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Interactive comment on "No observed effect of ocean acidification on nitrogen biogeochemistry in a summer Baltic Sea plankton community" *by* A. J. Paul et al.

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Author response to Reviewer #2 (Anonymous)

We thank the reviewer for the constructive comments on this manuscript. We have taken them on board and our responses to reviewer comments, including potential modifications to the manuscript, are detailed in the following:

REVIEWER COMMENT 1 by Referee #2 (Anonymous): Overall the results show little significant effect of CO2 except for phosphate availability, the implications of which should be considered more in the Discussion and Summary.

Author response: We acknowledge that CO2 seemed to have a minimal effect on the nitrogen cycling as indicated in the title of this manuscript, whereas phosphate availability seemed to be more affected. Please see an accompanying manuscript (Nausch et al., 2015) for coverage and in-depth discussion of the phosphorus pools and cycle, which is now available in Biogeosciences Discussion. We will update this reference.

REVIEWER COMMENT 2 by Referee #2 (Anonymous): Despite an interesting and novel approach to measuring nitrogen fixation in the latter part of the experiment, this was unfortunately confounded by contaminated isotopically-labelled dinitrogen. Frustrating as it is, particularly after what must have been a lot of hard work, the methodology and post-t21 results and interpretation on nitrogen fixation should be removed, as this contamination renders them unusable and confusing.

Author response: In accordance with the suggestions by Referee #2, we will remove Figure 2 and Figure 6 from the manuscript, along with the reference and discussion of these in the text body, and will put them as supplementary materials in the revised manuscript. Interpretation of N2-fixation rates is only made up until t21 where the data is not contaminated and afterwards any indication is from either P*, N pool sizes or abundance of A. flos-aquae. While these data is not conclusive on the effect of ocean acidification on N2-fixation for this study, these are indicators which we feel are reasonable to present and discuss in this manuscript in relation to N2-fixation for the period where the estimated rates from incubations were affected by the contamination issue.

REVIEWER COMMENT 3 by Referee #2 (Anonymous): Also, the reasons for the low nitrogen fixation rate and biomass of Aphanizomenon flos-aquae in the mesocosms relative to the surrounding water remain unclear. This may reflect an artefact of the mesocosms, or the possibility that, as the mesocosm water was initially filtered at 50um this may have removed some of the Aphanizomenon flos-aquae, particularly the colonial forms. This could then explain the observed low densities and nitrogen fixation rates relative to water outside the mesocosms.

Author response: Please refer to Author response to Comment 4 by Reviewer #2 below.

METHODS

REVIEWER COMMENT 4 by Referee #2 (Anonymous): Filtration at 50 μm may have excluded the large, colonial nitrogen-fixers. Aphanizomenon flos-aquae is a reasonably large filamentous cyanobacterium, particularly when in colonial form, and the low biomass in the mesocosms, below that of the surrounding water, may reflect removal of a proportion of the A. flos-aquae biomass during mesocosm filling.

Author response: Only the water used for the CO2 enrichment was filtered at 50 μm, whereas a net of 3 mm mesh size covered both ends of the mesocosm bag as the bottom end was lowered and the upper end pulled above the water surface during mesocosm closure. These details are described in depth in Paul et al. 2015. While we cannot exclude the possibility that this 3 mm net may have removed some of the A. flos-aquae biomass during the lowering of the mesocosm bags, this was done only at the beginning of the experiment when there were no filamentous cyanobacteria aggregates visible in the Tvärminne Störfjärden. It is more likely that the upwelling of phosphate-rich water outside the mesocosm around t17 encouraged growth of A. flos-aquae, rather than the removal of A. flos-aquae during mesocosm closure. This stimulation could not happen inside the mesocosms as they were tightly sealed and no phosphate-rich water was introduced and no nutrients were added.

REVIEWER COMMENT 5 by Referee #2 (Anonymous): The text should clarify that the nitrogen fixation techniques were modified from that of Mohr et al (2010).

Author response: We will change p. 17513 (line 14-15) in the revised manuscript to read: 'Incubations for determination of N2-fixation rates were carried out using an approach described by Mohr et al. (2010), with some modifications for the preparation of the 15N-N2 enriched seawater (see Section 2.3 for details).'

REVIEWER COMMENT 6 by Referee #2 (Anonymous): The replacement of 70-90mL

of water with degassed water and, to a lesser extent, the sampling & transfer of water samples, would have reduced the CO2 content and raised pH of the incubation samples. Was pH measured before or after the nitrogen fixation incubations?

