus on the biological parameters, rather than defining the chemical conditions in the cultures. In our study we have combined the accurate measurements of the carbonate system and those of the biological parameters studied. Therefore choices needed to be made on which parameters were measured, resulting in the current data set. We hope that you can accept the changes we suggest for this manuscript, and that you will allow us to submit a revised version of this manuscript. We are looking forward to your decision. Sincerely,

Astrid Hoogstraten, corresponding author

Response to comments of anonymous reviewer #1

We appreciate the comments of the reviewer, which aide to improve the manuscript. After reading the review, we do agree that the manuscript would benefit from restructuring

and the addition of information, such as a more elaborate introduction of Phaeocystis globosa and a more elaborate comparison to the papers by Wang et al. 2010 and Chen & Gao 2011. Furthermore, we do agree that the experimental approach should be described in more detail in a revised version of the manuscript. Here we give a short description of the setup in order to make the setup clearer and the data more relevant. The experimental setup consists of 6 L Polymethylmethacrylate (PMMA) vessels, which were water jacketed and placed in a climate controlled room in order to keep the temperature constant. The cultures were stirred with a Teflon® coated magnetic stirrer. Tubing and ports in the culture vessels, for sampling, addition of medium, and the air in- and outlet in the lid of the culture vessels were made of PFA tubing (Teflon®, Polyfluor Plastics, the Netherlands) an nylon (Swagelok, USA) respectively, to ensure gas tight connections. The gas supply and outlet had filters attached in order to prevent particles present in the cylinder to reach the culture vessels. The suggestion of implementing additional data, such as DMS production, is one that we certainly agree on. However, we do not have data available on this and the time-consuming and labour-intensive nature of these experiments make it difficult to repeat these and to obtain DMS data in a relatively short time span.

Minor remarks and suggestions:

- More detailed introduction of P. globosa. We do agree with the reviewer that the manuscript would benefit from a more thourough description of P. globosa in the introduction.

- p. 12355, l. 2: If I am not mistaken, “cells” should be single “as single cell” We have replaced single by solitary.

- p. 12355, l. 17: replace “as” by “into” The change have been made

- p. 12356, l. 4-9: Maybe it would be better to combine these sentences to have a better “reading flow”. We have rewritten the sentences and included extra information on the effects of increasing CO2 concentrations in the atmosphere.

- p.12356, l. 7: delete “AD” p.12356, l. 13: I strongly recommend referring to a more detailed/basic/expert work on ocean carbonate chemistry like e.g. Zeebe &Wolff-Gladrow, 2001. The work the authors refer to (Hoogstraten 2011) is not available yet and most likely not focusing on carbonate chemistry. We have replaced the reference by Millero, 1996.

- p.12356, l.19: The authors should provide more specific information what “this respect” means. We have changed the text after the comments of reviewer 2.

- Current knowledge and gaps to be investigated should be described more clearly in the introduction. We have discussed P. globosa in more detail and presented the updated current knowledge in the introduction.

- p.12356, l. 20: The authors could specify in more detail what the “socio-economic” relevance of this species is. We have changed this sentence

- p.12356, l. 22: Maybe the authors could exchange “environmental changes” with “CO2 and temperature” directly. The effect of CO2 and temperature is focus of this work and should not be “hidden” in parentheses. This has been changed.

- p.12356, l. 24: “clear yet”? Until now the authors did not present that this species was investigated in the context of global change at all. Please rearrange the structure of the introduction. The structure of the introduction has been rearranged in order to create a better flow for the reader.

- p.12356, l. 25: “as an accompanying anthropogenic effect” I suggest to mention and describe this in the paragraph about anthropogenic emissions and consequences (l.6-9 on this page). This has been changed.

- p. 12357, l. 2: Implementing a brief explanation how rising CO2 levels lead to a change in the light intensity in the paragraph about anthropogenic emissions and consequences (l.6-9 on this page) would be beneficial to the reader and clarify the intention for this experimental design. The section on anthropogenic emissions and

consequences has been changed and all extra info was added.

