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Interactive comment on "Dynamics of N_2 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.

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Dear Reviewer,

We thank you for the constructive comments and suggestions, which have improved the manuscript. We have addressed the concerns in a point by point response below (comments are copied with our replies below) and in a revised manuscript attached.

Best Regards,

Sophie Bonnet

Reviewer 2.

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

We agree with this comment and a sentence has been added page 4 line3: 'However, fairly little attention has been paid to sub-seasonal variability in N2 fixation and its biogeochemical drivers and consequences'. Moreover the previous sentence has been replaced by: 'Our goal was to study the high frequency temporal dynamics of N2 fixation over short time scales (sampling every day for 23 days), in relation to hydrological parameters, biogeochemical stocks and fluxes, and the dynamics of phytoplanktonic and bacterial communities in the same water mass'.

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.

Yes they were open to the air. This has been added page 5 line 12 and the word 'bag' has been replaced by the word 'enclosure': 'They consisted of large enclosures open to the air made of two 500 μm-thick films of polyethylene (PE)'

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?

Yes the Teflon pump has been set to a gentle flow rate for sampling in the mesocosms. We have been using this kind of pump for a long time and could check in past studies that fragile cells like diatoms or Trichodesmium colonies were not destroyed by this sampling strategy.

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N2 fixation rate measurements: were data from the mass spec corrected using low N content standards, as the del15N value typically changes when the mass decreases?

Yes of course, we often perform 'linearity tests' on our mass spec, i.e. we measure 3 replicates of a known IAEA reference molecule at various PN. At very low PN i.e. in ultra-oligotrophic regions (below 0.2 μM), the delta 15N is very variable and not reliable. The Vahine samples from the lagoon had by far higher PN values (between 0.6 and 1.2 μM) and were in the range in which delta 15N values are reliable and independent from the PN value.

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

This has been added in the section 2.2.3. Phenotypic characterization of UCYN in the water column and the sediment traps: 'Note that UCYN-A cannot be observed by standard epifluorescent microscopy'.

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

Our goal was to analyse the major diazotrophs at the time of the DDN experiment as well as the major groups of non-diazotrophic plankton to study the DDN transfer. As UCYN-C accounted for 90$\pm$29 % of bulk N2 fixation during that period, we specifically targeted UCYN-C for nanoSIMS analyses but we cannot exclude that some UCYB-B were analyzed as well despite they were present at very low abundances, i.e. almost two orders of magnitude less abundant than UCYN-C (Fig. 5) in the analysed samples. The following sentence has been added to the method section page 13 line 23: 'Diatoms were easily recognized on the CCD (charge coupled device) camera of the nanoSIMS, as were UCYN-C that formed large aggregates of cells, facilitating their

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recognition for nanoSIMS targeted analyses. However, we cannot exclude the possibility that some UCYN-B were analysed, despite being present at very low abundances, i.e., almost two orders of magnitude less abundant than UCYN-C (Fig. 5) in the analysed samples'.

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?

Actually P0, P1 and P2 were defined by previous companion papers in the SI based on C, N, P pools and fluxes (Berthelot et alk., 2015) and based on qPCR data on nifH (Turk-Kubo et al., 2015). When looking at our N2 fixation data, these 3 main periods could be defined and we used this terminology as all the other papers of the SI to ensure homogeneity and our descriptions. Exactly the same measurements were performed during the three periods. The sentence has been modified in the text page16 line 7 to clarify this point 'Based on our data on N2 fixation dynamics, we could identify three main periods during the experiments. These three periods were also defined by Berthelot et al. (2015b) based on biogeochemical characteristics and by Turk-Kubo et al. (2015) based on changes in abundances of targeted diazotrophs'..

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?

