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Interactive comment on "In depth characterization of diazotroph activity across the Western Tropical South Pacific hot spot of N_2 fixation" *by* Sophie Bonnet et al.

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Response to Referee #2

We thank Carolin Löscher for the time devoted to this review and for 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.

Bonnet and colleagues fill again one of the white spots on the maps of nitrogen fixation by presenting data from the OUTPACE cruise in 2015 to the western and central tropical South Pacific. N2 fixation rates from the photic are resented and complemented

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with a metadata analysis and a mining for the major diazotrophic clades Trichodesmium and UCYN-B. N2 fixation can be massive in that region, which is shown to be split in two different biogeochemical parts, and can reach up to 631$\pm$286μmol N/m/d. Those rates are extremely high and thus not only confirm the predicted importance of that region for N2 fixation but actually show that the N supply via N2 fixation is much higher than predicted. Moreover, they challenged the statistical approach of Luo and colleagues suggesting SST as a key regulating factor of N2 fixation in that region. This study now provides some realistic evidence for a limitation by either DIP or Fe. A discussion on the contribution of N2 fixation to primary and export production again emphasizes the importance of this region, which is from my point of view the best part of the manuscript. As in previous studies, I am missing direct C fixation rates, this would be easy to do in future studies and make your point of N2 fixation to primary production much stronger. Altogether, this is another piece of high-quality work of this group on this critical topic. The presentation is clear, the figures and tables are well chosen. I don't quite get, why there is no abstract, maybe it would be good to directly summarize your key findings, there. I only have minor comments and recommend publication in Biogeosciences.

Specific comments:

p. 2, l. 3 the biological carbon pump: the sentenced is phrase in a way that it sounds strange, here, also it deserves the Azam reference. Given your discussion on the contribution of N2 fixation to the carbon pump, this needs more than a sentence, please explain the connection between N2 fixation, C fixation and export production in some sentences.

The first paragraph of the introduction has been modified according to the suggestions as follows: 'In the ocean, nitrogen (N) availability in surface waters controls primary production and the export of organic matter (Dugdale and Goering, 1967; Eppley and Peterson, 1979; Moore et al., 2013). The major external source of new N to the surface ocean is biological N2 fixation (100-150 Tg N yr-1, (Gruber, 2008)), the reduction of atmospheric di-nitrogen gas (N2) dissolved in seawater into ammonia (NH3+). The

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process of N2 fixation is mediated by diazotrophic organisms that possess the nitrogenase enzyme, which is encoded by a suite of nif genes. These organisms provide new N to the surface ocean and act as “natural fertilizers”, contributing to sustaining ocean productivity and eventually carbon (C) sequestration through the N2-primed prokaryotic C pump (Karl et al., 2003; Karl et al., 2012). This N source is continuously counteracted by N losses, mainly driven by denitrification and anammox, which convert reduced forms of N (nitrate, NO3-, nitrite NO2-, NH4+) into N2. Despite the critical importance of the N inventory in regulating primary production and export, the spatial distribution of N gains and losses in the ocean is still poorly resolved’.

p. 2, l. 6. nifH is only one gene, and it codes for one subunit of the nitrogenase reductase

We agree and 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.4, l. 3 spell out HgCl2

It has been spelled: ‘fixed with mercuric chloride (HgCl2, final concentration 20 mg L-1)’

p.4, l. 8 and is throughout the text: submitted is capitalized, please change

We updated the references throughout the manuscript. Since the submission of this manuscript, most of the submitted ones are now under review or accepted. Anyways, when appropriate, we indicated ‘submitted’ without the capital S.

p.4, l. 10 As the paper is in review a brief description of the method would be helpful