- p. 12357, l. 5 and 7: I suggest to exchange the word “classified” by “referred to as”. This is changed.

- p. 12357, l. 8 and 9: Why were different CO2 concentrations reached for the intermediate treatments? It would be nice to see initial values in the methods and/or results section to compare starting conditions. Differences due to technical constraints or biological activity should be presented and thoroughly discussed in the results and discussion section. For the introduction, it might be sufficient to introduce HL and LL at low, intermediate and high CO2 values and the range of pCO2 in order to give a clear outline to the reader. The initial values of the CO2 concentration in these cultures did not vary due to biological activity, but due to technical issues. Unfortunately all DIC and AT samples were analysed after the experiment, because of time constraints it was not possible to do this during the experiment. As we have stated, these experiments are very time and labour intensive and it was impossible to analyse samples while running the experiment. Therefore we only discovered the large difference after the experiment. Both cultures were aerated from the same gas cylinder, with a similar flow rate and therefore concentrations should have been similar. For some, to us unknown, reason, the gas exchange in the supply vessel of the LL culture was less efficient than that of the HL culture. Initial DIC concentrations in the intermediate CO2 LL culture were approximately 100 μmol.kg-1 lower than those of the corresponding HL culture (1980 compared to 2090 μmol.kg-1). A similar difference was found in the supply vessels of those cultures, showing that it was indeed not biological activity, but a technical issue. Although the concentrations differ in these two cultures we have been looking at the gradients from low to high CO2 and for both of the light treatments these gradients were present. During the experiment, CO2 concentrations within each culture were constant, showing that the carbonate system in the cultures was well-controlled despite the initial difference in DIC and therefore CO2(aq) concentration. This shows that it is very important to monitor the carbonate system in cultures very well when working

on carbon perturbation experiments.

2 Material and Methods

- p. 12357, l. 17: “aged” seawater- The authors should specify time and conditions of “aging” as well as the purpose of the use of “aged” seawater. We have clarified this.

- p. 12357, l. 19 and l. 23: The authors should give the full description of methods and experimental design if the manuscript referred to (Hoogstraten 2011), is not available/published yet! We do agree with the reviewer and have changed the method section.

- p. 12358, l. 4: “Natural gas mixes” What does this mean? We have clarified this in the manuscript.

- p. 12358, l. 6 and table 1: Why are there such differences among the same CO2 treatments (intermerdiate CO2 at HL and LL)? If the variation is a result of algae activity I suggest providing target and initially reached values in the methods section, presenting the variation that occurred due to biological activity in the results section and if this variation has consequences for the interpretation of the data set, this issue should be argued in the discussion section. See above, the differences were not caused by biological activity

- 2.2 Inorganic carbon p. 12359, l. 14: delete “excel spreadsheet” and write e.g. “by the use of CO2sys”. This was changed

- 2.4.2 Chlorophyll a and 2.4.3 POC and PON p.12360, l.14 and p. 12361, l. 2: I don’t understand the procedure. Pore size of Whatman GF/F- filters is 0.7 μm (not 0.2 μm as given in the description), which is typically used for these parameters. The authors should explain precisely which filters they used. Sampling of particulate samples might be better described as to be filtered “on” the filter for analysis instead of “over”. Here a mistake was made. Indeed 0.7 μm Whatman GF/F- filters were used for the measurements. Filtered over is changed into filtered on.

3 Results General: The authors introduced the abbreviations HL and LL for the figures; I suggest doing so for the text as well. Additionally, the text might be easier to understand, if short and precise terms/abbreviations are introduced for the CO2-levels as well. Wang et al. 2010 is cited twice (as seen in the reference list) and the “a” and/or “b” should be added consistently also in the text. We have abbreviated the high and low light. We have corrected the citations for Wang et al. 2010.

