

## ***Interactive comment on “Response of *Nodularia spumigena* to $p\text{CO}_2$ – Part 3: Turnover of phosphorus compounds” by J. Unger et al.***

**J. Unger et al.**

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Reply to referee #2

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

Main comments:

1)Referee: While I find the results interesting I think the authors should make an effort to write the manuscript in a more comprehensive way. As it is now, the writing is not straightforward and the main points get diluted. There are numerous spots in the manuscript where I found myself stumbling over awkward sentence structure or confusing wording.

Reply 1): We are grateful for the advice. Although, we do not fully agree with the referees' comment that our main points get diluted we will carefully revise our manuscript including the referees' comments. Furthermore, we will intensively screen the revised manuscript for mistakes in sentence structure and wording as there are still some sentences in the manuscript which are not quite clear.

2.1)Referee: This is a part of the investigations accompanying two papers of Wannicke et al. (2012) and Endres et al. (2012) on response of *Nodularia* to pCO<sub>2</sub>. However, in this paper the authors had made no effort to synthesize their findings with those in other papers.

Reply 2.1): We agree that we have to synthesize our findings much more obvious with regard to the two accompanying papers of Wannicke et al. (2012) and Endres et al. (2012). In the revised version of the manuscript we will deepen this in the "Discussion section".

2.2)Referee: The author should be very careful with their discussion on the significant difference in observed pCO<sub>2</sub> with the targeted values; the range of pCO<sub>2</sub> was quite narrow (p.14715, L1-L3).

Reply 2.2): It is true that the difference between the low and the medium pCO<sub>2</sub> treatment was quite narrow, but as Wannicke et al. (2012) reported there was a significant difference between all three pCO<sub>2</sub> set-ups ( $p < 0.001$ ,  $n = 12$ , Supplement Table S2) for the calculated pCO<sub>2</sub>. For more details, look at the reply of main comment 2) to referee #1.

2.3)Referee: As the authors argued, they found no significant effect of variable pCO<sub>2</sub> on dynamics of the DOP compounds (p. 14732, L26-L27). Although the authors founds the significant variation in total DOP concentration with pCO<sub>2</sub> level, it is unclear if the DOP variation was directly due to potential change in metabolic functions of *Nodularia* with acidification. It could be that the variation of total DOP resulted from the difference in biomass observed between the low and high pCO<sub>2</sub> cultures.

Reply 2.3): Thank you for this comment. We thought we had highlighted it in the “Conclusion”-section where we wrote:

“Our results indicate that accelerated P turnover can be expected during the cyanobacterial growth period under the pCO<sub>2</sub> conditions predicted for the future Baltic Sea. This implies the faster utilization of DIP as well as DOP. We propose that the stimulating effect on P utilization by the filamentous cyanobacterium *Nodularia spumigena* is indirect, as it is mediated by elevated carbon fixation and is dependent on cyanobacterial growth, which induces a stronger P demand.”

But we will include this also to the “Results”-section to clearly state that the variation of total DOP is due to the difference in biomass between low and high pCO<sub>2</sub> cultures.

2.4)Referee: Finally, since the authors used an “aged” seawater, refractory and biologically less-available DOP components which had withstood biological breakdown during the “aged” phase might dominate DOP at the start of the culture experiment. However, the results of this paper suggest that the ambient DOP in the sweater could be rapidly utilized with DIP depletion. Do the authors have any idea why *Nodularia* could utilize such refractory DOP components?

Reply 2.4): That is a legitimate question. While we have not determined refractory or bioavailable DOP we can only venture a guess. (1) We can exclude addition of newly available DOP by inoculation of the *Nodularia* culture on day 0 because values of unamended aged Baltic Sea water and the starting culture were nearly the same ( $0.33 \pm 0.01 \mu\text{mol l}^{-1}$  DOP vs.  $0.35 \pm 0.03 \mu\text{mol l}^{-1}$  DOP, respectively). But (2) as the DOP concentration declined from day 0 to day 15 in all treatments ( $-0.10 \mu\text{mol l}^{-1}$  – low,  $-0.14 \mu\text{mol l}^{-1}$  – medium,  $-0.17 \mu\text{mol l}^{-1}$  – high), DOP seems to be not fully refractory. A possible explanation is the induced cell death by UV irradiation and the release of bioavailable DOP by the dying organisms which was still present at the start of the experiment.

