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Interactive comment on “Response of *Nodularia spumigena* to CO₂ – Part 2: Exudation and extracellular enzyme activities” by S. Endres et al.

Anonymous Referee #2

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This is an interesting manuscript, describing the potential effects of elevated CO₂ on the extracellular material associated with the cyanobacterium, *Nodularia spumigena*. The exudation and turnover of organic matter by phytoplankton is poorly understood and the subject area is clearly appropriate for publication in Biogeosciences. Whilst I think the manuscript is of merit, I have some major concerns about the robustness of the findings and the interpretation of data. In particular, I disagree strongly with the major conclusions of their manuscript.

1) Replication. The manuscript describes a single growth experiment with three CO₂ treatments. Although replication within the experiment was high (12 replicates per

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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.

2) Increased exudation. In the abstract the authors conclude that high CO₂ leads to increased exudation. However, when normalised 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.

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.

3) Enhanced recycling of organic nutrients promotes faster growth. In abstract (p5110 In 28) and the final paragraph of the discussion (p5131 In 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 in the high CO₂ treatment and the increased APA may just reflect this greater

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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.

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.

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.

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