- p.12362, l. 2: I suggest implementing the initial/target values for CO2 in table 1. I suspect the different values, especially for the intermediate CO2 treatment, are due to biological activity, but to evaluate this (e.g. DIC draw down, cell abundance, photosynthetic efficiency) it would be nice to see the initial CO2 concentration (compare comments above). We added the initial values of CO2 in the table. As mentioned above, the difference in the intermediate treatment was caused by a technical problem rather than by biological activity.

- p.12362, l. 8: exchange “larger” with “higher” This was changed

- p.12362, l.24: if the authors give the μ derived from linear regression of the natural logarithm of the cell abundance I suggest to simply write: The cells grew at μ =0.7 d-1. If you write dividing once a day, as you did, I would rather show growth dynamics as doubling time instead of μ. We have changed the sentence as suggested and changed it into “The cells grew at μ =0.7 d-1”. Indeed this results in a more uniform paragraph.

- 3.3 p. 12363, l. 10: I suggest giving a range of photosynthetic efficiency during this experiment. “High”, relative to what? We have given a range of FV/FM during the experiment.

- 3.5 p.12365, l. 12: I suggest a more neutral word like “stimulate” or “increase” instead of “positive” effect of CO2 on phosphate uptake. This is changed.

- 4 Discussion p. 12366, l. 1-9: I suggest moving these sentences to the introduction or simply delete them (see general comments). We have decrease the length of

this introductory paragraph in the discussion significantly, just stating the goal of this research: increasing CO2 and changing light regimes, and removed these sentences.

- p. 12366, l. 9: may “act” as? Missing word True, this should be act as, and has been changed.

- p. 12366, l. 10 ff: I don’t see a problem in stable CO2-concentrations within a culture, but among cultures of the same pCO2 (especially among the intermediate CO2 HL and LL treatment). Since there were only slight differences for cell abundances, Fv/Fm, and chl a between intermediate CO2 at HL and LL (table 2 and fig. 1) I do not understand what induced the difference in CO2 if nutrient media was aerated with the same commercially available gas. We have pointed out the difference between the intermediate CO2 high and low light cultures in the discussion and explained that this was due to technical problems rather than biological activity.

- p. 12367, l. 1 The authors highlight corroboration to results obtained by Wang et al. 2010 concerning the combined effect of light and CO2 on growth rates and the authors suggest consistency among different strains of P. globosa. I do not agree. If I am not mistaken, the authors compare data obtained at a light intensity of 240 μmol photons m-2 s-1 (HL-treatment in the present paper) with data obtained by Wang et al. at 80 μmol photons m-2 s-1 (comparable to the LL treatment in the present paper which showed no response). For me, this might indicates a strain specific response and should be discussed in more detail. We have written a more elaborate discussion and comparison on the differences between our results and those of the study by Wang et al., 2010.

- p. 12367, l. 3: It is the first time you mention strain specific responses of P. globosa. This issue should be introduced earlier (Introduction) to be discussed here. We have introduced strain specific responses in the introduction.

- p. 12367, l. 10: The authors argue the contradiction in cellular POC content to findings by Wang et al.2010a by differences between solitary and colonies forming cells. In

order to estimate the importance of different POC contents for solitary or colony forming cells the natural occurrence/dominance of both types of P. globosa in the ocean should be discussed. We have looked for data on occurrence/dominance single cell versus colonial P. globosa in the ocean and discussed this in the manuscript (introduction). The occurrence seems to be mainly regulated by light and nutrient conditions.

- The closing paragraph of the discussion deals with DMS production and global warming. Unfortunately the authors did not highlight, what new findings were obtained during their study and what the readers learn about the potential performance of P. globosa in a high CO2, high light ocean. An extra paragraph highlighting the finding of this study is added to the discussion.

