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Interactive comment on “Dynamics of N₂ fixation and fate of diazotroph-derived nitrogen in a low nutrient low chlorophyll ecosystem: results from the VAHINE mesocosm experiment (New Caledonia)” by S. Bonnet et al.

Anonymous Referee #2

Received and published: 17 February 2016

Manuscript describes mesocosm experiments to look at temporal changes in N₂ fixation and export. It's really great to see experiments like this being done in larger volumes than previously to get around the bottle issues that we all know exist in manipulation experiments. Researchers took care with the N₂ fixation measurements and I appreciate that they did a method intercomparison during the experiment. They were also careful to note the potential ¹⁵N₂ contamination issues, and I am satisfied that this was not an issue based on the analysis of their ¹⁵N₂ stock. This is a really interesting study and should be published, but I have some questions, detailed below.

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Discussion Paper



Page 5, Line 14-20: Could do a little more here to put your experiment in context with the spatial and temporal studies you site. This experiment is different in that it looks at temporal changes, but over a short, rather than annual cycle

Mesocosm description: perhaps a small thing, but were the enclosures open to the air? It sounds like they were from the rest of the description, but the term bag is throwing me off

Sampling strategy: The Teflon membrane air pumps described for sampling can be a little rough with the water if they are set to pump at a high rate, which might damage cells and affect the biogeochemical rates being measured. Were they set to a gentle flow rate?

N₂ fixation rate measurements: were data from the mass spec corrected using low N content standards, as the $\delta^{15}\text{N}$ value typically changes when the mass decreases?

UCYN microscopy: for clarity, it could be helpful to note here that UCYN-A will not be visible in this analysis

NanoSIMS: you mention that you analyzed diatoms and UCYN-C here. The diatoms can of course be identified with microscopy, but for the UCYN-C, did you identify them first with epifluorescence microscopy? And did you know that no UCYN-B was present because of the qPCR data? What about UCYN-A? Please clarify this aspect of the method description

Section 3.1: You define three periods of your experiment, P₀, P₁, and P₂. P₁ and P₂ have the line at ~ 15 days, but I don't really understand what that means. Did you use a different line between P₁ and P₂ for different measurements? Why is it not clearly defined?

Sediment trap data: methods say samples were collected daily. I see that you focus on the data from days 17 and 19 because the microscopy matches up with that, but were data for other days also analyzed?

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Page 20, lines 1-9: I'm not totally on board with this calculation of export efficiency. The cells that are in the water column on day 17 and 19 are not the same cells that will be in the sediment traps on those days, but perhaps the material from 1 or 2 days before or more. What do you think the sinking rate of these different types are and can you then look at the data from those days before? Or were the qPCR abundances similar in the previous days so that this is a real number? Seems that this is a more complicated issue than what you have done to calculate efficiency – maybe there is more you can do with information available or maybe you need to put some caveats in your explanation.

DDN transfer experiment: The N₂ fixation rates from the bulk values here are problematic to me. If you incubate bottles with isotope for 24, 48, and 72 hours, there should be an increasing total amount of fixed N in the bottles (as either PON or dissolved N). The hourly rates at the different timepoints don't entirely make sense then. What does it mean that the rate went up at the 72 hour timepoint? This value is averaged over the entire period; do you mean that because the 72 hour averaged value went up compared to the 48 hour value, that it must have actually gone up in the final 24 hours of the incubation? I'm not saying that the numbers don't have some sort of meaning, but that I think you need to make it more clear what they actually mean. I also question the calculation of the % gross N₂ fixation that is released as DN. Especially when considering the 72 hour incubation, some DN that was released is then taken up by other organisms (non-diazotrophs, as your data shows). So the TDN pool doesn't represent all of the DN release over the course of the incubation. The release of DN is likely quite a lot higher than what you have calculated because of this. Not sure how to deal with the issue, but it should be addressed.

Page 23, line 21: You can't exactly say that a specific cutoff for DIP turnover indicates DIP limitation, since limitation means control of productivity or biomass, and you haven't specifically done the experiment to compare these two values. You can say that more rapid cycling indicates deficiency. Also, the references for this 1 day value don't

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12, C9813–C9816, 2016

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Comment](#)

[Full Screen / Esc](#)

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[Interactive Discussion](#)

[Discussion Paper](#)



really fit the statement. I would look at Zohary and Robarts (L&O 1998) or Flonnes Flaten (DSR11 2005) for references that specifically address the bulk DIP turnover and how it relates to DIP limitation.

Page 25, line 25: Do you think that the UCYN-C grow as individual cells and then aggregated into the large clumps? Or do you think that as they divided, the presence of TEP kept the divided cells together in an aggregate? I suspect it is the latter, especially as you note that the currents were probably reduced, or maybe a little of both. UCYN-B in culture has aggregates grow in size over time. Also, could you calculate roughly what the sinking rates might be in comparison to the potential growth rate of the cells? Might help resolve this question.

Page 29, lines 14-16: Remember also that smaller cells with higher surface area to volume ratios will outcompete larger cells for the DIP available – I do not think that this is just related to DOP usage.

Page 30, line 6: are the aggregates forming because of the reduced currents in the bags? If so, is that representative of what happens in the natural system? Please address, maybe not here, but somewhere.

Page 30, lines 12-16: I'd like to see this on a stronger note – what are the implications of this finding?!

Figure 2: The standard ODV scale is a little hard to read, with the alternating bright and light colors. I suggest using one of the scales that goes from white to a color.

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12, C9813–C9816, 2016

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