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Interactive comment on "Aphotic N_2 fixation along an oligotrophic to ultraoligotrophic transect in the Western Tropical South Pacific Ocean" *by* Mar Benavides et al.

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Reviewer #3 The study by Benavides and coauthors report aphotic N2 fixation rates and identify diazotrophs present in the mesopelagic layer of the western tropical South Pacific. The paper is a significant contribution which increases the knowledge about aphotic nitro- gen fixation in a region which is highly interesting in terms of N-input from N2 fixation. Rates of N2 fixation were low but detected across all depths and stations. Shifts in diazotroph assemblages seemed to be mostly associated with depth. A distinct 1G phylotype was identified to coincide with the oxygenated Sub-Antarctic Mode Water. The paper is very well written and the methods used are well described,

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solid and established.

We thank this reviewer for his/her positive comments.

In my opinion the presentation of data could be improved by clearer links to different water masses. In the title the oligotrophic to ultraoligotrophic transect is highlighted but the way that this translates into sampling stations and different water masses is not evident to the reader from the figures. Further the nifH data is presented largely based on depth rather than sampling location/water mass.

In this study, our objective was to sample throughout the mesopelagic zone, not necessarily targeting any specific water masses. The depths sampled (200, 500, 650 and 800 db) were "arbitrarily" chosen according to water volume availability in deep casts during the OUTPACE cruise (note that we needed as much as 40 L per depth to perform all our analyses). Very interestingly, when examining the nifH sequencing results, it turned out that a specific phylotype was predominant in a given water mass (subcluster 1G in the SAMW). Unfortunately, the coverage of our samples throughout the mesopelagic zone is not enough to represent all the different water masses present and to identify patterns in N2 fixation activity or diversity of diazotrophs according to water mass distribution. This can be clearly seen in the T-S diagrams shown in Figure 1 (from the response to reviewers file).. On the left, we present a T-S diagram of the water masses sampled during the OUTPACE cruise (as displayed in Fig. 4a in Fumenia et al., this issue). According to this T-S diagram, our N2 fixation and nifH gene measurements (central and right figures) correspond to the lower part of the upper thermocline (t=24.7-25.4), lower part of the lower thermocline (t=26.5-26.7), and SAMW/AAIW (t=26.7-27.3). No measurements are available in the two water masses of the central thermocline.

The DOM analysis is valid but considering the low abundances these diazotroph groups are likely present in compared to other members of the microbial community establishing connections may be difficult. From the results section is not evident if differences in

DOM compounds were seen across the transect or different depths.

Our group investigated aphotic N2 fixation and its relationship with DOM in a couple cruises in the Solomon Sea (Benavides et al., 2015) and in the Mediterranean Sea (Benavides et al., 2016), in the frame of the project DIADOM https://cordis.europa.eu/project/rcn/187917_en.html In both cases we found positive correlations between labile compounds and N2 fixation. In the OUTPACE cruise we basically followed the same sampling strategy, but did not find significant relationships between DOM composition and aphotic N2 fixation. Although the FTICRMS data may itself not add much to the present study„ we decided to keep it for comparison with our previous studies and to reinforce the need for a mechanistic understanding of how non-cyanobacterial diazotrophs interact with DOM in the ocean.

Benavides, M., H. Moisander, P., Berthelot, H., Dittmar, T., Grosso, O. and Bonnet, S.: Mesopelagic N2 fixation related to organic matter composition in the Solomon and Bismarck Seas (Southwest Pacific), PLoS One, 10(12), 1-19, doi:10.1371/journal.pone.0143775, 2015.

Benavides, M., Bonnet, S., Hernández, N., Martínez-Pérez, A. M., Nieto-Cid, M., Álvarez-Salgado, X. A., Baños, I., Montero, M. F., Mazuecos, I. P., Gasol, J. M., Osterholz, H., Dittmar, T., Berman-Frank, I. and Arístegui, J.: Basin-wide N2 fixation in the deep waters of the Mediterranean Sea, Glob. Biogeochem. Cycles, 30, 1-19, doi:10.1002/2015GB005326.Received, 2016.

