

## 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.

## C. Löscher (Referee)

cloescher@geomar.de

Received and published: 1 February 2018

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 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  $\pm$  286  $\mu$ mol 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

C1

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, I. 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.
- p. 2, l. 6. nifH is only one gene, and it codes for one subunit of the nitrogenase reductase
- p.4, I. 3 spell out HgCl2
- p.4, l. 8 and is throughout the text: submitted is capitalized, please change
- p.4, I. 10 As the paper is in review a brief description of the method would be helpful
- p.4, I. 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.

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

p. 6, I.2 ff You follow your classical screening approach, here, and there is nothing bad about it. Still, I would be extremely curious seeing a nifH sequencing doe 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. 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 with a metadata

C3

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  $\pm$  286  $\mu$ mol 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, I. 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.
- p. 2, l. 6. nifH is only one gene, and it codes for one subunit of the nitrogenase reductase
- p.4, l. 3 spell out HgCl2
- p.4, I. 8 and is throughout the text: submitted is capitalized, please change

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

p.4, I. 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.

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

p. 6, I.2 ff You follow your classical screening approach, here, and there is nothing bad about it. Still, I would be extremely curious seeing a nifH sequencing doe 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

C5

for future studies to have a proper deep sequencing included.

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-567, 2018.