

## ***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 #1

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: 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.

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Discussion Paper



(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.

Reply 1): We agree with this comment and added the relevant references to the manuscript.

2)Referee: According to Wannicke et al. (2012, Biogeosciences), "the pCO<sub>2</sub> treatments differed significantly in pH and CT between mid and high pCO<sub>2</sub> treatment, as well as between the low and high pCO<sub>2</sub> treatment (p0.001, n=12, Supplement Table S2)." This means that pH and CT were not significantly different between the low and mid pCO<sub>2</sub> treatments. Hence, it appears that this experiment had two low pCO<sub>2</sub> and one high pCO<sub>2</sub>. However, all three papers (Endres et al., Under et al., Wannicke et al.) interpret the data based on the low, medium and high pCO<sub>2</sub> treatments. This definition may make the data interpretation complicated. Despite the original plan, they could not achieve the target pCO<sub>2</sub>. The authors need to clarify how to interpret the results with regard to the CO<sub>2</sub> manipulation. An example is (i) parameter X is statistically different between the low pCO<sub>2</sub> vs. the medium & high pCO<sub>2</sub> (e.g. low < medium = high), (ii) parameter Y is statistically different between the low & medium pCO<sub>2</sub> vs. the high pCO<sub>2</sub> (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 pCO<sub>2</sub>. How do the authors interpret them with regard to the pCO<sub>2</sub> manipulation?

Reply 2): We know that we have to be careful with our interpretation regarding pCO<sub>2</sub> manipulation. From the present point of view it would be better e. g. to compare the low with the high treatment. However, in consistency with the two accompanying publications of Wannicke et al. (2012) and Endres et al. (2012) we cannot completely change the interpretation of our results with regard to CO<sub>2</sub> manipulation. Even though we could not achieve the target pCO<sub>2</sub> values, we can clearly state that we have aerated the batch cultures continuously (once a day) with the respective pCO<sub>2</sub> gases of

Interactive  
Comment

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Interactive Discussion

Discussion Paper



180 ppm, 380 ppm, and 780 ppm. As mentioned in Wannicke et al. (2012) 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>. Furthermore, there were statistically significant differences between the low and the medium pCO<sub>2</sub> treatment in growth rate, and biomass specific C fixation. So, even the small mean difference between the two lowest pCO<sub>2</sub> treatments (median of 315  $\mu\text{atm}$  and 353  $\mu\text{atm}$ ) had an effect on biological parameters, despite the high overall variance. Additionally, carbonate chemistry might have been altered by cellular carbon uptake of *Nodularia spumigena*.

3)Referee: The authors used GF/F filters for inorganic nutrients, DOC, TDN, POC, and PON, while they used 0.2  $\mu\text{m}$  CA filters for total and dissolved phosphorus and POP (difference between TP and DP), dATP, dPL-P, dDNA, dRNA, and 0.2  $\mu\text{m}$  PC filters for 33P-PO<sub>4</sub> uptake by *Nodularia*. Provided that GF/F filters have a nominal pore size of 0.7  $\mu\text{m}$ , the difference between GF/F and 0.2  $\mu\text{m}$  filters may be trivial. However, this seems an inconsistency in the experimental analysis. the authors can clarify why they needed to use 0.2  $\mu\text{m}$  CA and PC filters for P-related measurements.

Reply 3): That is correct. Filters of different material were used in the experiment. In marine research, GF/F filters are predominantly used to distinguish between dissolved and particulate material. On the one hand, POC has to be measured on pre-combusted GF/F filters (due to their combustible matrix used for the element analyzer), while on the other hand, DOP is often measured in filtrates  $<0.2 \mu\text{m}$  (e.g., CA filters) because it represents the dissolved fraction more exact than GF/F filters (Karl and Björkman, in: Hansell and Carlson, 2002). However, Ruttenberg and Dyhrman (2005) as well as Raimbault et al. (2008) did not find differences between GF/F and 0.2  $\mu\text{m}$  filtrates. In own previous experiments the contribution of picoplankton in the fraction  $<0.8 \mu\text{m}$  to the phosphorus pool was below the detection limit of the method. However, picoplankton contains ATP, PL, DNA and RNA and concentrations of these compounds are orders of magnitude lower than total DOP. Here, bacteria can really interfere. Therefore, we decided to use 0.2  $\mu\text{m}$  CA filters. 0.2  $\mu\text{m}$  CA filters are more convenient than PC filters

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for filtration of higher quantities. We used PC filters for the [ $^{33}\text{P}$ ] samples because CA filters are not convenient due to non-specific absorption of  $^{33}\text{P}$ - $\text{PO}_4$  which is lowest by filtration on PC filters.

