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## ***Interactive comment on “Dynamics of N<sub>2</sub> 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 #1**

Received and published: 29 January 2016

This paper describes an experiment performed within coastal waters of New Caledonia and in addition to describing N<sub>2</sub> fixation rates over a 23 day period, details both the transfer of recently fixed nitrogen from diazotrophs to the wider microbial community and the vertical export of diazotroph carbon out of the system. This appears to be a well executed experiment and this paper provides some important information on the role and function of nitrogen fixers within biogeochemical cycles of the coastal South West Pacific.

At present though this manuscript requires some attention to its organisation, particu-

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larly within the methods section before it is publishable. 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.

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 N<sub>2</sub> fixation rates can be presented after 24 hours of incubation. Surely the premise of this experiment is that <sup>15</sup>N enriched nitrogen is being released into incubation bottles and being assimilated by the microbial community. How then, after 24 hours, do you differentiate between <sup>15</sup>N which has been recently fixed from N<sub>2</sub> from <sup>15</sup>N enriched DDN? Certainly the “N<sub>2</sub> fixation” rates presented at T72h and quite likely at T48h will be a combination of fixed <sup>15</sup>N-N<sub>2</sub> and assimilated <sup>15</sup>N-DDN.

A more thorough discussion of the P requirements for N<sub>2</sub> 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<sup>-1</sup>d<sup>-1</sup> in the lagoon and ~18 nmolN L<sup>-1</sup>d<sup>-1</sup> 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 N<sub>2</sub> fixation and DOP and N<sub>2</sub> fixation is counter intuitive and should be investigated further.

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

L11 favourable for N<sub>2</sub> fixation – and therefore probably not limited by P availability?

P19584 L16 22°28.855'S; 166°26.724'E or 22.481°S; 166.445°E ?

P19585, P19587 Please provide batch/lot number for <sup>15</sup>N-N<sub>2</sub> cylinder and details of

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how the (potential) contamination level was assessed.

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.

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

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

L9 inhibitors of what? Please describe this better

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

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

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.

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

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

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

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

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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) ??

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

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

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?

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

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?

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