Author response: This is an important point to consider when using this degassing method in ocean acidification studies. While pH was not measured in individual incubation bottles, the effect of degassing on the carbonate system was investigated during a different experiment also in the Baltic Sea (A. Paul, unpublished data). Dissolved organic carbon (DIC) concentrations were determined in samples before and after degassing. The reduction in DIC in water from the south-western Baltic Sea (S = 15.3, T = 22.5°C, TA = 1965.5 μmol kg-1) by the degassing system was on the order of 100 μmol kg-1, less than 10% of total DIC ($\sim$1800 μmol kg-1), using the same method as in the study presented here. Note that a water sample can be stripped of many dissolved gases relatively quickly. For CO2 however, it will take considerably longer as most DIC is present in the form of bicarbonate and carbonate.

In the incubations reported in this manuscript (northern, central Baltic Sea, S = 5.7, T $\sim$ 8.0– 15.9°C, TA $\sim$1520 μmol kg-1), about 70 mL of this degassed water with reduced DIC was added. Assuming a similar amount of DIC was removed (100 μmol kg-1), this corresponds to a calculated decrease in DIC of $\sim$3 μmol kg-1 in each incubation bottle. This is insignificant for fCO2 levels considering the range applied in our study. Due to the lower alkalinity present in this study, the amount of DIC removed through the degassing procedure may lead to decreased DIC in the incubation bottles of slightly more than 3 μmol kg-1 . However this would still be on the same order of magnitude as for the other study from the Baltic Sea, hence we do not consider that this would have a substantial effect on pH or CO2 content these incubations.

REVIEWER COMMENT 7 by Referee #2 (Anonymous): Figure 2 shows the 15N-N2 enriched seawater entering the overflow system & degassing. Should the 15N-N2 supply line connect to the airstone in the overflow system, rather than the 15N-N2 enriched seawater?

Author response: Figure 2 is correctly shown but will be moved in the revised manuscript to the supplementary materials in accordance with suggestions by Reviewer #2 (see also Author response to Comment 2 by Reviewer #2).

REVIEWER COMMENT 8 by Referee #2 (Anonymous): What was the final atom% 15N-N2 in the mesocosms following addition of isotopically labelled N2 at t21?

Author response: Peak enrichment of 15N in N2 after 2nd addition of the isotopically labelled N2 on t27 ranged between 0.53 and 0.57 atom%.

RESULTS

REVIEWER COMMENT 9 by Referee #2 (Anonymous): In Fig 1f both the key and the ammonium data are too small to read. As the key is important it should be larger, and ideally replicated on the other timeline figures.

Author response: The inserted legend will be removed and added to a separate panel in the figure to improve readability in the revised manuscript. The colour/symbol legend will be added to all figures in the revised manuscript.

REVIEWER COMMENT 10 by Referee #2 (Anonymous): In Fig 3a the increase in P* in Phase II occurs only in some of the treatments at the onset of Phase II, and otherwise Phase II is dominated by uniform concentrations, so the description is incorrect. The increase in P* in Phase III is similar to Phase II, if not more significant.

Author response: The statement in the manuscript refers to inorganic phosphate concentrations shown in Fig. 1d. We agree that this point was not clearly explained and thank Reviewer #2 for bringing this up. This variation may also be partly masked by the choice of scale so that the Baltic Sea data could also be shown. To clarify this point, this statement will be rephrased in the revised manuscript and will read (p.17517, line 22-23): 'Inorganic phosphate concentrations decreased during Phase I, followed by an increase at the beginning of Phase II. Concentrations in the ambient/control treatments remained higher than in the higher CO2 treatments in Phase III.'

REVIEWER COMMENT 11 by Referee #2 (Anonymous): "Nitrate concentrations increased throughout the experiment with a possible small drawdown after t39 in all treatments" – this drawdown is not really evident in Fig 3c.

Author response: This will be removed in a revised version of this manuscript to read (p. 17517, line 23-25): 'Nitrate concentrations increased slightly throughout the experiment, whereas NH4+ concentrations were variable'.

REVIEWER COMMENT 12 by Referee #2 (Anonymous): What is the source of the spikes in nitrate concentration?

Author response: This is likely related to the low concentrations observed. The nitrate concentrations were typically in the nanomolar range and therefore sampling and sample handling in the mesocosm environment is challenging. It is therefore likely that this variable is prone to unintended contamination during sampling and sample handling.

REVIEWER COMMENT 13 by Referee #2 (Anonymous): Perhaps combine Figs 1 and 3 to allow comparison, & also to reflect the text in the Results section. The rainfall data is not required as there was no relationship with measured variables.

Author response: As suggested, we will remove the panel with the rainfall data and instead combine the two panels from Figure 3.

REVIEWER COMMENT 14 by Referee #2 (Anonymous): "BSi in Phase II where a positive effect was detected ($p = 0.034$)" – why not include this in Table 1?

Author response: This data is presented in an accompanying paper (Paul et al., 2015) as cited on p. 17518 (line 18) and was thus not included in the table. Only new data and results of new statistical analyses presented here were included in Table 1. In the revised manuscript, these data (as well as PON, see also Comment 20 by Reviewer #2) will be included in Table 1. An asterisk (*) will be added to these data points to indicate the statistical analyses and data originate from Paul et al. 2015.