Karl, D. M. and Björkman, K. M.: Dynamics of Dissolved Organic Phosphorus, in: Hansell, D. A., and Carlson, C. A. (eds): Biogeochemistry of Marine Dissolved Organic Matter, Academic Press, Amsterdam, 249-366, 2002.

Ruttenberg, K. C., and Dyhrman, S. T.: Temporal and spatial variability of dissolved organic and inorganic phosphorus, and metrics of phosphorus bioavailability in an upwelling-dominated coastal system, *J. Geophys. Res.*, 110, C10S13, doi:10.1029/2004JC002837, 2005.

Raimbault, P., Garcia, N., and Cerutti, F.: Distribution of inorganic and organic nutrients in the South Pacific Ocean – evidence for long-term accumulation of organic matter in nitrogen-depleted waters, *Biogeosciences*, 5, 281-298, 2008.

4.1)Referee: The calculation of the transformation of  $\text{PO}_4$  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.

Reply 4.1): The explanations in the “Material and methods” section (P14720, L21-P14721, L3) “Filtrate 1 contained inorganic and organic bound [ $^{33}\text{P}$ ] $\text{PO}_4$ ; in filtrate 2, organic phosphorus was removed on activated charcoal, leaving only inorganic [ $^{33}\text{P}$ ] $\text{PO}_4$ . Organic, bound [ $^{33}\text{P}$ ] $\text{PO}_4$  was calculated as the difference between filtrates 1 and 2.” were difficult to understand. The paragraph in the “Material and methods” section was partly rewritten.

Changes within the text:

P14720, L21- P14721, L3: “The activity (cpm) on the filters is that [ $^{33}\text{P}$ ] $\text{PO}_4$  incorporated by *Nodularia*. The filtrate contained [ $^{33}\text{P}$ ] $\text{PO}_4$  which was not taken up (or released again) and [ $^{33}\text{P}$ ] released as DOP. To distinguish between these two dis-

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solved phosphorus forms, the method described by Ammerman (1993) for the uptake of dissolved ATP was applied. Activated charcoal absorbs dissolved organic matter including [ $^{33}\text{P}$ ]DOP, [ $^{33}\text{P}$ ]PO $_4$  remained in the dissolved fraction. To detect the total dissolved activity in the filtrate <0.2  $\mu\text{m}$  (filtrate 1), 1 ml was transferred into scintillation vials for counting. Activated charcoal (20 mg) and 1 ml 0.03N H $_2$ SO $_4$  were then added to the remaining 4-ml filtrate; the mixture was shaken for 15 min and then filtered through 0.45  $\mu\text{m}$  filters to remove charcoal with the absorbed DOP on the filters. One ml of the 0.45  $\mu\text{m}$  filtrate (filtrate 2) was counted again. Organic bound [ $^{33}\text{P}$ ]PO $_4$  was calculated as the difference between filtrates 1 and 2.”

The contribution of the fractions to the total activity has been calculated using following formulas:

[ $^{33}\text{P}$ ]DOP (%):

$$\left( \frac{\text{cpm (filtrate 1)} - \text{cpm (filtrate 2)} \times 25/100}{\text{cpm (filters + filtrate 1)}} \right) \times 100$$

[ $^{33}\text{P}$ ]Nodularia (%):

$$\frac{\text{cpm filter} \times 100}{\text{cpm (filters + filtrate 1)}}$$

dissolved [ $^{33}\text{P}$ ]PO $_4$  (%):

$$\left( \frac{\text{cpm (filtrate 2)} - \text{cpm (filtrate 1)} \times 25/100}{\text{cpm (filters + filtrate 1)}} \right) \times 100$$

4.2)Referee: In Table 5, it is unclear what "the quantity of DIP (nmol l $^{-1}$ ) 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.

Reply 4.2): We have revised the table heading. “Table 5. Quantity of DIP (nmol l $^{-1}$ ) occurred as DOP of *N. spumigena* calculated from the proportion of [ $^{33}\text{P}$ ]DOP to total [ $^{33}\text{P}$ ] at each sampling day using the initial DIP concentrations + DIP concentration added at day 3.”

