Biogeosciences Discuss., 12, C10130–C10137, 2016
www.biogeosciences-discuss.net/12/C10130/2016/

Biogeosciences Discussions
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Interactive comment on "Nitrogen isotopic evidence for a shift from nitrate- to diazotroph-fueled export production in VAHINE mesocosm experiments" *by* A. N. Knapp et al.

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Received and published: 9 March 2016

Overall quality of the manuscript:

Knapp et al. measured the concentration and isotopic composition of various N pools in response to an artificial addition of phosphate that deliberately induced nitrogen fixation in enclosed mesocosms. Based on the 15N-depleted signature of diazotroph derived N (DDN), the authors attempted to track the fate of N supplied by nitrogen fixers into other N pools. The main finding of this study is that DDN was rapidly channeled into sinking particles and showed no accumulation in dissolved N or suspended particulate N pools. Given that various diazotroph had bloomed and were active throughout

the experiments, the latter finding is puzzling. Because sinking particles were the only N pool that exhibited the 15N-depleted signal, the authors conclude that the best geochemical estimates of N-fixation can be achieved by monitoring the d15N of sinking particles. However, as also pointed out by the authors, it is unclear how the findings of the 15N budget determined for this mesocosm study apply to the open ocean.

General Comments:

As pointed out by the first reviewer, it is disturbing that for the same experiments, there are two different data sets reported for the same parameter (i.e., DON concentration). Due to the uncertainty, and lack of an explanation for why the data may differ, the DON concentrations estimated by both studies should be considered in the authors' interpretation of the 15N budget, and perhaps also as a correspondence (i.e., errata) by Berthelot et al., as their main findings stem from these controversial data.

How does N-fixed by diazotrophs immediately sink from the water column without existing as PNsusp? Conceptually, particles that sink from the surface must first be suspended; this would apply to growing populations of plankton. Given the sampling resolution of ca. every other day, the PNsusp reservoir does not appear to be large enough nor to turnover fast enough to obscure the passage of isotopically depleted DDN (up to 0.25 μM) through this reservoir before it sinks. As noted below, the insensitivity of d15N-PNsusp to input by N-fixation is in contrast to previous reports. The authors' final sentence raises a similar question regarding the bias of DDN toward sinking. Unfortunately, none of the companion studies of the VAHINE experiment have identified the composition (e.g., taxa, molecules, etc.) of sinking particles. This information seems to be well within the scope of the current study.

Coincidentally with increased N-fixation in the late stages of the experiment, Synechococcus were shown to be most responsive to the addition of DDN, exhibiting the most substantial increases in non-diazotroph biomass (i.e., more biomass than diatoms; Leblanc et al. 2016; Biogeosciences Discuss., doi:10.5194/bg-2015-605,

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2016). Given their small size and tendency to remain suspended in the water column, relatively to diatoms, it seems unlikely that DDN supporting the Synechococcus bloom would be channeled into PNsink on the rapid timescales invoked by the authors. Moreover, the trend of increasing Synechococcus biomass begins on Day 7, just after the DIP spike and well before the onset of the high N-fixation period of the mesocosm experiment. In summary, DDN uptake is interpreted with respect to how the taxa composition of suspended particles changed in response to the DIP spike and N-fixation, but corresponding information for sinking particles, where the 15N-depleted DDN accumulates, is largely unavailable.

The variability in d15N-PNsink during P2 is largely disregarded by the authors (Page 19915, Line 25; Page 19916, Line 14), who rather focus on the overall trend as the principal finding of this study. But there appears to be a consistent trend among the replicate mesocosms in pulses of 15N-enriched particles sinking on days 15-18 and again on day 20.

The Discussion section should be reorganized, with a brief discussion of the changes observed in community composition (currently in Section 4.2), followed by a discussion of the components of the 15N budget. The current Section 4.1 is too long and builds confusion. It should be divided into smaller digestible sections with appropriate titles (e.g., one section for DON and PNsusp and another for PNsink). The meaning of the current title of Section 4.1 is lost on me.

The discussion of the “missing P” among the mesocosm experiments does not appear to fit within the scope of this manuscript and should be removed. However, the authors could instead comment on how the presumably diazotrophic biofilms, which were proposed to account for “missing P,” could have biased the 15N budget.

The Conclusions section is far too long. Some of the authors’ points are even redundant within this section (e.g., Page 19923, Line 2-3 vs. Page 19924, Lines 19-21). References to other studies throughout this section make it difficult to identify the key

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findings of the current study. Most text following the first paragraph could be removed.

Specific Comments:

METHODS

Please provide more details for the method used to collect PNsusp. What was the pore size of the filter?

Was d15N-DON calculated by mass balance? Please state explicitly.

RESULTS

Page 19911, Lines 3-4 – The logic is not clear. Are you implying that lagoon water has mixed with the mesocosm? Or are there inherent methodological differences, such that the data reported by Berthelot et al. (2015) over the last five days of the experiment are perhaps invalid? What are the methodological differences? As DON is calculated by mass balance in both studies, which of the other parameters (i.e., TN, PN, nitrate, ammonia) were similar or different between these two studies? It seems that TN data for both studies was determined after persulfate oxidation. The values for PNsusp from the current study appear to be similar to those reported as PON by Berthelot et al. (2015). Page 19911, Lines 4-7 – This argument is not so convincing, given the decoupling of DOC and DON reported previously for regions of N-fixation (Abell et al., 2000, 2005), which should be presented together with this statement.