Other detailed comments:

1) p. 14714, L7-L8: Add the information on sampling depth.

Reply 1): We added the sampling depth.

“In preparation for the experiment, 1000 l of surface water (0-10 m) from the open Baltic Sea (54.22749°N, 12.1748°E) were collected. . .”

2) p. 14714, L8-L9: Add the information on condition of sample preservation.

Reply 2): We added information on the conditions of sample preservation.

“In preparation for the experiment, 1000 l of surface water (0-10 m) from the open Baltic Sea (54.22749°N, 12.1748°E) were collected and stored in a HDPE (high-density polyethylene)-tank under cool and dark conditions. Therein the water was aged for 4 months to allow the removal of inorganic nutrients by phytoplankton and bacteria.”

3) p. 14716, L7-L9: Add reference for chlorophyll a extraction and determination procedures.

Reply 3): We added the following reference for chlorophyll a extraction and determination procedures.

“HELCOM: Manual for Marine Monitoring in the COMBINE Programme of HELCOM, Part C.4., updated 2005: [http://www.helcom.fi/groups/monas/CombineManual/AnnexesC/en\\_GB/annex4/](http://www.helcom.fi/groups/monas/CombineManual/AnnexesC/en_GB/annex4/), 2001.”

4) p.14720, L8-L10: Add reference for the conversion factors from nucleic acids to P concentration.

Reply 4): We added a reference.

“Dissolved DNA and RNA concentrations were translated into P concentrations by multiplication by a factor of 2.06 nmol P for 1  $\mu$ g dDNA and 2.55 nmol P for 1  $\mu$ g dRNA, detected by DP determination in the microwave (Trinkler, unpublished).”

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5) p.14722, L25:  $4.69 \times 10^{-5} \pm 1.64$  should be  $(4.69 \pm 1.64) \times 10^{-5}$ .

Reply 5): We changed this.

6) p.14723, L28: Fig. 4a should be Fig. 3b.

Reply 6): We changed this.

7) p.14723, L28: The authors did not measure DOP uptake.

Reply 7): We agree and replaced “uptake” by “concentrations”.

8) p.14725, L2: Higher than what?

Reply 8): We added to the text:

“By this time, the proportion contributed by dPL-P to total DOP was higher in all three treatments (4.2, 7.6, and 9.3%, for low, medium, and high, respectively) compared to the starting proportion, mainly due to the decrease of total DOP.”

9) p.14726, 3.5 [33P]PO<sub>4</sub> uptake and transformation: The authors showed the results of only proportion of [33P] distribution in each fraction. Did the authors confirm conservation of the total activity throughout the incubation?

Reply 9). We are not sure, that we do understand the question properly. But we hope to give the right answer. <sup>33</sup>P has a half live time of 25 days and thus the activity changed within the incubation time. It is regarded in our calculations. The proportion in each fraction is always related to the total activity at each sampling time. The activity can be corrected by the decay, but, this is not necessary– the result is the same because the decay is the same in all fractions. According to comments of Ref. #1, the method is rewritten in more detail now.

10) p.14732, L9-L10: Add references for the turnover of DNA and other DOP.

Reply 10): We added references for the turnover of DNA and other DOP compounds to p. 14732, L9-10:

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“The turnover of dDNA (Paul et al., 1987) and other DOP compounds (e.g., ATP, Azam and Hodson, 1977; Björkman and Karl, 2005) is very fast...”

11) p.14732, L13-L14: Is there literature to support authors' assumption.

Reply 11): The sentence was not authors' assumption, it belongs to the reference of Paul et al. (1990) in the sentence before. We changed the beginning of the sentence to avoid confusing the reader:

“Furthermore, they assumed...”.

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**BGD**

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