4.3)Referee: The results were not discussed with regard to the pCO $_2$  effect in the

discussion section.

Reply 4.3): We have inserted a brief discussion of the results from Table 5 at the end of chapter 4.2 (P1429, L28). “Phytoplankton is assumed to be the main producer of DOP as supported by e.g. high DOP concentrations detected during a spring bloom (Lomas et al., 2010). Deduced from the [<sup>33</sup>P]-experiments, *Nodularia spumigena* released DOP in nanomolar concentrations (Table 5) under DIP depleted conditions, which can hardly be detected by pool size measurements. CO<sub>2</sub> dependent variations seem to be influenced rather by the P demand of *Nodularia* which was enhanced in the medium and high treatment compared to the low one at day 3. The higher formation of DOP in these treatments at day 15 could be explained by a possible progression of senescence, as visible by *Nodularia* growth, whereas in the low treatment DIP incorporated in *Nodularia* was still high (Fig.5). Thus, the influence of CO<sub>2</sub> on DOP formation seems to be of indirect nature”.

Lomas, M. W., Burke, A. I., Lomas, D. A., Bell, D. W., Shen, C., Dyrhman, S. T., and Ammerman, J. W.: Sargasso Sea phosphorus biogeochemistry: an important role for dissolved organic phosphorus (DOP), *Biogeosciences*, 7, 695-710, 2010.

Other detailed comments:

1) P14713, L9–L10: Please add reference for "it has long been assumed that the P cycle was not directly affected by rising ocean pCO<sub>2</sub>."

Reply 1): We rewrote the statement and added a reference.

“So far, there has been little research on the effects of elevated pCO<sub>2</sub> on the marine P cycle as stated in the review of Hutchins et al. (2009). Based on available literature it seems more likely that the P cycle is not directly affected by rising ocean pCO<sub>2</sub> (Hutchins et al., 2009 and literature therein).”

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

Full Screen / Esc

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Interactive Discussion

Discussion Paper

Reply 2): We added “axenic” to the text:

“Three weeks prior to the start of the experiment, axenic parent cultures of *Nodularia spumigena*. . .”

and started the subsection 2.1 with:

“The experimental design and preparation is described in detail in Wannicke et al. (2012). In the following we are giving a short overview.”

3) 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.

Reply 3): We removed this sentence.

4) 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).

Reply 4): We added the reference to the mentioned paragraphs and Fig. 1.

P14715, L21 – P14716, L3: “The carbonate system was characterized as described in Wannicke et al. (2012).”

P14721, L18 – P14722, L6: “As presented in Wannicke et al. (2012) average pH values. . .” Fig. 1: “. . .Values used are according to Wannicke et al. (2012, Table 1).”

5) 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).

Reply 5): We added the references to the mentioned paragraphs and replaced Fig. 2.

P14716, L5-L25: “A more detailed description of determining biomass and cells counts are given in Wannicke et al. (2012).”

P14722, L7-L19: We rewrote this paragraph.

“A detailed description of Nodularia abundance, filament length, number of heterocysts, and chlorophyll a (Chl a) in response to changing pCO<sub>2</sub> is given in Wannicke et al. (2012). Briefly summarized, the abundance of Nodularia spumigena increased by a factor of 2.5, 3.4, and 8.5 in the low, medium, and high pCO<sub>2</sub> treatment, respectively, until day 9. Afterwards, cyanobacterial growth under low and medium pCO<sub>2</sub> proceeded at a lower rate. At high pCO<sub>2</sub>, the abundance declined slightly. A similar trend was observed for Chl a in the 10 l batch and in the 0.5 l [<sup>33</sup>P] bottles (large and small, respectively) (Fig. 2). The Chl a concentrations increased by a factor of 6.1 (large) and 4.2 (small) at low pCO<sub>2</sub>, 5.9 (large) and 6.2 (small) at medium pCO<sub>2</sub>, and 10.2 (large) and 9.1 (small) at high pCO<sub>2</sub> until day 9 and then dropped, regardless of the CO<sub>2</sub> concentration. Accordingly, the period between day 0 and day 9 was considered to be the growth phase (Fig. 2). A comparison of the growth parameters in the large and the small bottles showed significant correlations ( $p < 0.001$ ,  $n = 36$ ) for Chl a ( $|R| = 0.691$ ), POC ( $|R| = 0.698$ ), PON ( $|R| = 0.682$ ), and Nodularia-P ( $|R| = 0.765$ ).“

Fig.2: Comparison of the Chlorophyll a distribution over incubation time and for the different pCO<sub>2</sub> treatments (low = white bars, medium = grey bars, high = black bars) in the 10 l batch bottles (a) (as shown by Wannicke et al., 2012; Endres et al., 2012), and in the 0.5 l [<sup>33</sup>P] bottles (b) (mean values and the respective standard deviation of 3 replicates).

