

## ***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.***

**Anonymous Referee #2**

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This manuscript describes a long-term mesocosm experiment examining the effect of ocean acidification on plankton community structure and nutrient cycling in low-nutrient water in a Baltic fjord. The experiment aimed to establish whether diazotrophy was stimulated by elevated CO<sub>2</sub> in a natural community, and whether any additional new nitrogen influenced phytoplankton biomass and composition. The paper is well-written, although some of the result descriptions are too generalised, and do not always reflect the actual results shown in the Figures. Overall the results show little significant effect of CO<sub>2</sub> except for phosphate availability, the implications of which should be considered more in the Discussion and Summary. Despite an interesting and novel approach to measuring nitrogen fixation in the latter part of the experiment, this was unfortunately

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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. 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 50µm 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. The manuscript should be revised, based upon these and the comments below.

### Methods

Filtration at 50µm 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.

The text should clarify that the nitrogen fixation techniques were modified from that of Mohr et al (2010)

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 CO<sub>2</sub> content and raised pH of the incubation samples. Was pH measured before or after the nitrogen fixation incubations?

Figure 2 shows the <sup>15</sup>N-N<sub>2</sub> enriched seawater entering the overflow system & degassing.

Should the <sup>15</sup>N-N<sub>2</sub> supply line connect to the airstone in the overflow system, rather

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than the 15N-N2 enriched seawater?

What was the final atom% 15N-N2 in the mesocosms following addition of isotopically-labelled N2 at t21?

#### Results

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.

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.

“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.

What is the source of the spikes in nitrate concentration?

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.

“BSi in Phase II where a positive effect was detected ( $p = 0.034$ )” – why not include this in Table 1?

“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

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.

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“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.

“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 Aphanizopyll spike on t15 be regarded as an artefact?

“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?

#### Discussion

“The only statistically significant, but very minor, correlation was a positive relationship between CO2 and PON concentrations” – why not include this in Table 1?

“This is due to the rather low A. flos-aquae biomass. . . . .” – might this reflect the 50 $\mu$ m filtration when filling the mesocosms?

“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.

“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

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would experience similar highly variable conditions. This caveat should be mentioned.

4.2 What is the explanation for the coincident increases in PON and nitrate from Phase II to Phase III?

Summary

Bearing in mind the only significant correlation with elevated CO<sub>2</sub> was a reduction in phosphate, the Summary should consider the implications of this for future nutrient budgets and productivity in the Baltic Sea.

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