- Figures: Fig. 1, Legend: I assume the lowest line supposed to be high CO2 LL Yes, this is a mistake and has been changed

Response to comments of reviewer #2, D. Hutchins

Thank you for your kind words concerning our manuscript and for your comments, which help us to improve the quality of the manuscript. We do agree that we should compare our findings to the results of studies on other Phaeocystis species in order to bring the results in a broader context. We will do so in the discussion of the manuscript. We will keep the first paragraph of the introduction as it is to introduce the other species for the discussion. As stated before in this response, we will compare the results of our study with those of the papers by Wang et al 2010 and Chen and Gao 2011 in more detail.

Specific comments:

- Abstract: The phrase “globally dominating phytoplankton species” seems to imply that it dominates everywhere. I know this is not what the authors mean, how about rephrasing it as “an ecologically dominant species in many areas around the world”, or something like that? Thank you for this suggestion, it is good to rephrase this sentence

and we have implemented your suggestion.

Introduction

- p. 12355, lines 19- 21- Since pCO2 is given throughout the paper as micromol kg-1, this should probably be kept consistent when discussing other papers too. The units could easily be converted from ppm here. We have chosen CO2(aq) as parameter for our results, since this is ecologically most relevant, phytoplankton can take up CO2(aq) passively without using carbon concentrating mechanisms. This parameter is calculated from DIC and alkalinity, as is pCO2. In order to make the comparison with other papers easier in the discussion we have given the calculated pCO2 from our experiments.

- p. 12356, lines 1-3- I agree with this statement, but what is missing is a consideration of how the experimental nutrient concentrations used here compare to typical in situ levels in natural P. globosa blooms. I assume the levels of 3.75 uM phosphate and 60uM nitrate (p. 12357) are considerably higher than natural levels, so is this important to consider in interpreting these results? The experimental nutrient concentrations have been chosen to be nutrient-replete, in order to look specifically for effects of CO2 and light. Natural nutrient concentrations are often lower and for example in the Dutch Wadden Sea phosphate limitation occurs. Of course this has an influence on the response of the phytoplankton. We have mentioned this in the discussion. Unfortunately it was beyond the scope of this study to test for nutrient-CO2-light co-limitation, due to the labour-intensive and time-consuming nature of the experiments.

- P12356, line 19- Actually there have been a number of other studies on HAB responses to pCO2 changes, these include Rost et al 2006 (Plant Cell Env, dinoflagellates), Fu et al 2008 (Harmful Algae, a dinoflagellate and a raphidophyte), Fu et al 2010 (AME, a dinoflagellate), a new paper in PLoS ONE (Tatters et al. 2012, Pseudonitzschia), as well as the two previous studies on P. globosa mentioned above. I’m not suggesting that all of these papers need to be referenced here, but this statement

is not strictly accurate. We were not aware of these studies and have changed the introduction.

- p. 12357, lines 5-7- How were these two light levels chosen? Was a complete light response curve available for this species to help choose appropriate suboptimal and saturating light levels, or were these just chosen arbitrarily and assumed to be limiting and saturating? We did not have a complete light response curve for this species, but we obtained this information from a study by Jahnke (1989) and mentioned this in the introduction, rather than in the discussion.

- p. 12358, results are presented on cell densities before the relevant methods are given in the text (not until the bottom of the following page). It was difficult to make a decision on how to write this down. In order to describe the experimental setup it was necessary to give cell abundance between dilutions and thereby describe the semi-continuous cultures. We have statde in line 10 of this page that the determination of cell abundance is described in paragraph 2.3, instead of in line 16.

- p. 12358, the cultures appear to have been fully acclimated to the experimental conditions during the 6 days pre-experimental growth period, but it would be helpful to readers to know how many cell divisions this represented. This information has been added.

- p. 12359- It is good that the added 60 uM silicate was considered in the CO2Sys calculations, but it is not clear why it was added to the medium at all, since Si is not a nutrient that is required by Phaeocystis. The silicate was added to the medium since it was part of the standard solution for the preparation of the medium, which is also used for diatom studies. Since silicate is important in the determination of the carbonate system, we have added it in our calculations.

