

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

Anonymous Referee #1

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General comments

This is an exhaustive and complete study in the West Tropical South Pacific Ocean, trying to elucidate which are the factors driving the microbial community structure in the photic layer (200 m). Authors did an amount of work measuring abundances of heterotrophic and phototrophic microorganisms, nutrients, . .etc, following a transect from mesotrophic to ultraoligotrophic area. Also, through established models they try to disentangle if microbes are top-down or bottom up controlled, and how is the role of limitation of nutrients and fixation of N₂. I am conscious that to explain all this without the reader losing attention it is not easy. But sometimes, due to small inconsistencies that I detail below, makes that the flow of the story to be lost.

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.

Specific comments

- 1) *Abstract Page 1, line 23: What abundant are Prochlorococcus? Notice that Synechococcus are quantified.*

Admittedly neglected to explicitly describe *Prochlorococcus* abundances. To incorporate this information, and in an effort to improve readability, we separated the description of physical characteristics describing "typical tropical structure" from the description of organism abundances and biomass. Lines 21 to 25 now read as follows:

At the most general level, we found a "typical tropical structure," characterized by a shallow mixed layer, a clear deep chlorophyll maximum at all sampling sites, and a deep nitracline. *Prochlorococcus* was especially abundant along the transect, accounting for 68 ± 10.6 % of depth-integrated phytoplankton biomass. Despite their relatively low abundances, picophytoeukaryotes (PPE) accounted for up to 26 ± 11.6 % of depth-integrated phytoplankton biomass, while *Synechococcus* accounted for only 6 ± 6.9 %.

Introduction and Material and Methods

- 2) *Page 2, line 22 the study was carried out from mesotrophy to ultraoligotrophy kinds of waters. In addition, in the introduction you are considering ultraoligotrophic areas and in the whole manuscript, you only refer to oligotrophic.*

Criteria for mesotrophic/oligotrophic designation were based on those used in Grob 2011. This designation does indeed conflict with terminology used in the abstract, and in other manuscripts in

the special issue that are based on Moutin et al. (2017). To resolve this, we now designate mesotrophic stations as **Melanesian Archipelago (MA)**, and oligotrophic stations as **Gyre (GY)**, based on Moutin et al. (2017). The definition of stations now reads as follows:

Following the designation used by Moutin et al. (2017), stations sampling the mesotrophic to oligotrophic waters of the Melanesian Archipelago (SD1 to SD12 and LDA) were classified as MA, while stations sampling the oligotrophic to ultra-oligotrophic waters of the western South Pacific Gyre (SD13 to SD15 and LDC) were classified as GY. Long duration station B was in the late stages of a phytoplankton bloom at the time of sampling (de Verneil et al., 2017). As such, it was analyzed independently of other stations on the transect and is simply referred to as LDB.

- 3) Page 3, line 34, I would move the definition of stations to field sampling section. Then all stations after LDB are oligotrophic including LDA and thereafter ultraoligotrophic?

Moved definitions to field sampling sections, as suggested. The text from the above point now follows page 2, line 20.

- 4) Page 3, line 15, Vázquez-Dominguez is missing in the reference list

Thanks for highlighting. Added to list

- 5) Page 4, line 11, arrange sub-index for phosphate, nitrate, and nitrite

Thanks for highlighting. Corrected formatting to NO₂, NO₃, PO₄.

- 6) Results Page 4, line 37, Fig. 2 do not show temperature

The reference to figure 2 was intended to only apply to the mixed layer depth. We agree, though, that the reference is somewhat unclear (and the thermocline description perhaps extraneous). Lines 14 to 18 on page 4 now read as follows:

Stations along the transect were characterized by warm sea surface temperatures (mean 29.4 ± 0.4 °C). The water column was strongly stratified along the entire transect, with mixed layer depths ranging from 21 ± 5 m for MA to 25 ± 8 m for GY (Fig. 2).

- 7) Page 6, line 31, Why do you not show HNF and ciliate profiles?

