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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.

Best Regards,

Sophie Bonnet

Reviewer 1.

Currently there is no flow to the description of experimental procedures and analytical protocols, which are randomly presented and make interpretation and understanding of the paper difficult. Please describe: 1) time series observations within the mesocosms; 2) DDN experiment and 3) Investigation of sediment traps, before detailing analytical methods.

The methods section has been reorganized as suggested by separating experimental procedures and analytical protocols. Some sentences have also been added to guide the reader.

If I understand the DDN transfer experiment correctly, and it is difficult at times to follow due to poor organisation of the methods, then I do not believe that N2 fixation rates can be presented after 24 hours of incubation. Surely the premise of this experiment is that 15N enriched nitrogen is being released into incubation bottles and being assimilated by the microbial community. How then, after 24 hours, do you differentiate between 15N which has been recently fixed from N2 from 15N enriched DDN? Certainly the "N2 fixation" rates presented at T72h and quite likely at T48h will be a combination of fixed 15N-N2 and assimilated 15N-DDN.

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 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.

A more thorough discussion of the P requirements for N2 fixation needs to take place with reference to other published work and likely N:P stoichiometry. Currently the discussion here suggests P limiting conditions in the lagoon and mesocosms prior to DIP addition, yet rates of 9 nmolN L-1d-1 in the lagoon and 18 nmolN L-1d-1 over the first few days of the experiment do not suggest a resource limited community of diazotrophs. 30 nM phosphate does not constitute "extremely low DIP". Presentation of N and P data as a figure or table should be included within this manuscript. The negative correlations observed between both DIP and N2 fixation and DOP and N2 fixation is counter intuitive and should be investigated further.

We agree and the term 'extremely' has been removed. Moreover, NO3-, DIP, DON and DOP data have been included to the paper and are now presented in Table 3 as average concentrations over the three main periods (P0, P1, P2). Diazotrophs can both be impacted by the DIP concentrations and have an impact on DIP via assimilation. We believe that the correlations observed between N2 fixation and DIP concentrations are not necessarily unexpected. N2 fixation rates increased while DIP concentrations were still relatively high. During spring blooms in temperate waters, the uptake of nitrate is negatively correlated with the nitrate concentrations, and nitrate concentrations become limiting at the end of the bloom. We have modified the sentence page 20 line 12 as follows: 'Yet, in all three mesocosms, N2 fixation rates were negatively correlated with DIP concentrations and DIP turnover time and positively correlated with APA (Table 2). Below, we describe the scenario that likely occurred in the mesocosms, which likely explains these correlations'.

P19581 L2 How is a spatial analysis enabled from a mesocosm experiment?

We agree and the word 'spatial' has been removed

L11 favourable for N2 fixation – and therefore probably not limited by P availability?

We removed 'indicating that the waters surrounding New Caledonia are particularly favorable for N2 fixation' as it was redundant with the previous sentence. The sections related to the effect of DIP on N2 fixation have been expanded later in the MS so no more details are given in the abstract.

P19584 L16 22_28.855'S; 166_26.724'E or 22.481_S; 166.445_E ?

The real position was 22°28.855'S; 166°26.724'E. The minutes have been added to the text.

P19585, P19587 Please provide batch/lot number for 15N-N2 cylinder and details of how the (potential) contamination level was assessed.

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 Methods 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'.

P19588 L4, L22 The DDN experiment has not been introduced at this point, and so please detail the DDN experiment earlier or re-phrase this section.

With the reorganization of the methods section, this kind of problem does not occur anymore.

L23 This is the first mention of 13C. Methods and reason for use should be introduced previously.

The DDN experiment is now presented before this mention of 13C and refers to 13C labelling in our bottles: 'Full bottles were immediately amended with the dissolved 15N2

gas (98.9 atom% 15N, Cambridge Isotopes Laboratories, Inc) as described above (dissolution method), and with 1 mL of 80 g L-1 NaH13CO3 solution (99 atom% 13C, Cambridge Isotopes Laboratories, Inc) and incubated in situ. . .'

P19589 L2 Identify here that het-1 and het-2 are the DDAs mentioned elsewhere, as this is not specified explicitly.

We have added 'the two DDAs Richelia associated with both Rhizosolenia (het-1; (Church et al., 2005)) and Hemiaulus (het-2; (Foster et al., 2007)) diatoms. . .'

L9 inhibitors of what? Please describe this better

We have added the following precisions: 'Extracts were tested for the presences of PCR inhibitors, compounds sometimes present in DNA extracts from the environment or introduced in the extraction process that reduce PCR efficiency, using either the UCYN-B or the UCYN-C assay. If recovery of the spiked standard template in the sample extract was <98%, the sample was considered inhibited, and diluted 1:10 with 5 kD filtered milliQ water'.

L19 Which UCYN-C bloom? The reader does not know about this yet!

The UCYN-C bloom was mentioned in the Introduction section. In the current version it is also now mentioned in the Experimental procedures section before its mention in the DDN experiment.

L25 ..at the appropriate sampling depth? Isn't this always 6m as stated on L20?

Yes they were incubated at 6 m. It has been replaced 'and incubated in situ on the mooring line at 6 m-depth close to the mesocosms'

P19590 L1 How was "DDN released in the dissolved pool" determined? This whole section needs some reorganisation to group analytical methods together and experimental procedures together. Methods for N2 fixation and qPCR have already been presented, methods for DDN determination, cell counts and nanosims follow.

This was misleading in the previous section as this section was not separated from the 'experimental procedures'. A full dedicated section is now provided in the section 2.3 Analytical protocols (i.e. section 2.3.3 Quantification of the net release of DDN to the dissolved pool during the DDN transfer experiment).

