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**Biogeosciences Discussions**

# *Interactive comment on* "Response of Nodularia spumigena to pCO<sub>2</sub> – Part 2: Exudation and extracellular enzyme activities" *by* S. Endres et al.

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Reply to Referee #2

We thank the anonymous reviewer for his/her comments on our manuscript. Below are the point by point replies to comments and suggestions.

1) REFEREE: Replication. The manuscript describes a single growth experiment with three CO2 treatments. Although replication within the experiment was high (12 replicates treatment), I am extremely concerned that no attempt has been made to replicate the findings of this single experiment. This can be difficult for certain studies (long term evolutionary studies, field studies, etc) but for a short term laboratory study there is a



clear need to ensure the findings are reproducible and robust. Given that laboratory studies of the effects of elevated CO2 on Nodularia have already produced conflicting results (Czerny et al, 2009), surely there is a requirement to ensure that the findings presented here are sufficiently reproducible? Clearly, the authors do not need to replicate their extensive analysis in its entirety but they must present an indication of the robustness of their major findings.

REPLY: The referee raises concerns on the reproducibility of our results. We ensured robustness through the high number of replicates. The setup of another such experiment was beyond the possibilities of the project because of the high work load. As the referee admits, replication in our study was high and the detected effects were statistically significant, so we may conclude that our findings are robust. Moreover, the experimental set-up was explained in detail and accepted within the first of the three companion papers by Wannicke et al. 2012 in this journal. Unpublished work in small experiments previous to the one described here gave similar results in terms of stimulation of nitrogen fixation. We found a stimulating effect on Nodularia spumigena growth and N2 fixation due to increasing CO2 as described in Wannicke et al., 2012. Our findings are in well accordance with earlier findings, e.g. Hutchins et al., 2007; Kranz et al., 2010; Barcelos e Ramos et al., 2007. The mentioned study of Czerny et al. is not completely comparable to ours as they cultured Nodularia in phosphorus-repleted semi-continuous batch cultures (pre-bloom conditions, medium with 5.4 $\mu$M PO4) while we simulated a typical bloom situation were phosphorus (initial concentration 0.5 $\mu$M PO4) is limiting. Moreover the movement of incubation bottles may have affected the study of Czerny, while here the bottles were only manually rotated once or twice a day. This was also reflected in different growth rates in both studies. Organisms might react different to pCO2 depending on their nutritional status or the phase of the bloom. However, Czerny et al. or other studies did not investigate the effect of CO2 on Nodularia mucus production or phosphatase activity so there is no reason to consider conflicting results in our manuscript in great detail.

2) REFEREE: Increased exudation. In the abstract the authors conclude that high CO2 leads to increased exudation. However, when normalized to biomass (POC) the total concentration of mucinous substance is not correlated to CO2. Surely this means that exudation has not increased and that the increased in exudates measured is explained by the increase in biomass? The authors do state that 'cell-specific rates do not change' and again in the discussion they mention that 'we cannot confirm a stimulating effect of elevated pCO2 on exudation'. Therefore, it is not clear what their conclusions are. Does exudation increase or not with increasing CO2? This has to be clearly addressed as it is a major conclusion of the manuscript.

REPLY: We agree that some sentences concerning exudation might be misleading and we will clarify these in our manuscript. We did not measure exudation directly. We find in total more mucinous substances per liter over time and in the high pCO2 treatments. During the growth phase, biomass was increasing faster than mucinous substances accumulated. Then, from day 9 on, more mucus was produced or accumulated while biomass was decreasing which indicates higher exudation or cell lysis. This was pronounced at high pCO2. Therefore, our conclusion is that cell-specific production of mucinous substances was not effected by CO2 but due to higher biomass we found more mucinous substances in the high pCO2 treatments. In the future ocean this may increase aggregation of filaments, export of biomass to deeper waters and also effect bacterial growth as mucus is a suitable substrate for marine bacteria. These results confirm earlier studies of Engel 2002 on natural communities of nitrogen fixers in the Baltic Sea.

3) REFEREE: In the abstract, the authors state that more mucinous substances accumulated in the growth phase' but they present the data for the concentrations reached on day 15, after the growth phase. This should be clarified.

REPLY: We refer to both sampling days: "accumulation of mucinous substances during the first 9 days (growth phase) significantly higher in high pCO2 treatment compared to low pCO2 treatment (p = 0.039, see p.5122 ln 2 and Figure 3a)". Afterwards this effect



was even more pronounced and we give the concentrations reached on day 15 (p. 5121 ln 25, Figure 1b). Therefore, we conclude that in total more mucus accumulated in the high pCO2 treatments.