Please see response to Reviewer 1: Sediment traps were collected daily with the main objective to measure for POC, PON and POP export and make budgets in our mesocosms. When the UCYN-C bloom occurred, we discovered (by microscopic analyses onboard) that cells were aggregated in the water column and decided to collect some aliquots of the traps at the height of the UCYN-C bloom to figure out whether or not these large aggregates could be exported to the traps. So we only have qPCR analy-

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ses in traps at that period (days 17 and 19). Of course they were taken at the height of the UCYN-C bloom, and it is likely that the contribution of UCYN-C to total POC export was lower at other periods of the experiment.

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.

The sinking rates of UCYN-C were not measured in the study but were estimated by the model presented by Gimenez et al., (2015, Vahine SI) to be 10 m/day at the end of the experiment when the UCYN-C bloom occurred. As sediment traps from day 19 (for example) integrate the export between day 18 and day 19, we agree that it is more accurate to consider water column qPCR data from day 18 than from day 19 in our calculations. This is what we did, which changes slightly the results. The paragraph is now: 'Using the volume of each mesocosm (Bonnet et al., 2016) and the total nifH copies for each diazotroph phylotype in the sedimenting material and in the water column the day before the collection of the sediment traps (Turk-Kubo et al., 2015) (assuming a sinking velocity of ∼10 m day-1, Gimenez et al. (2016)), we estimated the export efficiency for each phylotype. For UCYN-C, 4.6 % and 6.5 % of the cells present in the water column were exported to the traps per 24 h on day 17 and 19, respectively (assuming one nifH copy per cell). For het-1, 0.3 and 0.4 % of cells were exported into the traps on day 17 and 19, for het-3, 15.5 % and 10.5 % were exported, and for UCYN-B, 37.1 % and 15.5 % of UCYN-B were exported on day 17 and 19, respectively'.

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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.

Please see response to reviewer 1: We agree that the way the release was presented was misleading. We have thus changed Figure 5a and the 15N2 uptake data are now presented as cumulative uptake over the experimental study period (72 h). 15N2 uptake includes both N2 fixation and the uptake of 15N-labelled DDN by non-diazotrophic plankton, especially after 24 h. Consequently, we no longer talk about N2 fixation in the framework of the DDN experiment but about 15N2 uptake. Similarly, the 15DDN measured in the TDN pool either come from direct release during N2 fixation, and/or from remineralization of diazotrophic biomass or biomass grown on 15DDN. We thus no longer talk about release but about 'DDN quantified in the TDN pool'. The results and discussion sections as well as the legend of Figure 5 have been modified accordingly. Moreover, the 15DDN measured in the TDN pool does not reflect the release by diazotrophs that may be higher as a part of this DDN has been uptaken by surrounding planktonic communities. This has been added to the discussion section page 25 line 3: 'The amount of DDN measured in the TDN pool during the 72 h DDN transfer

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experiment is higher than that reported for culture studies of Cyanothece populations (1.0±0.3 to 1.3±0.2 % of gross N2 fixation (Benavides et al., 2013; Berthelot et al., 2015a)). The DDN measured in the TDN pool reflects the DDN release by diazotrophs during N2 fixation and is likely underestimated here as a fraction of this DDN has been taken up by surrounding planktonic communities'.

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

We replaced the previous sentence by 'In all cases, the increase in UCYN-C abundance coincided with low DIP turnover time, indicative of DIP deficiency (Berthelot et al., 2015b; Moutin et al., 2005)'

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. UCYNB 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.

We believe that both are possible, i.e. UCYN-C cells grew as individual cells and aggregated afterwards, likely at the start of the bloom. It is also likely that divided inside the aggregates themselves. Please also see response to the comment above regarding sinking rates.

Page 29, lines 14-16: Remember also that smaller cells with higher surface area to

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volume ratios will outcompete larger cells for the DIP available – I do not think that this is just related to DOP usage.

The section has been modified as follows: 'We hypothesize that picoplankton were more competitive for DDN under low DIP conditions as small cells with high surface to volume ratios are known to outcompete larger cells for the available DIP (Moutin et al., 2002). Moreover, some prokaryotes from the 0.2-2 μm size-fraction can utilize DOP compounds (Duhamel et al., 2012)'.