HNF profiles were included with other plankton groups as Fig. 2E, and citation of Fig 2 E is now included in this sentence. Ciliate profiles were not included mainly because these profiles were previously published in Dolan (2016) and we only use the data in the discussion (i.e. not as a main result of the present study).

- 8) Page 6, line 39, Gasol (1994) is missing in the reference list.

Thanks for highlighting. Added to list

- 9) Another way to corroborate this top-down bottom-up issue from the point of view of bacteria could be the application of Ducklow 1992 equation, relating bacterial biomass and bacterial production, but for that you will need bacterial production measurements. Did somebody measure it during this cruise? Ducklow H. 1992. Factors regulating Bottom-up control of bacterial biomass in open ocean plankton communities. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* 37: 207-217

France Van Wambeke did indeed measure bacterial production for the transect, and we did attempt to apply Ducklow's model to this data. The slopes of the model I regression were calculated on the log (BB) = f (log (BP)) relationship, based on BB in µg C l⁻¹, estimated from abundances based on a constant conversion factor for BP as used in our MS (i.e. 11.5 fg C per cell) and BP expressed in µgC l⁻¹ h⁻¹ and based on the leucine technique, using 1.5 kg C mole⁻¹. The slopes (± se) were 0.41 ± 0.030 for MA, 0.61 ± 0.049 for LDB, and 0.49 ± 0.071 for GY based on the maximum layer depth sampled (0-200 m). Biogeochemical condition was determined to have a significant effect on regression slopes (ANCOVA, p < 0.01). However, in Z_u, slopes were insignificant for MA, site LDB, and GY. In Z_i, slopes were insignificant in MA, 0.324 ± 0.015 in LDB

and 0.381 ± 0.116 at GY. By the criteria described in Ducklow 1992, this would correspond to "strong" bottom-up control at LDB, and "moderate" bottom-up control at MA and GY if we consider the 0-200m layer, but no, or weak bottom-up controls when focusing on Z_u or Z_l alone.

We added a sentence to address these results following line 15 on page 11. The new text reads as follows:

Plotting bacterial abundances against the bacterial production data reported by Van Wambeke *et al.* (this issue, in press), and interpreting regression slopes using the criteria described by Ducklow *et al.* (1992) in the Z_u layer, we found no evidence for bottom-up control of bacteria populations at MA, LDB, or GY.

- 10) Page 6, Fig. 6. Very interesting the obtained results related with bottom-up and top-down issue. Although as the authors say there were no measures of grazing rates. Perhaps you might have a look on the paper of Lara *et al.* (2017) that for near stations to this study, during the MALASPINA cruise, they measured grazing and viral mortality rates (Lara, E., D. Vaqué, E.L. Sà, J.A. Boras, A. Gomes, ..., R. Massana, T.S. Catalá, G.M. Luna, S. Agustí, M. Estrada, J.M. Gasol & C.M. Duarte (2017) *Unveiling the role and life strategies of viruses from the surface to the dark ocean Sci. Adv.* 3: e1602565, doi: 10.1126/sciadv.1602565 3: 1-1).
- 11) Another paper from the same cruise that deals with this top-down, bottom-up issue from the point of view of bacteria could help you as is: Morán, X.A.G, J. M. Gasol, M. Pernice, J.-F. Mangot, R. Massana, E. Lara, D. Vaqué & C.M. Duarte (2017) *Temperature regulation on marine heterotrophic prokaryotes increases latitudinally as a breach between bottom-up and top-down controls Global Change Biol.* 23:3956–3964. doi: 10.1111/gcb.13730 23: 3956-3964.

Thanks for the very useful references. We added a sentence at line 33 on page 11 to acknowledge the importance of viruses in regulating bacterial abundance, citing the Lara *et al.* paper. The new text reads as follows:

Finally, viruses undoubtedly contribute to the observed variation in bacterial abundances, with previous studies reporting viral lysis to be an equally, if not more important factor in controlling prokaryote mortality than grazing alone in the surface waters of the open ocean, with protistan grazing only becoming dominant in the DCM layer (Lara *et al.*, 2017). Indeed, our d values were significantly reduced in Z_l (including the DCM) compared to Z_u in all the three regions investigated in the WTSP. However, there is a large degree of scatter along the 1:1 line in the relationship presented by Lara *et al.* (2017) between protist-mediated mortality and virus-mediated mortality, making it difficult to infer how viral lysis might have contributed to the reported differences in d .

