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Biogeosciences Discussions

Interactive comment on "Response of *Nodularia spumigena* to pCO_2 – Part 3: Turnover of phosphorus compounds" *by* J. Unger et al.

Anonymous Referee #1

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This paper is a part of the results from a pCO2 perturbation experiment, and focuses on P dynamics in Nodularia culture. Two accompanying papers are reporting growth, stoichiometry, production and nitrogen fixation of Nodularia (Wannicke et al., 2012, Biogeosciences, 9, 2973–2988), and exudation of organic matter and extracellular enzyme activity of Nodularia (Endres et al., 2012, Biogeosciences Discussions, 9, 5109-5151). Unger et al. report responses of DIP, DOP and particulate P in Nodularia culture under different pCO2 treatments. With regard to DOP, they analyzed 4 compounds (ATP, DNA, RNA, and phospholipids). Using radioisotope technique, uptake of PO4 and its transformation into DOP and particulate P were determined in another Nodularia culture. This paper is a valuable contribution to better understand the effect of increasing pCO2 on nitrogen fixing phytoplankton with regard to P dynamics.

Main comments

As the authors mentioned, this paper is part of two other investigations based on the same experiment. I understand that certain data need to be presented in different paper in order that each paper is independent. But even in such a situation, the authors should carefully cite the reference which firstly presented the data. As I listed below, several data, which have already been published in Wannicke et al. (2012) and/or Endres et al. (2012), are presented in this paper without any relevant reference. The authors should acknowledge that such data presentation may be an issue of double data presentation.

According to Wannicke et al. (2012, Biogeosciences), "the pCO2 treatments differed significantly in pH and CT between mid and high pCO2 treatment, as well as between the low and high pCO2 treatment ($p \leq 0.001$, n=12, Supplement Table S2)." This means that pH and CT were not significantly different between the low and mid pCO2 treatments. Hence, it appears that this experiment had two low pCO2 and one high pCO2. However, all three papers (Endres et al., Under et al., Wannicke et al.) interpret the data based on the low, medium and high pCO2 treatments. This definition may make the data interpretation complicated. Despite the original plan, they could not achieve the target pCO2. The authors need to clarify how to interpret the results with regard to the CO2 manipulation. An example is (i) parameter X is statistically different different between the low pCO2 vs. the medium & high pCO2 (e.g. low < medium = high), (ii) parameter Y is statistically different between the low & medium pCO2 vs. the high pCO2 (e.g. low = medium < high), and (iii) parameter Z is statistically different between all the three treatments (e.g. low < medium < high), whereas both pH and CT were not statistically different between the low and medium pCO2. How do the authors interpret them with regard to the pCO2 manipulation?

The authors used GF/F filters for inorganic nutrients, DOC, TDN, POC, and PON, while they used 0.2 μm CA filters for total and dissolved phosphorus and POP (difference between TP and DP), dATP, dPL-P, dDNA, dRNA, and 0.2 μm PC filters for 33P-PO4

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uptake by Nodularia. Provided that GF/F filters have a nominal pore size of 0.7 μm, the difference between GF/F and 0.2 μm filters may be trivial. However, this seems an inconsistency in the experimental analysis. the authors can clarify why they needed to use 0.2 μm CA and PC filters for P-related measurements.

The calculation of the transformation of PO4 into DOP by Nodularia is interesting (P14727, L9–L12, Table 5). However, the authors did not explain the hypothesized mechanism and the calculation in Materials and methods. In Table 5, it is unclear what "the quantity of DIP (nmol lˆ-1) transformed into DOP" exactly means. Please clarify if it means the amount at a given day or total amount accumulated by a given day (by day 3, day 9, 15 day) or else. The results were not discussed with regard to the pCO2 effect in the discussion section.

Other detailed comments

P14713, L9–L10: Please add reference for "it has long been assumed that the P cycle was not directly affected by rising ocean pCO2."

P14714, L12: According to Wannicke et al. (2012), the culture of N. spumigena was axenic.

P14715, L9–10: Without mentioning the correspondence between the actual time and the light/dark cycle in the incubator, the description of the sampling time (08:00 and 09:00 am) is not really informative.

P14715, L21–P14716, L3 & P14721, L18–P14722, L6 & Fig. 1: The data on carbonate chemistry have already been presented in Wannicke et al. (2012). The authors should cite Wannicke et al. (2012).