L22 What denitrifying bacteria? First time these have been mentioned. Denitrifier method doesn't appear until L26.

The paragraph has been reordered as follows: 'The concentration of the resultant NO3- (i.e., TDN + the POR-associated N blank) was measured by chemiluminescence (Braman and Hendrix, 1989), after which the TDN isotopic composition was determined using the 'denitrifier method', wherein denitrifying bacteria that lack N2O reductase quantitatively convert sample NO3- to N2O (Casciotti et al., 2002; Sigman et al., 2001). The denitrifying bacteria (see below) are extremely sensitive to pH; care was thus taken to lower sample pH to 7-8 after POR oxidation via the addition of 12N ACS grade HCl'.

P19591 L11-15 I assume this describes procedure for picoplankton analysis? This should be stated.

Yes, this was indicated in the title of the section. We have also modified the first sentence as follow to state that: 'After each incubation period, 3.6 mL from each 15N2-lableled 4.5 L bottle were subsampled into cryotubes, fixed with paraformaldehyde (2 % final concentration), flash frozen in liquid N2, and stored at -80°C until analysis. Picoplankton analyses were carried out at the PRECYM flow cytometry platform. . .'

L23-24 two subsets of bacteria (: : :: : :: : :: : :: : :..) were optically (Gasol et al., 1999). Something is missing here.

The sentence has been replaced by 'Based on these criteria, two subsets of bacteria (referred to low- and high nucleic acid-containing, or LNA and HNA, respectively) were optically resolved in all samples based on their green fluorescence intensity (Gasol et al., 1999)'.

P19597 L15 onwards Why is sediment trap data only presented for days 17 and 19? Surely this biases your conclusion that UCYNs are more efficiently exported than DDAs as this was a period of UCYN dominance? If sediment trap data is available for other days it should be included to allow comparison of export rates between the different periods of the experiment.

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 analyses 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.

L24 Something cannot be described as “often” when the analysis is only described on 2 days. How about: UCYN-B was detected in all mesocosms on both days (except for M1 day 19) ??

We agree and the sentence has been replaced as advised: ‘UCYN-B was detected in all mesocosms on both days (except in M1 day 19)....’

P19598 L3 sediments exist on the sea bed, change to sedimenting material (or similar description).

The term ‘sediment’ has been replaced by ‘sedimenting material’

P19598 L27 There is no previous mention of 13C analysis by nanosims and no method Presented

The following section has been modified accordingly in the section ‘2.3.5 NanoSIMS analyses and 13C 15N assimilation rates during the DDN transfer experiment’: ‘All

scans were first corrected for any drift of the beam during acquisition, and C and N isotope ratio images were created by adding the secondary ion counts for each recorded secondary ion for each pixel over all recorded planes and dividing the total counts by the total counts of a selected reference mass. Individual cells were easily identified in nanoSIMS secondary electron, 12C-, 12C14N-, and 28Si images that were used to define regions of interest (ROI) around individual cells (28Si data are not presented here). For each ROI, the 15N and 13C enrichments were calculated’.

P19600 L 15 The main difference between the mesocosms and lagoon was the modified DIP, however rates in the mesocosms were approximately twice those measured in the lagoon before DIP was added. How is this explained?

In this sentence, we meant that N2 fixation rates were twice those measured in lagoon waters but this is over the whole 23 days of the experiment and not during P0. This was mentioned in the results section but we also modified the sentence in the discussion for clarity as follows: ‘Averaged over the 23 days of the experiment, N2 fixation rates in the mesocosms were $\sim$ 2 fold higher (18.5$\pm$1.1 nmol N L-1 d-1) than those measured in lagoon waters (9.2$\pm$4.7 nmol N L-1 d-1)’ However, N2 fixation rates were indeed higher in the mesocosms compared to lagoon waters during P0. We added the following possible explanation page 20 line 18: ‘During P0 (day 2 to 4), N2 fixation rates were higher in the mesocosms than in the lagoon waters, possibly due to the reduction of turbulence in the water column facilitated by the closing of the mesocosms (Moisander et al., 1997) and/or to the reduction of the grazing pressure in the mesocosms as total zooplankton abundances were slightly lower (by a factor of 1.6) in the mesocosms compared to the lagoon waters (Hunt et al., 2016)’.

P19603 L15-16 Sentence does not make sense. Something like: : : :in our experiments did not utilise diazocytes to separate diazotrophy from photosynthesis.

The sentence has been modified as advised ‘Here, UCYN-C cells fixed both 13C and 15N proportionally, which suggests they did not utilize diazocytes to separate diazotro-

phy from photosynthesis in our experiments'.

P19605 L7-9 This conclusion is possibly biased, as on days 17 and 19 when sedimenting material was collected UCYN-C was the dominant diazotroph. The carbon export potential is a significant factor, with great relevance. Is it possible that these aggregations were influenced by stress of containment within mesocosms?

We agree with this comment and the following sentence has been added page 23 line 12 to nuance our results in mesocosms: 'Aggregation processes may have been favored by the low turbulence in the mesocosms and it would be necessary to confirm that such processes also occur in the open ocean'. Another sentence has been added in the conclusion section: 'Here, we demonstrate for the first time that UCYN can efficiently contribute to POC export in oligotrophic systems, predominantly due to the aggregation of small (5.7$\pm$0.8 μm) UCYN-C cells into large aggregates, which increase in size (up to 500 μm) with depth. Our results suggest that these small (typically 3-7 μm) organisms should be considered in future studies to confirm if processes observed in mesocosms are applicable to open ocean systems'.

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