Page 19911, Lines 15-19 – The comparison of 15N-DON to other studies should be moved to the discussion section.

Page 19912, Lines 11-13 – Please point out here that there was much higher variability in d15N-PNsink during P2 compared to the earlier phases of the experiment.

DISCUSSION

Page 19913, Line 6-8 – It remains unclear how PNsusp is decoupled from the N source that fuels export production, particularly when there are significant inputs of “new N.”

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This conclusion is in conflict with previous reports, which have identified PNsusp as a responsive reservoir to changes in N source, as suggested by the variability in d15N-PNsusp in regions of high N-fixation (Mino et al., 2002; Montoya et al., 2002; Mahaffey et al., 2003; Meador et al., 2007). The authors should include these findings and address this discrepancy, especially if the suggestion is that the changes in PNsusp documented by these previous studies are rather attributable to "recycled N."

Page 19914, Line 11-13 – In order to balance the There must be a fraction of PNsusp that is depleted in 15N toward P2 balance the

Page 19914, Line 24 – It would be useful to also note that Trichodesmium is known to produce 15N-depleted DON (Meador et al., 2007).

Page 19914, Line 26-27 – The rapid uptake of DDN by N-limited non-diazotrophs suggests that DDN enters the PNsusp pool, which conflicts with the authors' conclusion that DDN did not accumulate as PNsusp. (see next comment)

Page 19915, Line 11-15 – The addition of 0.25 μM DDN to the DON pool may not alter d15N-DON, but is difficult to explain how DDN could enter the N budget via unicellular n-fixers without existing as PNsusp, which is a smaller reservoir of N (i.e., ca. 1.3 μM during P2). Furthermore, the 0.25μM addition of DDN is similar to the increase in PNsusp concentration between P1 and P2 (Table 1), and would represent a significant fraction of PNsusp (ca. 20%).

Page 19916, Line -14 – The logic is not clear. Is this a continuation of the caveats outlined in the previous paragraph?

Page 19917, Line 17 – The logic is not clear. Please clarify how the "more than half" estimate was derived.

Page19917, Line 20-23 – As above, if PNsusp increased with N-fixation rates from P1 to P2, why isn't the low d15N value of the N supplied to the system imparted to the PNsusp pool? In other words, what is supporting the increase in PNsusp if not the

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supply of isotopically depleted N?

Page 19918, Line 6-10 – It is difficult to envision either of these mechanisms of DDN export without DDN existing, at some point, as PNsusp.

Page 19918, Line 15 – Given that Mino et al. (2002) and Meador et al. (2007) both observed that 15N-PNsusp appeared to be sensitive to N derived from N-fixation, it is difficult to know if this claim extends beyond the scope of the mesocosm study. For example, the "short timescales" referred to here represent a couple of weeks following an artificially induced diazotroph bloom, whereas the signals recorded by PNsusp in the open ocean integrate supply of N on seasonal timescales.

Page 19920, Line > 15 – Most of the text summarizing the P imbalance of the mesocosm does not seem applicable to the current study. The "missing P" observed during this study is independent of the authors' conclusion related to this topic, i.e., that DDN uptake by non-diazotrophs would yield sinking particles that carry an N:P similar to the Redfield ratio. As there is no attempt to balance an N* budget, or a plot C:N:P stoichiometry of the different organic matter pools, I don't understand the need to identify or explain the "missing P." The conclusion derived from this discussion cannot be confirmed and has no application for the mesocosm study as a model for the open ocean. Save for the sentence beginning Page 19920, Line 29 ("Similarly, the N and C sinking fluxes..."), which could be appended to end of the previous paragraph, the discussion beginning here and continuing to the Conclusions section, as well as Fig. 3, could be removed without affecting the impact of this paper.

Page 19921, Line 13-20 – What is the basis for the assumptions of biofilm thickness or coverage of the mesocosm surface area? What are the implications of biofilms comprised by diazotrophs for the 15N budget?

CONCLUSIONS

Page 19924, Line 14-16 – The phrase "strongly suggests" is not well supported by

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authors' inference of diazotrophic DON production, which is largely a result of the lack of a depleted 15N-signal in any organic matter pool other than PNsink, and/or the observations reported in companion studies.

Page 19924, Line 26-27 – I can't think of a better way of answering this question than analyzing the molecular and taxonomic composition of the sinking particles, or repeating the mesocosm experiment.

REFERENCES

Abell, J., S. Emerson, and R. G. Keil. 2005. Using preformed nitrate to infer decadal changes in DOM remineralization in the subtropical North Pacific. Global Biogeochem. Cycles 19, GB1008, doi:10.1029/2004GB002285.

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Interactive comment on Biogeosciences Discuss., 12, 19901, 2015.

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