The (relatively) high N2 fixation rates at station 13 are curious and could be given some more attention in the discussion. From Fig 2. it appears like the diazotroph composition from station 13 differs largely between the depths and clusters away from the other samples. I find it very intriguing that this suggests that several different groups may be responsible for similar rates at the different depths. It is mentioned that high concentrations of chlorophyll were observed at this station. Did this coincide with high photic N2 fixation rates?

Indeed, it is very interesting that aphotic N2 fixation rates were highest at station 13. These high rates coincided with a patch of chlorophyll at the surface (de Verneil et al., 2017; this issue), and the high photic N2 fixation rates (see figure 2e in Bonnet et al. (2018), same issue). We hypothesized that labile organic matter exported from the photic zone fuels aphotic N2 fixation below, as we previously observed in another cruise in the WTSP (Benavides et al., 2015). Within station 13, the 200 and 650 dbar samples clustered closely with other samples from the same depths. It was interesting that at 500 dbar, the majority of the nifH sequences were from cluster 3Q, and at 800 dbar, from 1O. These high proportions of the community are due mostly to specific OTUs (denovo18755 and denovo6047, respectively), which are included in Figure 4.

Benavides, M., H. Moisander, P., Berthelot, H., Dittmar, T., Grosso, O. and Bonnet, S.: Mesopelagic N2 fixation related to organic matter composition in the Solomon and Bismarck Seas (Southwest Pacific), PLoS One, 10(12), 1-19, doi:10.1371/journal.pone.0143775, 2015.

Bonnet, S., Caffin, M., Berthelot, H., Grosso, O., Benavides, M., Helias-Nunige, S., Guieu, C., Stenegren, M. and Foster, R. A.: In depth characterization of diazotroph activity across the Western Tropical South Pacific hot spot of N2 fixation, Biogeosciences, (January), 1-30, doi:10.5194/bg-2017-567, 2018.

de Verneil, A., Rousselet, L., Doglioli, A. M., Petrenko, A. A. and Moutin, T.: The fate of a southwest Pacific bloom: Gauging the impact of submesoscale vs. mesoscale circulation on biological gradients in the subtropics, Biogeosciences, 14(14), 3471-3486, doi:10.5194/bg-14-3471-2017, 2017.

Other comments: The presentation of average N2 fixation rates and relation to % of photic N2 fixation is unclear and values in abstract and text appear to be different. (Abstract Lines 33-34 and Discussion Lines 20-23)

We agree. The overall contribution of aphotic N2 fixation (across the whole transect) ranges between 6 and 88% (as said on the abstract). In the discussion we split the

contribution to the two regions (Melanesian Archipelago -MA-, and subtropical gyre -GY-), with average contributions of 13 and 51%, respectively (according to the regional values provided in Bonnet et al., 2018).

Bonnet, S., Caffin, M., Berthelot, H., Grosso, O., Benavides, M., Helias-Nunige, S., Guieu, C., Stenegren, M. and Foster, R. A.: In depth characterization of diazotroph activity across the Western Tropical South Pacific hot spot of N2 fixation, Biogeosciences, (January), 1-30, doi:10.5194/bg-2017-567, 2018.

The Bray-Curtis distances in Figure 2 might be more meaningful if done on a level with higher resolution. Currently the variations in phylotypes is largely "hidden" in the 1G subcluster. A rarefaction to equal sampling depth would further improve this analysis.

The clustering was conducted using the method by Frank et al. (2016), which uses the subclustering to the lowest level of 1G. This method thus does not allow going to a higher resolution with subcluster 1G. By resampling to only 15,000 reads (average 89,000, std dev 42,500), there is no significant difference in Shannon or Simpson diversity indices. Additionally, we have previously compared similar sequence data analyzed via resampling to the same sequencing depth and the results were not appreciably different. Additional resolution of distributional patterns within Cluster 1G is shown in the OTU based analysis shown in Figure 2.

Frank, I. E., Turk-Kubo, K. A. and Zehr, J. P.: Rapid annotation of nifH gene sequences using classification and regression trees facilitates environmental functional gene analysis, Environ. Microbiol. Rep., 8, 905-916, doi:10.1111/1758-2229.12455, 2016.