6) P14717, L1–L12 & P14723, L9–L10 & Table 1: The data on DIP/PO<sub>4</sub> concentration have already been presented in Wannicke et al. (2012). The authors should cite Wannicke et al. (2012).

Reply 6): We added the reference to the additional paragraphs and to Table 1.

P14717, L1-L12: “Inorganic nutrients were determined as reported in Wannicke et al. (2012). Briefly, . . .”

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9, C7365–C7380, 2013

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Discussion Paper





P14723, L9-L10: "From day 3 onwards, DIP concentrations were below the detection limit (Wannicke et al., 2012, Table 2; this publication, Table 1)."

Table 1: "...Values of DIP and DOP are according to Wannicke et al. (2012)."

7) 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).

Reply 7): We added the reference to the additional paragraphs and Table 1.

P14717, L13- P14718, L7: "Organic matter analyses were processed as described in Wannicke et al. (2012). A brief description is given below."

P14723, last paragraph: "DOP concentrations (Wannicke et al., 2012, Table 2; this study, Table 1) decreased from day 0 onwards."

Table 1: "...Values of DIP and DOP are according to Wannicke et al. (2012)."

8) 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.

Reply 8): Only 4 out of 22 data sets tested were normally distributed. So we decided to use the Spearman's rank test.

9) 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 ( $|R| = 0.741, 0.86, 0.841, \text{ and } 0.888, p < 0.001, n = 36$ )."

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 ( $R^2 = 0.74, 0.83, 0.88 \text{ and } 0.88, p < 0.01, n = 12$ )."

(P2979, the left column).

Reply 9): We removed this sentence to avoid double data presentation. Furthermore, we rewrote the paragraph (see "other detailed comments - 5").

10) 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?

Reply 10): Although the Nodularia culture was originally axenic, we cannot completely exclude the possibility of contamination during experimental handling. Therefore, we have checked the culture by counting free living bacteria in a flow cytometer. Wannicke et al. (2012) reported heterotrophic bacteria cell counts were below the blank value of 1000 cells l<sup>-1</sup> at the start of the experiment. So, we can assume that the initial cultures were free of bacteria. In addition, abundances were very low in course of the experiment as they never exceeded 1% of cyanobacterial biomass (Wannicke et al., 2012). Endres et al. (2012) discussed that if bacteria were attached to each other, to Nodularia or to gel particles we might have underestimated the bacterial abundance by flow cytometry. Furthermore, Wannicke et al. (2012) mentioned the possibility of staining non-viable bacteria cells with SYBR GREEN which might have been also included in the enumeration. In conclusion, we think that the determined bacteria cell counts (average of  $4.65 \pm 1.37 \cdot 10^5$  cells l<sup>-1</sup>) had only less or no influence on our measurements.

We added additional information to the “Results” section 3.2: P14722, L23-27: “Heterotrophic bacteria cell counts at the start of the experiment were below the blank value of 1000 cells l<sup>-1</sup> and never exceed 1% of cyanobacterial biomass in course of the experiments (Wannicke et al., 2012). There was no significant increase of heterotrophic bacteria cell numbers over time. Cell numbers on average were  $4.69 \pm 1.64 \cdot 10^5$ ,  $4.54 \pm 1.59 \cdot 10^5$ , and  $4.73 \pm 1.28 \cdot 10^5$  cells l<sup>-1</sup> for the low, medium, and high treatment respectively (Wannicke et al., 2012, Table 2). In Endres et al. (2012) it is discussed that

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if bacteria were attached to each other, to Nodularia or to gel particles we might have underestimated the bacterial abundance by flow cytometry. Furthermore, Wannicke et al. (2012) mentioned the possibility of staining non-viable bacteria cells with SYBR GREEN which might have been included in the enumeration, too. Thus, we assume that heterotrophic bacteria had only less or no influence on our measurements.”

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

Reply 11): We added the following sentence including the reference of Endres et al. (2012) to P14723, L20: “The determination of APA is described and discussed in detail in Endres et al. (2012).”

