

Interactive comment on “In depth characterization of diazotroph activity across the Western Tropical South Pacific hot spot of N₂ fixation” by Sophie Bonnet et al.

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Response to Referee #3

We thank reviewer 3 for the time devoted to this review and for his/her constructive comments. Below are copied the comments in regular font with our point by point responses in blue. Changes in the manuscript appear in ‘track change’ mode.

The manuscript presented by Bonnet et al. reports N₂ fixation rate measurements and diazotroph abundances from the Western tropical South Pacific. Complementary single-cell measurements of the two most abundant diazotrophs reveal the biogeo-

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chemical importance of each of these organisms in this region. Since this manuscript reports a subset of data collected on the OUTPACE cruise, these measurements are analyzed in correlation to a comprehensive set of nutrients, including dissolved iron, and other biogeochemical parameters. The manuscript is well written and the conclusions of this manuscript are appropriately based on the presented data. This new data is a nice addition of N₂ fixation rate measurements in relation to biogeochemical parameters and a contribution of individual organisms (not solely based on abundances) that will ultimately help confine the extent and magnitude of N input by N₂ fixation into the global ocean. I only have a few comments (please see below).

Abstract: Maybe the authors could add a little more discussion/conclusions to the abstract as it currently reads almost like results only.

The abstract has been extended in the new version of the manuscript and now includes more discussion/conclusions.

p 3, l 5: I would use either ammonia (NH₃) or ammonium (NH₄⁺)

Totally right, this has been fixed

p 3, l 6: Isn't this 'nif genes' rather than 'nifH genes'?

Yes, the sentence has been changed as follows: 'The process of N₂ fixation is mediated by diazotrophic organisms that possess the nitrogenase enzyme, which is encoded by a suite of nif genes'.

p 3, l 7: anammox uses nitrite and ammonium as substrates, maybe those could be added to the fixed N?

I had added 'anammox' just before submission thanks to a suggestion of a co-author, so yes of course, I have added these substrates in the new version of the manuscript.

Section 2.2: As far as I understand, the authors used the time-zero samples rather than incubated controls as the natural abundance value in the N₂ fixation rate calculation.

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In many cases, this is OK; however, I have also seen quite large changes in the natural abundance over time in incubated samples/bottles without the addition of stable isotope. These are usually the result of fractionation during the incubation time, e.g. due to the uptake of residual nitrate or remineralization of organic material. The fractionation effects can lead to higher or lower d15N values of the natural abundances. In the absence of incubated controls, the detection limits of N₂ fixation might be a bit worse than if those values are available. I would therefore recommend that the authors add their detection limits to the manuscripts, such as the minimum change in d15N that was used as a cutoff for a significant ¹⁵N enrichments or reporting the actual d15N values measured in their incubations and the time-zero values. This would also be coherent with general criticisms brought up in the recent paper by Gradoville et al. (2017; DOI: 10.1002/lno.10542).

We acknowledge that isotopic fractionation may occur during the incubation period and that it would be generally more correct to use the natural abundance value after incubation. In the present case, the rates were so high so the impact of fractionation is probably negligible. However it may impact the quantification limit, and we have added a sentence regarding the minimum quantifiable rates in section 2.2: 'The minimum quantifiable rates (quantification limit, QL) calculated using standard propagation of errors via the observed variability between replicate samples measured according to Gradoville et al. (2017) were 0.035 nmol N L⁻¹ d⁻¹.

Section 2.4: Was the at% ¹⁵N in the N₂ pool measured here as well?

It was measured in triplicates at every station but only in the bottles dedicated to bulk N₂ fixation measurements and this value was used for the group-specific rate calculations as the same methodology was used (same bottles, same amount of ¹⁵N₂ added) for both types of measurements.

Section 3.4.: Was primary production measured on this cruise? Based on the description in this section, it sounds to me as if N₂ fixation somewhat scales with productivity

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and/or turnover of organic material.

Yes PP was measured using the ¹⁴C labeling method (not ¹³C). It appears in the correlation table but not in text. We have thus added PP and bacterial production in this section (which are both correlated with N₂ fixation). The sentence is now: 'Regarding the main biogeochemical stocks and fluxes measured during the cruise, N₂ fixation rates were significantly positively correlated with dissolved Fe, dissolved organic N (DON), phosphorus (DOP) and carbon (DOC), particulate organic N (PON), particulate organic carbon (POC), biogenic silica (BSi), Chl a concentrations, primary and bacterial production (p<0.05), and significantly negatively correlated with NO₃⁻, NH₄⁺, DIP and silicate concentrations (p<0.05)'.

p 15, l 5: I assume that the “%” dropped from the 9.7? If not, does that mean that more organic matter is exported than produced at a given point in time?

Yes, this is 9.7 %, I have added the % in the new version of the manuscript.

p 15, l 14: With respect to the structure of the sections, I would almost move the entire section 4.2 here, as the rest of the discussion nicely scales from a more detailed and organism-centric discussion to a more system-oriented discussion. This would also have the side effect that the end of your discussion is not quite as focused on so many references that are related to other OUTPACE data which are not actually presented here.

I tried to do that and the structure of the manuscript did not seem coherent anymore to me. As the 4.2 section discusses the N₂ fixation results, which are the first presented, it was not consistent for me to present detailed group-specific data before presenting the big picture. However, I acknowledge that section 4.4 Ecological relevance of N₂ fixation in the WTSP contains many references of the OUTPACE SI not really related to the present study. Therefore, I propose to merge this section and the conclusion section to set our study in the general context of the OUTPACE study.

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Table 1: Any idea on why the d15N value is so low for stn 11 (i.e. -7.05 ‰). Do you have any depth distribution of the d15N values for PN?

We measured d15N of PN at only 2 depths (the surface, 5 m and the deep chlorophyll maximum) so it's not a very good resolution. I was also wondering why such low values at SD 11. I know that this is the area where we have seen some high Fe inputs likely coming from underwater volcanoes. This may alter the isotopic composition of plankton. . .

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