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Biogeosciences Discussions

Interactive comment on "Response of Nodularia spumigena to pCO₂ – Part 2: Exudation and extracellular enzyme activities" *by* S. Endres et al.

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Reply to Referee #1

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

1) REFEREE: The article often refers to a twin article by Wannicke et al., especially about the experimental set-up. The review process of Wannicke et al. raised some points I agree with about the early stages of the experiments, in particular about the lack of achievements of desired CO2 concentrations. For this reason I also suggest not to refer to past, present and future CO2 levels (e.g. page 5110, lines 14-15),

but the authors should name their experimental conditions with the actual CO2 level (or mean among replicates), or (as often written in the ms) low, medium, high CO2. Furthermore, since this paper is highly connected to Wannicke et al. which I guess is in the revision stage, I recommend the authors to follow any change (relatively to experimental description, carbonate chemistry and other data, when possible) applied to the twin manuscript.

REPLY: We agree with this suggestion. We followed the revision process of Wannicke et al. and will update our manuscript in terms of carbonate chemistry and experimental description. We will change page 5110, lines 13-15 to: "Batch cultures of Nodularia spumigena were grown for 15 days under aeration with low (median 315 μatm), medium (median 353 μatm), and high (median 548 μatm) CO2 concentrations."

2) REFEREE: Heterotrophic bacteria: in general I understand that heterotrophic bacteria did not 'grow'. In lines 4-6 of page 5121 it is stated that cell number remained around 5x10ˆ5 cells l-1. The authors need to be more precise about this, adding an error value or describing more in details this lack of growth for each treatment. Although it is stated that variability among replicated was high (p5123, l28-29) these data are important. I am convinced that degradation processes are mainly carried out by Nodularia, but 5x10ˆ5 bacteria per liter in a batch culture can in theory be responsible alone for the Vmax values detected in this experiment. The fact that cell numbers did not increase along with time, suggest that they were not particularly active (as discussed by the authors), but I would not stress the main role of Nodularia too much, since it is not proven. This for example in p5123, l 11-13; p 5127 l 14-16; p 5129 l 27-29 (in this case the author cannot state that the bacterial community was not active because (i) Nodularia is a bacterium and (ii) they have no data about heterotrophic bacterial activity).

REPLY: Bacterial cell numbers were in average 4.7 $\pm$ 1.6 x10ˆ5 in the low, 4.5 $\pm$ 1.6 x10ˆ5, in the medium and 4.7 $\pm$ 1.3 x10ˆ5 in the high pCO2 treatment. The bacterial cell counts per treatment and sampling day are shown in Table 2 in Wannicke et al.

(2012). Standard deviations are relatively high and cell numbers were varying between replicates and over time probably due to methodological constraints. If some bacteria were attached to each other or to gel particles we might have underestimated the bacterial abundance in some replicates by flow cytometry. On the other hand, we might have also measured non-viable, but SYBR green stainable bacteria since also extracellular nucleic acids and dead, DNA containing, cells will be stained by the dye as discussed in Wannicke et al. (2012). No growth of heterotrophic bacteria was detectable as cell numbers were varying but not continually and significantly increasing. In the end of the experiment we filtered the cyanobacteria cells out (2.7 μm GF/D filter). Extracellular enzymes in the medium however cannot be removed by this filtration. We determined extracellular enzyme activities of the filtrate and found that 1-2 % of the APA and 2-59% of the LAP activity remained. Thus, we conclude that (1) AP was mainly attached to Nodularia cell surface, (2) LAP was mainly released to the medium, (3) degradation of DOP was driven by Nodularia while heterotrophic degradation of DOP is negligible and (4) we cannot ascribe LAP activity solely to Nodularia spumigena but to a certain degree heterotrophic bacteria might have contributed although they did not build up biomass. We will include this data and considerations in our manuscript.

3) REFEREE: As for the previous comment I think that specific APA values related to chlorophyll can be misleading (APA attribution to Nodularia is a very plausible speculation, not an evidence). Since the authors do not deeply discuss sAPA data, and they are not among the major findings of the experiment I suggest to remove them.

REPLY: As explained above and in our discussion section, we conclude from our data that the APA can be primarily referred to Nodularia, but we agree with the Referee that the chlorophyll based specific APA values might be just a rough assumption and therefore we will remove them in the revised version of our manuscript.

4) REFEREE: p 5127 l 6: the four tested enzymes are not the 'major' enzymes, although they are among the major enzymes. Lipolytic and chitinolytic activities in the field, for example, are very often faster than glucosidases.

REPLY: We are grateful for this annotation. We will change the sentence to clarify this: "To determine the turnover rates of organic matter due to enzymatic cleavage, extracellular enzyme activities of four key enzymes in carbon and nutrient cycling (alkaline phosphatase, a- and b-glucosidase, and leucine aminopeptidase) were followed over time in all treatments."

5) REFEREE: Unger et al (p 5128, l29) is not reported in the reference list.

REPLY: Thank you, we will add this reference.

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