In Figs. 2 and 3 data is presented as depth but in Fig. 1 as pressure [dbar]

It should be dbar. Figs. 2, 3 and 4 have been corrected accordingly.

Fig. 1 Please adjust the scale so that the circles are not cut for stations 1 and 15

In order to address the comments of Reviewer #2, we have changed Fig. 1 providing aphotic N2 fixation rates as sized dots (as we find it very visual and easy to spot where

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activity is higher), but with actual rates superimposed in coloured numbers (see Figure 2 from the response to reviewers file).

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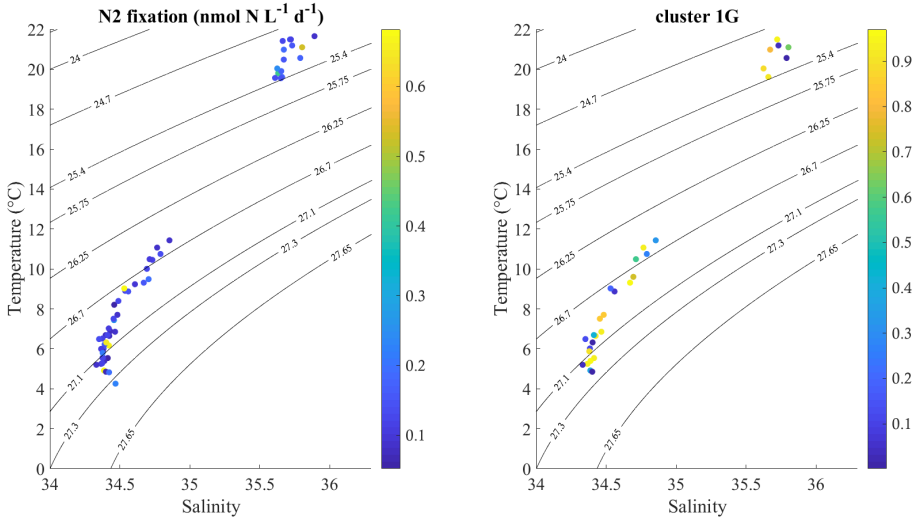

Fig. 1.

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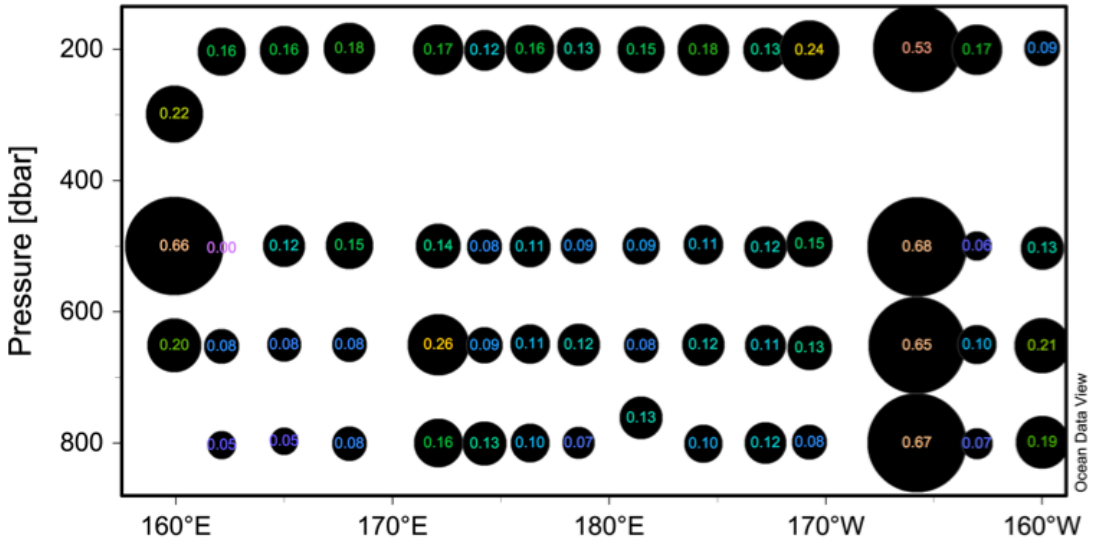

Fig. 2.

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