12) 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 CO<sub>2</sub> manipulation.

Reply 12): We agree with this objection. We had to deal with some problems. (1) At the beginning of the analysis, we unfortunately had phospholipid contaminated chloroform and had to eliminate the first set of samples (including the start concentration on day 0). (2) The standard deviation between the remaining three samples per pCO<sub>2</sub> treatment was nearly as high as the measured concentrations. We hope the explanations are acceptable. Even though the data are not fully verified, we think it gives first indications of the behavior of phospholipids. Additionally, we added the standard deviation to the initial values ( $6.7 \pm 5.7$ ,  $10.5 \pm 4.3$ , and  $15.2 \pm 9.2$  nmol l<sup>-1</sup>).

13) P14727, L3–L4: The sentence should be rewritten to clarify why P-turnover was faster under medium and high than under low pCO<sub>2</sub> conditions. and 14) P14727, L7: “The phosphorus decrease of 6%” means “the Nodularia-P”? Please specify.

Reply to 13) and 14):

The sentences were replaced by:

P14727, L3-L4: “Thus, the transition of Nodularia to the senescent state occurred rather under the medium treatment compared to the other treatments.”

P14727, L5-L8: “The 81% decrease in Nodularia-P detected in the medium treatment was combined with a release of 78% as DIP and 3% as DOP. The decrease of Nodularia-P by 6% in the high treatment consisted of 5% as DIP and to 1% as DOP.”

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

Reply 15): We added an explanation to the “Material and methods” section. For the changes please look at “Main comment 4.1”.

16) 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 pCO<sub>2</sub> compared to the medium pCO<sub>2</sub>.

Reply 16): We rewrote the paragraph (P14728, L5-L12) to clarify this.

“In the [<sup>33</sup>P]-experiments, nearly all of the DIP (95–98 %) was converted to biomass during the growth phase of Nodularia. The uptake seemed to be faster in the medium and high treatment compared to the low treatment, but a higher sampling frequency is necessary to get a clear answer. The subsequent release from Nodularia was lower in the low and high treatment compared to the medium treatment. But the mechanisms behind the delay in P release may be different in both treatments. In the low pCO<sub>2</sub> treatment, growth and [<sup>33</sup>P]PO<sub>4</sub> incorporation were slower than in the high pCO<sub>2</sub> treatment such that senescence, in which P is released, might not have been reached. In contrast, in the high treatment, the greater P demand of Nodularia could have caused the persistence of P in the cells.”

17) P14728, L17–L19: Please add reference for the current pCO<sub>2</sub> in the central Baltic Sea.

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Reply 17): We added the reference of Schneider et al. (2006) to the sentence.

18) P14729, L8–L9: For the enhanced P-demand of Nodularia with increasing pCO<sub>2</sub>, statistically higher P uptake by Nodularia in the high pCO<sub>2</sub> compared to the low pCO<sub>2</sub> should be straightforward.

Reply 18): We agree and removed the sentence.

19) 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?

Reply 19): We added an explanation for the meaning of positive and negative correlation to the “Data and statistical analyses” subsection 2.5.

P14721, L15: “A positive correlation means that both parameters either increase or decrease in concert. A negative correlation implies that one parameter increases while the other one decreases.”

For the importance of uncharacterized DOP for P nutrition of Nodularia it means on the one hand that the uncharacterized DOP decreases while APA increases. This can be seen as an indication for enzymatic degradation of this fraction. On the other hand the positive correlation of uncharacterized DOP and total DOP indicates that the uncharacterized DOP fraction is a favored utilized proportion of total DOP. For clarification we rewrote the passage.

P14729, L24: “The importance of the uncharacterized DOP derives from the fact that it accounted for the bulk of P-nutrition in Nodularia in this study. On the one hand the positive correlation with DOP ( $|R| = 0.932$ ,  $p < 0.001$ ,  $n = 36$ ) hypothesized that the uncharacterized DOP fraction is favored to satisfy the P demand. On the other hand this is supported by the negative correlation with Nodularia-P and APA ( $|R| = -0.82$  and

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-0.681,  $p < 0.001$ ,  $n = 36$ ) which is an indication for the need of P for growth and the enzymatic degradation of this pool.”

20) 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?

Reply 20): We added to P14730, L3: “In the laboratory experiment described herein, the DOP concentration and the composition of aged and UV-light treated Baltic Sea water. . .”.

