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Interactive comment on "Microbial community structure in the Western Tropical South Pacific" by Nicholas Bock et al.

Anonymous Referee #2

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Journal: BG Title: Microbial community structure in the Western Tropical South Pacific
Author(s): Nicholas Bock et al.
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Special Issue: Interactions between planktonic organisms and biogeochemical cycles across trophic and N2 fixation gradients in the western tropical South Pacific Ocean: a multidisciplinary approach (OUTPACE experiment)

This MS describes picophytoplankton abundance in Western tropical south Pacific, where such information is strictly limited. I hope that my comments are helpful to improve the MS.

We would like to thank the reviewer for their constructive comments and suggestions, which have contributed to improve the manuscript. Please find below the reviewer's comments copied in italics and our response in regular font, with reference to changes in the manuscript in bold.

1) ANOVA results 1-a: Please show the summary table of Two-way ANOVA. Authors analyzed two-way ANOVA, but I am not sure two factors, and the aim of the analysis. If authors will show the summary table in the Result section and mention the strategy of two-way ANOVA in Materials and Methods section, these are helpful for readers

Summary table is included below and added to manuscript as Table 2. To clarify, the goal of the analysis was to quantify vertical and longitudinal differences in parameters measured along the transect. Categorical variables used in doing this were biogeochemical condition (mesotrophic, LDB, oligotrophic) and the region of euphotic zone (upper: Z_u or lower, Z_l). Interpreting results using Tukey's HSD, two-way ANOVA was useful in separating between-condition differences in each section of the euphotic zone. Although the same could have been accomplished by subsetting the data to include only upper or lower euphotic zone samples and then performing one-way ANOVA on parameter values * condition, two-way ANOVA was a more straightforward approach.

1-b: ANOVA assess the difference among the multiple assemblages (groups) and cannot determine which is higher than others. However, authors sometimes mentioned that one assemblage is significantly higher than others. For example, "values for %HNA were significantly greater at LDB relative to the mesotrophic and oligotrophic stations (ANOVA, p < 0.01)" in lines 6-7 of page 6. In the Materials and Method section, please describe the multiple comparison after ANOVA.

Thanks for catching this! Comparisons were made using Tukey's Honest Significant Differences, by way of the R TukeyHSD package. To clarify, we added the following sentence to the methods:

Tukey's Honest Significant Difference post-hoc test was used to compare group means when two-way ANOVA indicated significant between-group differences.

2) Bottom-up and top-down control of microbial communities Authors analyzed top-down or bottom-up control of microbial communities using counting data of various planktonic groups. In the analysis, author used heterotrophic bacterial counting data. However, I wonder that Prochlorococcus should also be included for this analysis, as the Prochlorococcus was numerically abundant, and the cell size is also overlapped with heterotrophic bacteria. Is there any data that heterotrophic flagellates grazed only on heterotrophic bacteria?

One of the limitations of the Gasol model is that it assumes HNF to feed only on bacteria, which is very certainly not the case, as has been documented by a large number of studies. The sentence starting on line 29, page 11 is intended to address this, although perhaps does not go far enough. Regardless, adding *Prochlorococcus* to the analysis does indeed increase *d* values across all biogeochemical conditions, as shown in the figure below. However, values for *d* at the oligotrophic stations remain significantly greater than those at mesotrophic or bloom stations, and so the results are not affected qualitatively. Regardless, to clarify the likelihood of HNF feeding on cyanobacteria, we modified the sentence starting on line 29, page 11 to read as follows:

Given that the HNF abundances predicted by the model are calculated on the assumption that HNF only graze on heterotrophic bacteria, the increase in *d* at GY may reflect increased grazing on cyanobacterial prey. Indeed, previous studies have reported HNF to graze on cyanobacteria, generally at rates similar to those reported for grazing on heterotrophic bacteria (Christaki, 2001; Cuevas and Morales, 2006; Ferrier-Pagès and Gattuso, 1998).

Also added the following sentence following at the end of the paragraph starting on line 25, page 11:

Moreover, including abundances of both heterotrophic and autotrophic bacteria when calculating values for *d* does not qualitatively affect the comparison between sites or layers as described above.

3) phagotrophy of PPE I think it is too much to say the possibility of phagotrophy of PPE under nitrate limited condition. Authors cannot make any concrete conclusion, I recommend that last two paragraphs (lines 16-32 of page 11) should be shorten.

Second to last paragraph (lines 16-32 of page 11) was shortened as recommended. Paragraph (which was also combined with final paragraph) now reads as follows:

By reducing bacterial abundances relative to those of HNF, the reduction of *d* reported in Z_l may also result from phagotrophy by PPE. Feeding experiments in the North Atlantic have demonstrated small plastidic eukaryotes to account for up to 90 % of bacterivory in nutrient-limited waters (Zubkov and Tarran, 2008), while laboratory and field studies have demonstrated increased feeding rates specifically in response to P limitation (Christaki et al., 1999; McKie-Krisberg et al., 2015).

4) NO2, NO3, PO4 Page 3, line 33, PO4, NO2, and NO3. Authors used PO4, NO2, NO3 in other sentences. Also, nitrate and nitrite are used instead of NO3 and NO2. Please correct them appropriately.