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.

We agree with this comment and have added the following sentence in the section '4.3 UCYN aggregation and export': 'Aggregation processes are probably enhanced by the low turbulence in the mesocosms and it would be necessary to confirm that such processes also occur in open ocean systems'.

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

The whole conclusion section has been modified. In particular the end of the discussion has been amended as follows: "Moreover, the experimental and analytical approach used in this study allowed for the quantification of the actual transfer of DDN to different groups of non-diazotrophic plankton in the oligotrophic ocean. Our nanoSIMS results coupled with 15N2 isotopic labelling revealed that a significant fraction of DDN (21 ± 4 %) is quickly (within 24 h) transferred to non-diazotrophic plankton, which increased in abundance simultaneously with N2 fixation rates. A similar nanoSIMS study performed during a Trichodesmium bloom (Bonnet et al., Accepted) revealed that diatoms were the primary beneficiaries of DDN and developed extensively during and after Trichodesmium spp. blooms. Diatoms are efficient exporters of organic matter to depth (Nelson et al., 1995). These studies show that plankton grown on DDN in the

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oligotrophic ocean drive indirect export of organic matter out of the photic zone, thus revealing a previously unaccounted for conduit between N2 fixation and the eventual export to depth of DDN from the photic zone'.

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.

Figure 2 has been redrawn using a different color code (please see attached).

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C10023/2016/bgd-12-C10023-2016-supplement.pdf

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Interactive comment on Biogeosciences Discuss., 12, 19579, 2015.

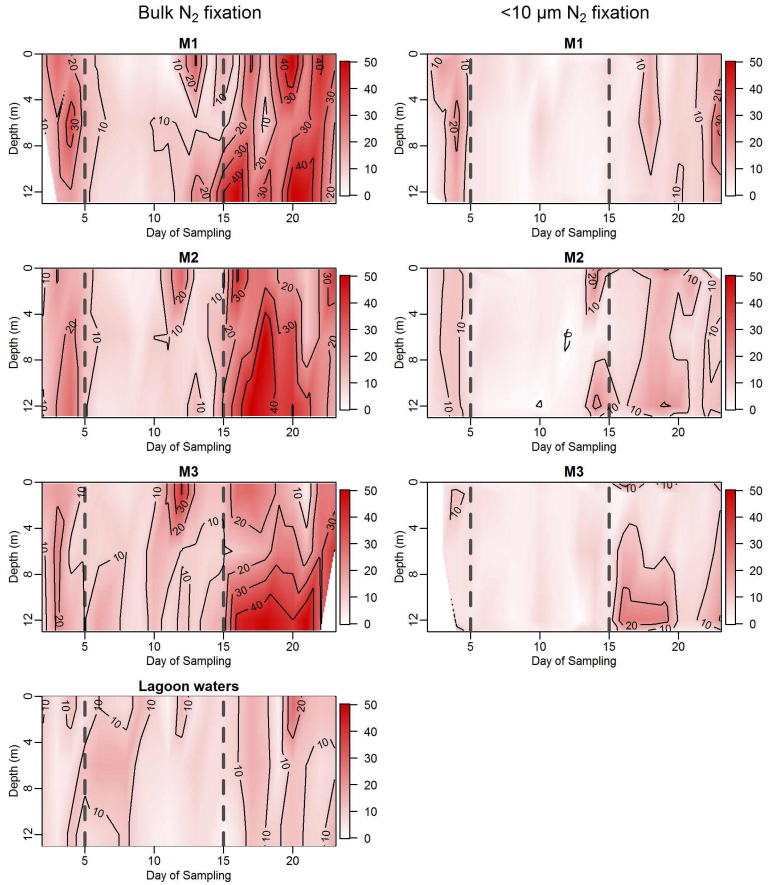

Fig. 1. Figure 2

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