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Biogeosciences Discussions

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 3.

As I understood, the scope of the manuscript and experiment is to provide a time series and temporal variability in N2 fixation rates. This should be mentioned already in the abstract.

This is now mentioned in the first sentence of the abstract 'N2 fixation rates were measured daily in large (∼50 m3) mesocosms deployed in the tropical South West Pacific coastal ocean (New Caledonia) to investigate the temporal variability in N2 fixation rates in relation with environmental parameters and study the fate of diazotroph-derived nitrogen (DDN) in a low nutrient, low chlorophyll ecosystem'.

What does the abbreviation VAHINE stand for? Please add!

This has been added page 4 line 6: 'In the framework of the VAHINE (VAriability of vertical and tropHIc transfer of diazotroph derived N in the south wEst Pacific) project. . .'

1) Page 19584, line 7: Short term fate of to me <24 hours.

The term 'short' has been removed and this whole section modified (see response to comment 2 below).

How do you distinguish between direct 15N2 fixation and recycling and re-uptake of 15N derived from of N2 fixation?

Please see comment to reviewer 1 and 2: First, the way the release was presented was misleading. We have thus changed Figure 5a and the 15N2 uptake data are now presented as cumulated 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 taken up by surrounding planktonic communities. This has been added to the discussion section: 'The amount of DDN measured in the TDN pool during the 72 h DDN transfer 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 uptaken by surrounding planktonic communities'.

2) Please add a list of accompanied manuscripts which deal with the VAHNE mesocosm experiment and their individual scope (I understand that there were a couple more).

There are 16 articles in the Vahine SI (please see http://www.biogeosciences.net/special_issue193.html). We have modified this introduction section to introduce the papers dealing with DDN transfer. The new section is now: 'Over the course of this 23-day mesocosm experiment, diatom-diazotroph associations (DDAs) were the most abundant N2 fixers during the first half of the experiment (days 2 to 14), while a bloom of the unicellular N2-fixing cyanobacteria from Group C (UCYN-C) occurred during the second half of the experiment (days 15 to 23) (Turk-Kubo et al., 2015). Berthelot et al. (2015b) described the evolution of the C, N, and P pools and fluxes during the experiment and investigated the contribution of N2 fixation and DON uptake to primary production and particle export. They also explored the fate of the freshly produced particulate organic N, i.e., whether it was preferentially accumulated and recycled in the water column or exported out of the system. Complementary to this approach, Knapp et al. (2015) report the results of a

δ15N budget performed in the mesocosms to assess the dominant source of N (i.e., NO3- versus N2 fixation) fueling export production during the 23-day experiment. In the present study, we focus specifically on the fate of DDN in the ecosystem during the UCYN-C bloom by studying i) the direct export of diazotrophs into the sediment traps, and ii) the transfer of DDN to non-diazotrophic plankton using high-resolution nanometer scale secondary ion mass spectrometry (nanoSIMS) coupled with 15N2 isotopic labelling during a 72 h-process experiment'.

3) Please structure analytical methods and experimental procedures together.

As suggested by Reviewer 1, the methods section has been reorganized by separating experimental procedures and analytical protocols. Some sentences have also been added to guide the reader.

4) Did you clean the walls of the mesocosm - cell wall growth can be a major difficulty and introduce errors in the overall element budget.

We did not clean the walls of the mesocosms during the experiment has it would have introduced artificial export of organic matter. However it is true that at the end of the experiment a biofilm started to be visible on the walls of the mesocosms. The way this biofilm may affect the elemental budget has been fully discussed in the companion paper Knapp et al., 2015 (Vahine SI). We believe that it does not affect the results presented in the present paper and is not discussed here.

5) A schematic overview concerning samples taken and sub experiments done would be useful maybe put Fig. 1 in supplements and add it here.

We have fully restructured the Methods section, which should now be clearer for the reader. Consequently, we did not include a new Figure describing the protocols.

6) How did you calculate DIP turnover?

The DIP turnover time was calculated as the ratio of DIP concentration and uptake as described in Duhamed et al., (2006). The full DIP turnover time is presented in the

companion paper Berthelot et al., (2015, Vahine SI).

7) Page 19581, line 10: The authors state, that their values are in the upper range of rates reported for the global ocean- that is not surprising as they added DIP to fuel production.

We modified the sentence as follows: 'These later rates measured after the DIP fertilization are higher than the upper range reported for the global ocean'.

8) What was the batch number of 15N2 gas used? 9) Page 19587, line 16:- Please give details on how you testes for contamination.

The batch number has been added. The potential contamination level was assessed by the Dadundo group on one of our batches. The method is very long to explain and is not in the scope of this MS. However, some details have been incorporated in the method section as follows: 'To verify this, one of our 15N2 Cambridge Isotopes batches (18/061501) was checked for contamination following the method described in Dabundo et al. (2014); it was 1.4 x 10-8 mol of 15NO3- per mol of 15N2 and 1.1 x 10-8 mol NH4+ per mol of 15N2. The application of this contamination level to our samples using the model provided by Dabundo et al. (2014) indicates that our rates may only be overestimated by $\sim$0.05 %, confirming that our present results were unaffected by possible 15N2 stock contamination'.

10) 15N enrichment in bottle done for the bubble method- why did you not analyze the 15N enrichment using MIMS like you did for the Mohr method and use measured value in the calculation instead of the theoretical one?

We agree that it would have been better to measure the 15N enrichment value when using the bubble method as we did for the 15N2 enriched seawater method but unfortunately we did not. We did that on a recent cruise and will be able to compare the theoretical value to the actual measured one for future studies.

11) How did you identify organisms in the NanoSIMS picture- by additional microscopic

identification and marking with laser?

Please see response to Reviewer 2: 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: '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 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'.

12) Please add a table with abundances measured. 13) Figure 3- What sustained C-fixation in A1 below 200 m and was there any light available at that depth? 15) Fig. 1. SSHA is not an acronym for Aviso sea level anomaly- please correct! 16) Fig. 3: Please enlarge numbers and legends- it s hard to read. 17) Fig. 6. Please delete repetition of "N2 fixation and O2 and N2 fixation and O2" I think these five comments do not refer to our paper. . .

14) Page 19605, line 9. Please explain the calculation of e ratio in methods.

The definition of the e ratio has been directly included to the sentence: 'This observation was further confirmed by the e ratio, which quantifies the efficiency of a system to export POC relative to primary production (e ratio = POC export/PP), and was significantly higher ($p<0.05$). . .'

Please also note the supplement to this comment:

http://www.biogeosciences-discuss.net/12/C10033/2016/bgd-12-C10033-2016-supplement.pdf

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