4) REFEREE: Enhanced recycling of organic nutrients promotes faster growth. In abstract (p5110 ln 28) and the final paragraph of the discussion (p5131 ln 18), the authors state that their results reflect enhanced recycling of organic nutrients. However, there are no data presented in the manuscript to support this conclusion. For example, APA was greatest in the high CO2 treatment at day 9. However, biomass is greatest is the high CO2 treatment and the increased APA may just reflect this greater biomass. Indeed, APA demonstrated a positive correlation with POC and chl a, but did not show a correlation with CO2. Furthermore, there was no significant difference in DOP concentration between CO2 treatments, so there is no evidence for increased DOP uptake at elevated CO2. How then, can the authors conclude that elevated CO2 leads to enhanced recycling of organic nutrients which in turn promotes growth? As the cultures were P-limited this is one possible explanation, but their results may simply demonstrate that the increased availability of carbon at high CO2 leads to enhanced growth.

REPLY: The decrease in DOP concentration during the growth phase differed significantly between low and high pCO2 treatments (p=0.038, p. 5123 ln 21). We conclude that this decrease is due to enzymatic hydrolysis and uptake by Nodularia. This assumption is supported by significantly higher AP activities. The question then is whether higher biomass lead to higher enzyme activities or whether higher enzyme activities supported growth of Nodularia. Generally, enzyme activities show strong pH dependency because changes in hydrogen ion concentration modify the three-dimensional structure of the active site of the enzyme. The alkaline phosphatase has its optimum between pH 7.5 and 10 (e.g. Healey & Hendzel 1979, Münster 1992) depending on origin and composition of the enzyme assemblage. Other enzymes have been shown to have their optimum at a pH below present seawater pH (e.g. Münster 1992, Grossart et al. 2006, Piontek et al. 2010). Therefore, we conclude that pH had a

direct stimulating effect on APA. This facilitated to overcome P-limitation and increased Nodularia growth which in turn may have increased the expression of more AP. This point needs further examination, because it cannot be resolved completely with our results. We will clarify this in our manuscript.

5) REFEREE: The authors also state that elevated CO2 leads to faster growth of Nodularia. However, they do not measure growth rates and for the data presented it appears that growth rate is very similar initially, but at elevated CO2 the cells grow to a higher density.

REPLY: We measured growth rates based on different biomass parameters as described in detail in the accompanying publication (Wannicke et al. 2012, Figure 5): "Calculated growth rates ($\mu$) per day based on changes in abundance, chlorophyll a (Chl a), particulate organic nitrogen (PON) and particulate organic carbon (POC) for the three pCO2 treatments. Compiled growth rates based on all parameters were significantly different between the pCO2 treatments ($p < 0.05$ and $p = 0.001$), with the highest growth rate at high pCO2 ($0.212 \pm 0.018$ $d-1$)." We will add a short summary of the determined growth rates to our manuscript.

6) REFEREE: In summary, I think the data presented in the manuscript does not support the authors' conclusions and because of this I think the manuscript in its current format is potentially misleading. There is not sufficient evidence for increased exudation or increased uptake of organic nutrients, and there is little evidence to suggest that these processes are responsible for increasing growth at elevated CO2. These issues have to be addressed before the manuscript is suitable for publication.

REPLY: We hope that we could convince Referee #2 that CO2 stimulated Nodularia growth under P-limitation by enhancing the enzymatic hydrolysis of organic phosphorus. In the revised paper we will carefully elaborate all above mentioned arguments and re-phrase misleading sentences. Overall we want to show that the increased accumulation of mucinous substances at high pCO2 did not directly contribute to growth



but was a result of it.

Additional references:

Healey, F. P. and Hendzel, L. L.: Fluorometric measurement of alkaline phosphatase activity in algae, Freshwater Biology, 9, 429–439, 1979.

Münster, U., Einiö, P., Nurminen, J., and Overbeck, J.: Extracellular enzymes in a polyhumic lake: important regulators in detritus processing, Hydrobiologia, 229, 225–238, 1992.

Wannicke, N., Endres, S., Engel, A., Grossart, H.-P., Nausch, M., Unger, J., and Voss, M.: Response of Nodularia spumigena to pCO2 – Part 1: Growth, production and nitrogen cycling, Biogeosciences, 9, 2973-2988, doi:10.5194/bg-9-2973-2012, 2012

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Interactive comment on Biogeosciences Discuss., 9, 5109, 2012.