For the Baltic Sea, so far as we know, are no in situ dPL-P concentrations available. Own measurements of field samples are showing a range of 1.95 nM P to 12 nM P (unpublished data) for several Baltic Sea stations (1-20 m). In situ dPL-P concentrations for other locations are given in Table 3. Suzumura and Ingall (2001, 2004) collected samples from Tokyo Bay, Japan and Corpus Christi Bay, USA as well as from four locations in the Pacific Ocean and determined in situ dPL-P concentration ranging from 0.7 to 6 nM P and from 4 to 17.9 nM P, respectively. Even though, Suzumura and Ingall determined the dPL-P concentration by filtering through  $<0.7 \mu\text{m}$  GF/F, our values ( $0.2 \mu\text{m}$  CA) are comparable to our data as stated above (Main comment 3) and fit the range very well.

21) 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 M of  $\text{PO}_4$  was added on day 0 and day 3).

Reply 21): We agree that “uptake” is misleading. We replaced it by “decrease”. The sentence is now: “This and the observed decrease of dDNA-P in our study lead to the assumption that dDNA-P is rapidly used within hours, as it could not be detected with our sampling strategy.” As samples were taken before another 0.35  $\mu\text{M}$   $\text{PO}_4$  were added, we assume that Nodularia cultures were P limited at that point as  $\text{PO}_4$  concen-

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trations were near the detection limit (Wannicke et al., 2012, Table 2; this manuscript, Table 1).

22) P14731, L18–L20: Please clarify this sentence.

Reply 22): We rewrote the passage.

P14731, L12-22: “Our results demonstrate that the various compounds within the DOP pool developed differentially over time and with pCO<sub>2</sub>. The dynamic of dATP-P seems to be not or only marginally influenced by pCO<sub>2</sub>. Dissolved ATP-P did not differ significantly between pCO<sub>2</sub> treatments at day 3 and day 9, despite a trend towards higher release at low pCO<sub>2</sub> than at medium and high pCO<sub>2</sub> (by a factor of 1.03 and 1.16, respectively). From day 9 to day 15, the decline in dATP-P (by a mean of  $3.04 \pm 0.22$  nmol l<sup>-1</sup>; Fig. 4e) followed the trend of total DOP (by a mean of  $40.04 \pm 14.64$  nmol l<sup>-1</sup>). At this stage (day 15), Nodularia cells were in stationary phase, exhibiting the first signs of decay. With beginning of decay the release of DOP, including dATP, would be expected. Instead, the opposite was observed which suggests the possibility of DOP and dATP utilization in this phase.”

23) 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.

Reply 23): It was not derived from our study. We rewrote the passage and added references for the turnover of dDNA and other DOP compounds.

“The turnover of dDNA (Paul et al., 1987) and other DOP compounds (e.g., dATP, Azam and Hodson, 1977; Björkman and Karl, 2005) is very fast, occurring within hours, so that shorter sampling intervals would have been necessary to estimate the variations.”

In addition, as some confusion arose to referee #2, we changed the beginning of the sentence in L13 from “The authors assumed...” to “Furthermore, they assumed...”

24) 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.

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Reply 24): We appreciate this advice and changed it within text to:

“Over the course of the experiment dRNA-P release was highest in the low treatment whereas it was lowest in the high treatment (41.7 and 12.8 nmol l<sup>-1</sup> dRNA-P, respectively),...”

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

Reply 25): We agree to this comment and changed the text to:

“Overall, DOP is a viable P source besides DIP and a certain intracellular P-pool.”

26) Fig. 1: It is difficult to understand the statistical differences between the pCO<sub>2</sub> treatments. Please indicate the statistical results in the figure. For example, using alphabets (a, b, c), the same alphabets mean statistically insignificant difference.

Reply 26): We are grateful for this advice and included it in figure 1. In contrast to the given example we added symbols and highlighted the statistically significant difference.

The figure legend is now: “Box plot (n=12) of the carbonate system for three CO<sub>2</sub> treatments (low, medium, high). Range of the measured values: (a) pH, (b) CT, and of the calculated values: (c) pCO<sub>2</sub>, (d) AT. The box plots show the range from the start to the end of the experiment (showing each outlier). Solid lines represent the median. Dashed lines represent the mean value. Statistically significant differences are highlighted using symbols: (#) between the low and the medium, (\*) between the medium and the high, and (+) between the low and the high pCO<sub>2</sub> treatment. Values used are according to Wannicke et al. (2012, Table 1).”

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