Reviewing at the Morán *et al.* paper, we also added the following after line 15, page 11 to account for the very low slopes that they reported in the South Pacific when using the Ducklow model. The new text reads, which immediately follows the text added in response to comment 9, reads as follows:

This is similar to the results obtained by Morán *et al.* (2017), who applied the Ducklow model to data collected in the South Pacific during austral summer and reported very weak bottom-up control at all sampling sites, calculating regression slopes around 0.2 for samples between the surface and 4000 m. The authors likely would have obtained still lower slopes had their analysis been restricted to surface data alone, as we found for Z_u .

- 12) Page 7, line 5, About the interpretation of the large distance of d when using data from the Z_l in the oligotrophic stations, since *Prochlorococcus* are pretty abundant, as well as *Synechococcus* and both could be a prey for HNF, I am wondering, which would be the result if you sum up all bacteria (heterotrophic and phototrophic) and apply the model again? At the time that Gasol 1994, did the model it was very difficult to detect *Prochlorococcus* in the epifluorescence microscope, so, perhaps some of them were counted as heterotrophic bacteria after DAPI staining.

Redefining Bac as Bac+Pro+Syn did not seem to have any real impact on the model outputs. While both mean log(abundance) and d values increased across all biogeochemical condition, upper euphotic zone (Z_u , where the bias to dim fluorescent *Prochlorococcus* cells is the greatest) values for d remained significantly greater at GY compared to MA or LDB. Differences between MA and LDB remained insignificant. To address the concern, we added the following sentence at the end of the paragraph starting on line 25, page 11:

To account for the possibility that *Prochlorococcus* cells were erroneously identified as heterotrophic bacteria at the time of the model's formulation, we repeated the analysis including abundances of *Prochlorococcus* on the x-axis. Although doing so increased d values across all biogeochemical conditions, it did not qualitatively affect the relationship as described above.

In summary, I consider this manuscript a relevant piece of work describing the microbial abundance and biomass through a gradient from meso to oligotrophic in a not explored tropical area of the Pacific Ocean, and I think that few changes will contribute to make it clearer.

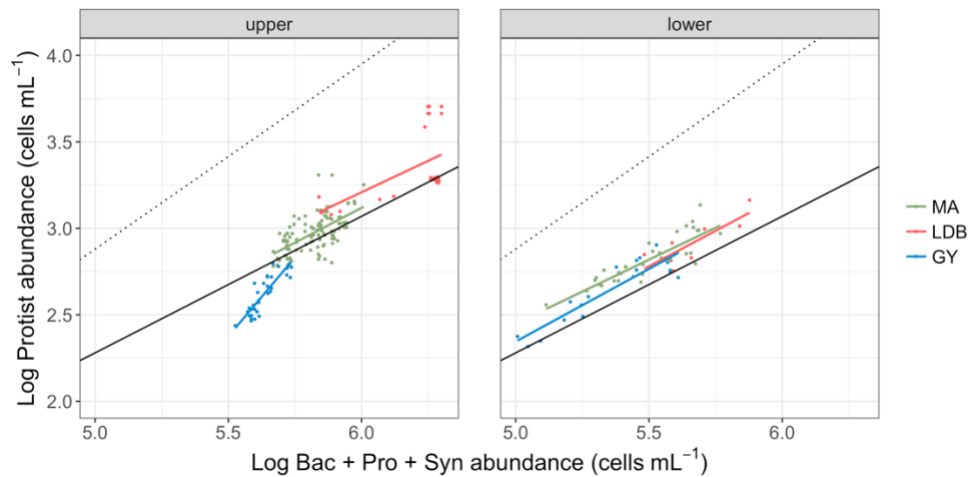


Figure: Gasol model analysis repeated using abundances of heterotrophic bacteria, *prochlorococcus*, and *Synechococcus* rather than heterotrophic bacteria alone