REVIEWER COMMENT 15 by Referee #2 (Anonymous): "A. flos-aquae abundances

. . .. were highest in Phases II/III and lowest in Phase I" – sporadic spikes in certain treatments were higher in Phase III than Phase I, but overall Fig 5d shows similar A. flos-aquae abundances in Phase I & III.

Author response: This statement was made based on the abundances of A. flos-aquae determined by microscopy counts (Fig. 5a). Nonetheless it is correct that the temporal variation in abundances of A. flos-aquae and the Aphanizophyll marker pigment concentrations do not fit exactly together. This may be in part influenced by missing data points in Aphanizophyll concentrations on t35 and t39 where the microscopy counts indicate peaks in A. flos-aquae biomass. In a revised figure, we will remove the connecting lines in the Aphanizophyll marker pigment panel (1c) so this issue becomes clearer.

REVIEWER COMMENT 16 by Referee #2 (Anonymous): Although the 15N-N2 addition to the mesocosms (from t21) is interesting, the authors identify that these results are unusable due to gas contamination, and so the methodology and results (including Figure 6) should be omitted from the paper, as they do not assist the discussion and interpretation.

Author response: Figure 6 will be moved to the supplementary materials in the revised manuscript. Please also see Author response to Comment 2 by Reviewer #2.

REVIEWER COMMENT 17 by Referee #2 (Anonymous): "This indicated potential input of atmospheric N with a low d15N into particulate matter via N2-fixation. . .". The authors should consider that this could alternatively reflect the uptake of ammonium depleted in 15N produced during ammonification.

Author response: Ammonification is a process which likely occurred in this study, although this was not quantified. Thus, this may have influenced the δ15N signal in particulate matter reported here through production of ammonium depleted in 15N and consequent assimilation into the PON pool. We thank Reviewer #2 for commenting on this and will add this point to the revised manuscript to p. 17520, line 5-6 to read:

'This indicated either potential input of atmospheric N with a low δ15N into particulate matter via N2-fixation during this period or potential uptake of ammonium with a δ15N signature depleted through ammonification.

REVIEWER COMMENT 18 by Referee #2 (Anonymous): "This was one day after the mesocosm walls were cleaned indicating that there were likely diazotrophic species and diatoms attached to the mesocosm walls". Was this the only time the mesocosm walls were cleaned (in Paul et al, 2015, it mentions "Mesocosm bags were cleaned occasionally inside and outside throughout the experiment"), If not, were other trap samples affected on other days? Should the Aphanizophyll spike on t15 be regarded as an artefact?

Author response: The mesocosm bags were cleaned for the first time on t16 with cleaning occurring more regularly after this (t22/23, t29, t36 and t42) as detailed in Paul et al. 2015 (refer to Figure 3). Hence the effect of wall-growth on the material collected in the sediment traps was probably more important on t17 ($\sim$2.5 weeks of growth) than on the other sampling days after cleaning.

REVIEWER COMMENT 19 by Referee #2 (Anonymous): "The assessment for between t23 and t43 is based on the premise of continued elevated . . .. – why not just do this comparison up to t21 to remove any uncertainty?

Author response: As suggested, we calculated the N input up until t21. Using the same method as for between t23 and t43, a mean N2-fixation rate of 0.57 nmol N L-1 day-1 over 24 days (t-3 – t21) and an assumed 50% exudation of DON/NH4+, leads to a calculated N input of 27.55 nmol N. This is a little higher than the estimate from isotope uptake incubations of 20 nmol N. Nonetheless we believe that this is still in reasonable agreement with the original estimation, particularly considering the variability between mesocosms in both N2-fixation rates and Aphanizomenon flos-aquae abundances.

DISCUSSION

REVIEWER COMMENT 20 by Referee #2 (Anonymous): "The only statistically significant, but very minor, correlation was a positive relationship between CO2 and PON concentrations" – why not include this in Table 1?

Author response: This data will be included in Table 1 in the revised manuscript. Please also see Author response to Comment 15 by Reviewer #2 above.

REVIEWER COMMENT 21 by Referee #2 (Anonymous): "This is due to the rather low A. flos-aquae biomass . . .. . ." – might this reflect the 50 μm filtration when filling the mesocosms?

Author response: A net with a 3 mm mesh size was used to exclude larger particles and organisms during mesocosm closure, whereas the 50 μm gauze was used only for the water for CO2-enrichment. Please also see Author response to Comment 4 by Reviewer #2 above.

