

Interactive comment on “Transfer of diazotroph-derived nitrogen to the planktonic food web across gradients of N₂ fixation activity and diversity in the Western Tropical South Pacific” by Mathieu Caffin et al.

Anonymous Referee #2

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In this manuscript Caffin et al. examine transfer of diazotroph-derived (DDN) through the foodweb using ¹⁵N stable isotope probing, comparing sites dominated by *Trichodesmium* with a site dominated by UCYN-B as the dominant diazotroph. They find that over 48h in the UCYN-B dominated station, no DDN was detectable in the dissolved pool, whereas a significant fraction was detectable in the *Trichodesmium* stations. They further characterize DDN to different microbial and zooplankton groups, and find differences between the stations. These results have major ecological implications for our understanding of DDN fate. Overall, I thoroughly enjoyed the manuscript,

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and highly recommend it for publication. I do have a few general questions and suggestions regarding the interpretation of the results and the context those results are put in. I recognize that putting these results in context of the other research done on the same cruise is difficult to carve out one piece to focus on, but I think the manuscript could use some focusing.

- Regarding whether *Tricho* releases recalcitrant N and UCYN-B releases labile N, I'm not sure the data really tells us this. It might mostly be a matter of semantics, and how you define labile and recalcitrant. But for me those terms imply different molecules released by the diazotrophs. From the data I don't think we can rule out that *Tricho* and UCYN-B release the exact same molecules of N, but because of the difference in both the amount of N released and the composition and metabolic state of the resident community, you see different DDN transfer and efficiency. In fact, I think it's interesting, although maybe expected, that you see higher efficiency in the ultra-oligotrophic location, implying that that maybe that community have higher affinity responses and uptake relative to the resident community in the *Tricho* stations. *Prochlorococcus*, for example, is likely to be better at high affinity uptake than *Synechococcus* because of its smaller surface area to volume ratio and adaptation to oligotrophic environments. Maybe this knowledge could help us predict, by knowing community composition and amount of N fixed, how efficient DDN transfer will be?

- One of the points that the authors emphasize is novel is that this is the first open ocean study. But I am not getting the full context for moving to the open ocean-what do the authors expect will be different, other than diazotroph identity? If this is the focus, it would be nice to include an expectation in the introduction—do they expect the open ocean DDN transfer to be different from the other studies of coastal or mesocosms performed before by this group? Or the same? For example, P.4 line 15-what was expected, different or similar to what found for coastal? Also P.4 lines 25-27. Then, I think these experiments help give us a context to predict DDN transfer through the food web, so I would like some more discussion in that context at the end: i.e. Will we need

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to know both diazotroph identity and nutrient conditions to predict DDN transfer? Or other factors? In some ways focusing on "first time in the open ocean" might actually even sell the results a little bit short-is this maybe the first full food web study in this manner as well?

I also have some specific questions and suggestions:

P.8 lines 1-8-Flow sorting before analysis-I would like more information on this method included, when I looked up the referenced Bonnet et al, 2016b, it didn't include flow sorting-is there another paper with these details? If not, more information should be provided in this manuscript in order to verify that you had what was expected on the filter, and the NanoSIMS analysis was on the expected cells. For example, was there any correlated imaging of the filters (i.e. with fluorescence or SEM) to verify and map the cells other than the CCD camera on the NanoSIMS? It would be good to include some more raw data in supplemental with some examples of the NanoSIMS ion and secondary electron images for each group with examples of how ROIs were drawn. Particularly, it seems like the bacteria may have come through in the other sorts, was that a problem and were those identifiable in the NanoSIMS? Prochlorococcus and bacteria for example, might would look similar in the CCD camera?

P.8 line 24-25, a table of ROIs per sample in supp would help, i.e. n for each analysis

p.8 line 32-UCYN-B cell diameters from NS images-interesting and not typical-an example in supp would help, was it correlated with other imaging? (i.e. fluor or SEM?).

P.11 line 5-20 3.3-I couldn't find the information on the T0 values, how many and how analyzed? Everything is relative to the T0 but unclear what the n is.

P.11 Line 15-Sentence "For the three experiments.." -I don't get what this statement means and not sure how it relates to Figure 3

P.11 line 26-Again, like the T0, how was the prelabelled plankton measured? NanoSIMS or IRMS? what is the n?

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P.12 lines 3-4-when the error is bigger than the reported number, I worry this becomes meaningless to report-how else can the data be described?

P12-Because averaging to T0, lose some information about total N-fixation. Maybe Zoo4 is only different because lower total enrichment?

P13 line 4-5: but the DDN in the dissolved pool doesn't show release by UCYN-B, the results do imply release because you see DDN transfer but then shouldn't this statement be in the next section?

P15 Line 29-Not clear what that 50-95

P16 line 8 "The DDN transfer efficiency was more important..." not sure what is meant by "more important" more important how?

P.16-last paragraph is a bit confusing and tangential to me. This is just a suggestion, but I would prefer more of a wrap-up on what this data presented means in the context of DDN transfer prediction, e.g. does this help to reconcile the differences between the culture and field studies, or coastal vs. open ocean? What are the implications from the results for predicting transfer through the food web in other areas?

Figure 1: I think in the figure legend "secondary electrons channel of UCYN (e)" should be (c)? Also, does f correlate with anything? Is there a NanoSIMS image of Prochlorococcus cells?

Figure 4: The left pie charts numbers I think should correspond to P.11 lines 19-20 numbers-but they don't-how much N stays with the diazotrophs? Is it 50, 79 and 85

Technical corrections:

P3 Line 15-16-this sentence is confusing to me, lower than what? In the field?

P9 line 21-22 after "Plus an additional..." add "Zoo-2", if that is what that experiment is, confusing.

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