P14716, L5–L25 & P14722, L7–L19 & Fig. 2: The data on N. spumigena abundance and chlorophyll concentration have already been presented in Wannicke et al. (2012). The authors should cite Wannicke et al. (2012).

P14717, L1–L12 & P14723, L9–L10 & Table 1: The data on DIP/PO4 concentration

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have already been presented in Wannicke et al. (2012). The authors should cite Wannicke et al. (2012).

P14717, L13–L14718 & L7 P14723, the last paragraph & Table 1: The data on DOP concentration have already been presented in Wannicke et al. (2012). The authors should cite Wannicke et al. (2012).

P14721, L14–L15: It is unclear why the authors used Spearman's rank test for correlation analysis. If the normality of the data is accepted, Pearson correlation test can be applied.

P14722, L17–19: The authors mention "During the total time of the experiment, Nodularia abundance positively correlated with Chl a, Nodularia-P, POC, and PON ($|R|$ = 0.741, 0.86, 0.841, and 0.888, $p < 0.001$, $n = 36$)." This sentence seems similar to the one mentioned by Wannicke et al. (2012): "Nodularia abundance correlated significantly positive with chlorophyll a, POC, PON and POP (R2 = 074, 0.83, 0.88 and 0.88, $p < 0.01$, $n = 12$)." (P2979, the left column).

P14722, L23–L27: Please specify if the authors counted free-living bacteria and/or Nodularia-attached bacteria. According to Wannicke et al. (2012), Nodularia culture was originally axenic. This may suggest that the bacterial contamination occurred during the experimental set up. However the authors mention that there was no significant increase in abundance of bacteria during the experiment. Do the authors any comments on the timing of contamination and no significant increase of bacterial abundance during 15 days?

P14723, L18–L20: APA measurement was not explained in Materials & methods, but fully described in Endres et al. (2012, Biogeosciences Discussions).

P14724, L19–L20: The difference in concentration of dPL-P seems unexpectedly large between the treatments on day 0, despite the same experimental set-up except for the CO2 manipulation.

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P14727, L3–L4: The sentence should be rewritten to clarify why P-turnover was faster under medium and high than under low pCO2 conditions.

P14727, L7: "The phosphorus decrease of 6%" means "the Nodularia-P"? Please specify.

P14727, L10–L12: Please explain how the authors calculated DIP transformed into DOP by Nodularia in this experiment.

P14728, L6–L8: It is difficult to understand how differences in growth rate between the treatments can explain high amount of 33P retention in the low and high pCO2 compared to the medium pCO2.

P14728, L17–L19: Please add reference for the current pCO2 in the central Baltic Sea.

P14729, L8–L9: For the enhanced P-demand of Nodularia with increasing pCO2, statistically higher P uptake by Nodularia in the high pCO2 compared to the low pCO2 should be straightforward.

P14729, L24–L28: Please clarify what significant correlation between two parameters suggest, and what kind of significant correlation supports the importance of uncharacterized DOP for P-nutrition in Nodularia in this experiment. A negative correlation between uncharacterized DOP and APA and a positive correlation between uncharacterized DOP and DOP, how to interpret them together?

P14730, L1–L22: The authors should acknowledge that they measured dPL-P in aged, UV-light irradiated, filtered Baltic seawater. How much does it represent in situ dPL-P?

P14731, L4: Uptake of dDNA-P by Nodularia was not directly measured in this study. It seems that the authors suggest "the observed uptake of dDNA-P by Nodularia" from the decrease of dDNA-P from day 0 to day 3. However, Nodularia were likely not P-limited during this period (note that 0.35 μM of PO4 was added on day 0 and day 3).

P14731, L18–L20: Please clarify this sentence.

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P14732, L9–L10: Please clarify if "the turnover of dDNA-P and other DOP compounds is very short" is derived from this study or other study.

P14732, L22–L24: It is unclear why the authors used "dRNA-P production" in the low treatment and dRNA-P "release" in the high treatment.

P14732, L26–27: Even if DIP is limited, DOP will be produced by cell death or viral lysis in Nodularia culture.

Fig. 1: It is difficult to understand the statistical differences between the pCO2 treatments. Please indicate the statistical results in the figure. For example, using alphabets (a, b, c), the same alphabets mean statistically insignificant difference.

Interactive comment on Biogeosciences Discuss., 9, 14709, 2012.