Thanks for highlighting. Fixed subscript on page 3, line 33 and replaced nitrate and nitrite as needed.

5) HNF abundance Authors mentioned that depth integrated abundances of HNF were greater in the Zu than the Zl at mesotrophic and oligotrophic stations (lines 15-16 of page 6). But, as far as I understood, HNF abundance in oligotrophic site is low in Zu (0.41) than that in Zl (0.43, Table 1)

This was admittedly an oversight. We rewrote the sentence to more accurately reflect values provided in Table 1, and repeated analysis using ANOVA for consistency with other results. Lines 15-17 of page 6 now read as follows:

Depth integrated abundances were significantly greater in Z_u than Z_l at MA and GY (ANOVA, $p < 0.01$), while there was no significant change in HNF abundances with depth at GY.

6) Reference list Below papers are not included in the reference list;

Thanks for highlighting. All indicated papers added to reference list

7) Below two papers*, which are in the reference list, are not cited in the MS. Moutin et al., 2017 Tillson et al., 2004*

Thanks for highlighting. Papers removed from reference list

8) Figures Fig. 3 are hard to see. It is preferred that axis color is changed to black, and size of labels is larger than the present. In my printing environment, axes of Fig. 4 are almost invisible.

Thanks for highlighting. We will thicken axes on figures 3 and 4 and will increase label size on figure 3 to match that of other figures in the manuscript.

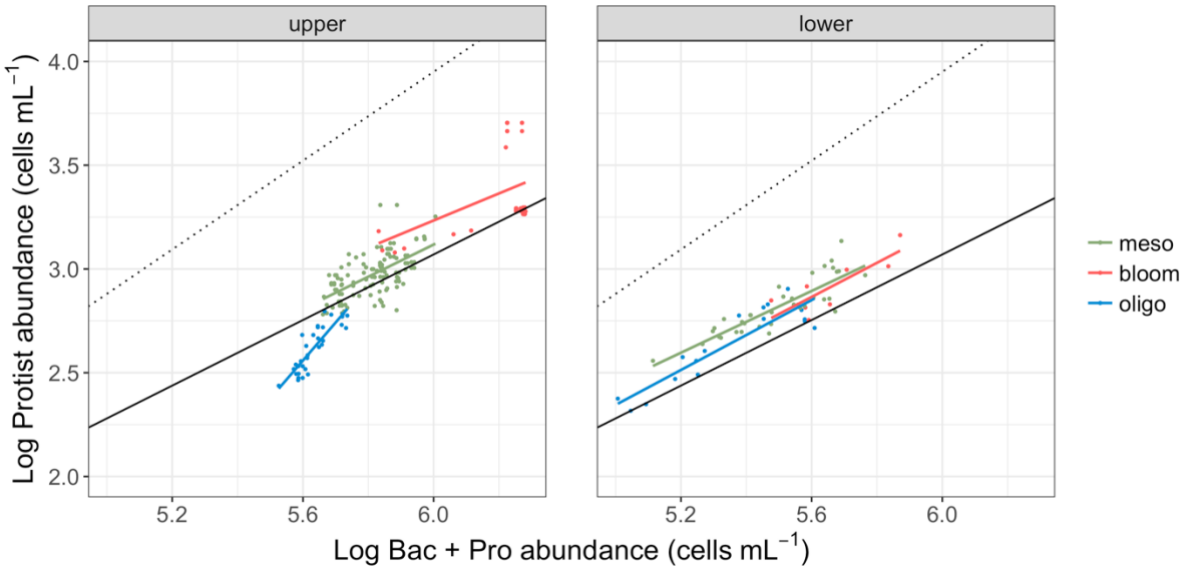

Figure: Gasol model analysis repeated using abundances of both heterotrophic bacteria and *prochlorococcus* rather than heterotrophic bacteria alone

		Pro		Syn		PPE		Bac		%HNA		HNF		NOx		PO4	
	DF	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Euphotic layer	1	49.23	< 0.01	23.35	< 0.01	68.08	< 0.01	88.29	< 0.01	5.36	0.021	24.66	< 0.01	42.38	< 0.01	40.6	< 0.01
Area	2	2.94	0.06	25.88	< 0.01	3.15	0.054	4.57	0.017	16.90	< 0.01	6.041	0.005	12.62	< 0.01	113.2	< 0.01
Interaction	2	7.03	< 0.01	15.78	< 0.01	0.85	0.436	5.97	0.005	0.69	0.501	11.31	<0.01	11.88	< 0.01	3.01	0.057

Table: Summary table of two-way ANOVA results for parameters analyzed in this study. Row 1 (euphotic layer) tests for significant differences between mean parameter values across different layers of the euphotic zone (Z_u vs Z_l). Row 2 (condition) tests for significant differences between mean parameter values across different biogeochemical areas (MA vs LDB vs GY) on mean parameter values. Row 3 (interaction) tests for combined effect of euphotic layer and biogeochemical condition on mean parameter values. Relationships for Pro, Bac, HNF, NO_x, and PO_4 calculated from depth-integrated abundances; Relationships for %HNA calculated from raw values.