- p. 12361- It is too bad that particulate organic phosphorus was not measured as well as carbon and nitrogen, it is a relatively simply spectrophotometric measurement and would have avoided having to calculate cellular N:P ratios based only on phosphate

drawdown. Methods- Likewise, measurements of DMS/P would have greatly added to the biogeochemical relevance of the study. We do regret not having the data on organic phosphorus and DMS/P. Especially the latter would indeed have added a lot of information. In our experiments, we have chosen to make accurate measurements of the carbonate system, in order to ensure that this was well-defined. The problem with many other studies is that it is not clear from the article how and if parameters of the carbonate system were measured. For carbon perturbation experiments this is a critical point. We have found that for example high cell densities will decrease the pCO2 of the culture vessels dramatically, and that therefore low cell abundance is necessary. These results can only be obtained when the carbonate system is well defined and monitored. Since this is not easy to do and you need a relatively large sample volume, choices need to be made on the amount of parameters measured. These choices have results in the current data set.

- p. 12363- Lines 5-8- Pointing out a trend and then saying it is not significant is probably unnecessary. We have changed the sentence.

- lines 17-18- This statement is redundant to the previous sentence, which already gives the information that no effect on Fv/Fm was observed except in the high light cultures. We have removed the sentence.

- p. 12364 and Fig 3- Is Fig 3 really needed, considering that there are no significant trends shown on any of the three panels? This could be easily said in the text instead. We have replaced figure 3 by adding the data to table 3.

- p. 12364, lines 17-20- Obviously, the changes in C:Chl ratios were driven entirely by changes in Chl a cell-1, since POC quotas didn't change. The authors say this in the discussion but it could be pointed out first here. We have pointed out that this is due to the Chl a.cell-1 in the last sentence.

- p. 12365, lines 3-4- Since this paper doesn't have an excess of data in it, is there a need to relegate some results to supplementary tables? Why not show these data in

the paper? Because we have received comments in the past concerning the amount of data in a different manuscript, we have decided to put the tables with the inorganic carbon and nutrient measurements in the supplementary files and use the averages in the paper. It is always difficult to make a decision on this, but we felt that it was necessary to add this data, but that it was too much to put the tables in the main manuscript.

- p. 12365, lines 22-25- Again, it is a bit odd to say that N:P ratios were highest in the low light cultures, then follow in the next sentence with a statement that "There was no significant effect of the two different light intensities on the N:P ratio". We have changed the sentence, and mentioned the averages from the high light cultures.

- p. 12366- The statement that "this trend was opposite to the results presented by Kim et al. (2006)" is confusing without first telling readers what their results were. And why the difference in your study? Some additional explanatory text is needed here. We have add more text in this section and explained what Kim et al (2006) found and why this might be important. In short: Kim et al (2006) found a significant effect of POC on total alkalinity, therefore they state that all calculations made from this total alkalinity will be biased. This seems to be a species specific response however. They found different rates of increase in AT with the [POC] of different phytoplankton cultures. Furthermore, they worked with relatively high [POC] in comparison to our study, most likely explaining why we did not find an effect of [POC].

- p. 12367, lines 16-19- How much higher are your C:N ratios than the ones reported from single cells from previous work? The text needs to be a little more specific and quantitative here. We have added the data from literature.

- p. 12367, bottom- Here is where discussion of some of the many previous studies of irradiance effects on P. antarctica would be appropriate. We have added a comparison of our results with different Phaeocystis species to the discussion.

- p. 12368, top- Whether these growth rates would allow them to outcompete diatoms

obviously depends on the diatom, all diatoms do not grow at the same rate. We have removed this sentence

- p. 12368, bottom- The concluding sentences illustrate the reason why some direct measurements of organic sulfur pools in this experiment would have added greatly to its biogeochemical relevance. We completely agree with this point. However, as mentioned before we do not have this data available.

Interactive comment on Biogeosciences Discuss., 8, 12353, 2011.