The following paragraph has been added in section 2.1: ‘Samples for determining dissolved Fe concentrations were collected and analyzed as described in Guieu et al. (Under review). Briefly, samples were collected using a Titane Rosette mounted with 24 teflon coated 12 L GoFlos deployed with a Kevlar cable. Dissolved Fe concentrations

were measured by flow injection with online preconcentration and chemiluminescence detection according to Bonnet and Guieu (2006). The reliability of the method was monitored by analyzing the D1 SAFe seawater standard (Johnson et al., 2007), and an internal acidified seawater standard was measured daily to monitor the stability of the analysis'.

p.4, l. 13 ff and discussion: I see and understand why you chose for the bubble method here and I respect that you give me an argument to not start this discussion, again. I assume my opinion on the method issue is anyway clear by now, and seeing your discussion shows how reflective and aware of the importance of that topic you are. For the sake of having one day a truly comparative method, let me add a very pragmatic argument: I used both methods excessively, as a practical conclusion I can conclude (and I am sure you know what I am talking about) shaking the bottles with the gas bubbles in them for 100 times each is much more exhausting then pre-dissolving the gas in a bag with water. This limits the number of bottles you can handle, and with that the spatial resolution you will get. In addition, if you try shaking on a culture of Trichodesmium, you kill parts of the culture as they seem to dislike the shaking even more than I do. This would lead to a possible underestimation in addition to the one caused by the method. I appreciate the text you added to the discussion, but I feel it is a bit of an overkill. I would recommend to shorten it, but leave the justification with the DOM and Fe contamination potential in, so that everyone can understand why you chose for it. In addition, Grosskopf et al showed, and this is actually what plays into your cards, that the bubble method is mostly problematic for low rates- you have massive rates, so you don't really run into that problem.

Yes I know what you are talking about ïĄŁ I will not give more details here as I can see that we understand each other. Ideally, we should have done proper comparisons on-board but as long as we have MIMS measurement, we can correct our data. Anyways, we shortened the discussion as suggested to keep only the 'contamination' argument, the rest is dedicated to the MIMS measurements during the in situ versus on-deck in-

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cubations. We also now make reference to a new study by Wannicke et al., (2018) and have added the following sentence: 'Moreover, a recent extensive meta-analysis (13 studies, 368 observations) between bubble and enriched amendment experiments to measure 15N2 rates reported that underestimation of N2 fixation is negligible in experiments that last 12-24 h (e.g. error is -0.2 %); hence our 24 h based experiments should be within a small amount of error (Wannicke et al., 2018)'.

p. 5, l.5: Could you add a sentence on what a trapezoidal integration is?

We have added the following sentence in the new version of the manuscript: 'Discrete N2 fixation rate measurements were depth-integrated over the photic layer using trapezoidal integration procedures. Briefly, the N2 fixation at each pair of depths is averaged, then multiplied by the difference between the two depths to get a total N2 fixation in that depth interval. These depth interval values are then summed over the entire depth range to get the integrated N2 fixation rate. The rate nearest the surface is assumed to be constant up to 0 m (JGOFS, 1988)'.

p. 6, l.2 If You follow your classical screening approach, here, and there is nothing bad about it. Still, I would be extremely curious seeing a nifH sequencing done on those samples- how else can we know who is doing the N2 fixation, who could do it if conditions change? In the results it seems, your single cell rates add up to the bulk at station SD2 (I am referring to table 3), not at the others- so there must be something else. In this context, I appreciate the gene expression assay and indeed it could be that the het groups fill the gap. But obviously, the microbes you don't look at will not show up. I see, you are referring to Stenegren et al., 2017, but it would be beneficial for future studies to have a proper deep sequencing included.

I totally agree with this comment. Indeed we cannot totally reconcile the single-cell rates of dominant groups with bulk rates, which mean that there are other players. Of course, DDAs quantified by Stenegren et al. are part of the story. We also observed some Katagnymene spiralis during the cruise, which despite scarce are really big and

may contribute to N2 fixation, together with the heterotrophs mentioned in Benavides et al. (same issue) and probably fungi etc.. so yes, deep sequencing is really needed to fill this gap. . .

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