REVIEWER COMMENT 22 by Referee #2 (Anonymous): "Diazotrophic organisms typically have slower growth rates than other organisms. Hence any potential influence of ocean acidification on their physiology may take longer to become apparent in biogeochemical parameters sampled in larger-scale field studies." As growth rates will be the same in the field and the lab, the difference in the response of nitrogen fixation to CO2 from reported lab experiments results more likely reflects ecosystem interactions (grazing, competition, nutrient availability) in field studies.

Author response: It is difficult to say that the growth rates would be the same as in the lab as it is difficult to exactly replicate macro/micro-nutrient availability and light conditions. However we agree that these ecosystem interactions will likely modulate the physiological response to CO2 observed in laboratory studies. To reflect this, we will modify p. 17523 (lines 10-12) in the revised manuscript to read: 'Hence any potential influence of ocean acidification on their physiology may take longer to become apparent in biogeochemical parameters sampled in larger-scale field studies where most sampled parameters such as PON are a mixture of organic compounds of vari-

ous origin and isotopic composition. In addition, the overall response to CO2 observed in such field studies is a combination of the pure physiological response, which can be observed in laboratory experiments, with trophic interactions such as grazing and competition between species for nutrients and light.'

REVIEWER COMMENT 23 by Referee #2 (Anonymous): "Hence natural exposure to highly variable carbonate chemistry conditions..." this is an interesting idea, but does not explain why most of the papers reporting CO2 enhancement of marine N fixation showed it in Trichodesmium (see Hutchins et al papers) which would experience similar highly variable conditions. This caveat should be mentioned.

Author response: We thank the reviewer for pointing out this interesting caveat. We would like to reiterate that the filamentous, heterocystous cyanobacteria species found in the Baltic Sea such as A. flos-aquae have generally shown the opposing physiological response to tropical/subtropical species such as Trichodesmium sp. under high CO2 (as summarised in the Introduction, p17510, lines 1 – 11), despite both being aggregate forming species. Currently, to the best of our knowledge, there is no clear explanation for this inconsistent response to elevated CO2 between cyanobacteria morphologies or species in physiological single-strain culture studies although a variety of hypotheses exist. In addition, we are not aware of any published study on the microenvironments in Trichodesmium aggregates which could be used as a comparison. Hence, to avoid any potential for confusion here and refocus the discussion on the A. flos-aquae, for which there is data on aggregate microenvironments, we will remove the reference to Trichodesmium sp. in the revised manuscript on p.17524 (line 7-14) to read: 'In addition, filamentous cyanobacteria in the Baltic Sea form characteristic surface aggregations. Inside aggregations of A. flos-aquae, microenvironments can create substantially different conditions compared to the surrounding water with large diurnal fluctuations in pH (7.4 vs. 9.0) and O2 concentrations ($\sim$ 150–450 μmol O2 L-1) and thus also inorganic carbon availability (Ploug, 2008). '

REVIEWER COMMENT 24 by Referee #2 (Anonymous): 4.2 What is the explanation

for the coincident increases in PON and nitrate from Phase II to Phase III?

Author response: Perhaps the reviewer means DON here, rather than PON? We do not have a clear reason or mechanism as to why either variable (DON or nitrate) increased over time as no rate measurements were made. We can only speculate that some DON was released through 'sloppy feeding' by zooplankton who were abundant at the time (see Lischka et al., 2015) or nitrate was produced by nitrification in the water column (0 - 17m). We consider it unlikely that these increases are directly related to each other as the increase in nitrate (in the range of tens of nanomol per liter) is an order of magnitude smaller than the apparent increase in DON (in the range of a few hundred nanomol per liter).

SUMMARY

REVIEWER COMMENT 25 by Referee #2 (Anonymous): Bearing in mind the only significant correlation with elevated CO2 was a reduction in phosphate, the Summary should consider the implications of this for future nutrient budgets and productivity in the Baltic Sea.

Author response: As mentioned in Author response to Comment 1 from Reviewer #2, please note that discussion of the phosphorus pools and cycle are discussed in an accompanying paper (Nausch et al., 2015). We also consider that any speculation here, with reference to nutrient budgets and diazotrophic cyanobacteria in the future, is not justified considering the lack of response observed.

REFERENCES:

Lischka, S., Bach, L. T., Schulz, K.-G., and Riebesell, U.: Micro- and mesozooplankton community response to increasing CO2 levels in the Baltic Sea: insights from a large-scale mesocosm experiment, Biogeosciences Discussions, 12, 20025 – 20070, doi:10.5194/bgd-12-20025-2015, 2015.

Nausch, M., Bach, L., Czerny, J., Goldstein, J., Grossart, H. P., Hellemann, D., Hor-

nick, T., Achterberg, E., Schulz, K., and Riebesell, U.: Effects of CO2 perturbation on phosphorus pool sizes and uptake in a mesocosm experiment during a low productive summer season in the northern Baltic Sea, Biogeosciences Discussions, 12, 17543 – 17593, doi:10.5194/bgd-12-17543-2015, 2015, 2015.

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