

## ***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.***

**Anonymous Referee #3**

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### Summary and Evaluation

This paper by Knapp et al. investigates the nitrogen budget of VAHINE mesocosms experiments by analyzing the nitrogen isotopic composition ( $\delta^{15}\text{N}$ ) and concentration of various nitrogen forms in the water and trap samples. They showed that the  $\delta^{15}\text{N}$  values of the sinking particulate nitrogen (PN<sub>sink</sub>) at 15 m depth decreased during the 23 day experiments. In contrast,  $\delta^{15}\text{N}$  values of the suspended PN (PN<sub>susp</sub>) and dissolved organic nitrogen (DON) did not show significant changes. Based on these results, they suggested that the main fate of fixed nitrogen from increased  $\text{N}_2$  fixation stimulated by DIP fertilization was the PN<sub>sink</sub>, not PN<sub>susp</sub> nor DON. In addi-

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tion, based on the results of community composition of phytoplankton and diazotrophs by concurrent studies, they discussed the possibility that nitrogen transfer occurred through dissolved phase from diazotrophs to non-diazotrophs, which could be an important pathway for the nitrogen transport from the surface ocean to the subsurface ocean. I think that, although this paper showed interesting and important datasets, several points listed below need to be amended or considered before publication in Biogeosciences.

### General Comments

1. The authors should note that the term “PN<sub>sink</sub>” in this paper indicates a different thing from that usually used in field studies of oligotrophic oceans. The sampling water depth of PN<sub>sink</sub> of this paper is 15 m, which is much shallower than usual field sampling of PN<sub>sink</sub> in oligotrophic oceans (e.g., ~150 m at St. ALOHA). PN<sub>sink</sub> at 150 m is expected to reflect export flux out of nutrient-depleted euphotic zone via nitracline, but PN<sub>sink</sub> at 15 m may not represent it. The term “export production” would need some caution as well, because PN<sub>sink</sub> at 15 m would only reflect processes in the very upper part of euphotic zone but not in the lower part of euphotic zone. Such limitation of the experimental setup should be clearly stated in Abstract and Introduction. I also think that “export production” is not a suitable term for interpretation of the results of this paper. Readers may be confused by two different “export production” used in the paper: general term “export production” in the euphotic zone of the ocean (~150 m in the usual oligotrophic ocean) vs. special term “export production” in the upper 15 m of this VAHINE experiment.

2. I request the authors to show their individual data as tables (not only figures and averages) in Supplementary Materials (or anywhere else). Because the authors analyzed many samples and obtained interesting data sets, it would be beneficial for research community and future readers of this paper. In addition, because some symbols in the figures of this paper overlap each other and they are difficult to resolve, supplementary tables would help readers to understand the results.

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3. The possibility of the assimilation of DIP by the biofilms (which is discussed in 4.2) is important, and it likely affects the interpretation of d15N budget of this study. If the assimilation of DIP by the biofilms is the primary sink for the “missing” DIP in the mesocosms, as concluded by the authors, it means that large amount of nitrogen was also assimilated by the biofilms (recycled N or N<sub>2</sub> fixation).

A) Assimilation of recycled N by the biofilm: In the early part of 4.1, the authors discussed that DDN did not accumulate in the PNsusp pool in the mesocosms, based on the roughly constant d15N values of PNsusp. However, the d15N values of PNsusp (~3 permil) could be also explained by the addition of heterotrophic biomass which assimilated DDN and experienced trophic nitrogen isotopic fractionation by heterotrophic degradation of organic nitrogen and release of 15N-depleted ammonium (or anything else). Then, this 15N-depleted ammonium would be assimilated by the biofilms. Therefore, I think that, without closing the nitrogen budget of the mesocosms by analyzing d15N value and nitrogen quantity of the biofilm, accumulation of DDN in the PNsusp pool cannot be excluded.

B) N<sub>2</sub> fixation by the biofilm: If significant amount of N<sub>2</sub> fixation was conducted by the biofilm, it also does not support the conclusions of this study that, for example, “the primary fate of newly fixed N in the VAHINE mesocosms experiments was to be converted in to the PNsink flux” (Page 19918 Lines 5–6). Therefore, the authors should estimate the amount of possible assimilation of N by the biofilm as well as P, and should discuss its effects on the discussion and conclusion in 4.1

#### Specific Comments

Page 19910 Lines 16 – Page 19911 Lines 12: Which parameter (TN, PNsusp, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, or else) is likely the main cause of the discrepancy of calculated DON concentration between the two studies? Specifying the main cause may be useful to understand the discrepancy. I’m also wondering whether the cutoff size of PN and DON filtering is same between the two studies.

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Page 19911 Line 21–23: What was the N source for the increased PNsusp concentration? While the authors concluded that DDN was not the N source for the increased PNsusp concentration, it seems that the authors did not suggest alternative N sources. Nitrate is suggested as the origin of the elevated d15N values of PNsusp, but it would not explain the increased PNsusp concentration in the mesocosms, because the water in the mesocosms was depleted in nitrate.

Page 19939: Figure 4 would be more suitable for Supplementary Materials. For me, the biofilms are not so obvious in the photos.

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