

## ***Interactive comment on “Heterotrophic prokaryote distribution along a 2300 km transect in the North Pacific subtropical gyre during strong La Niña conditions: relationship between distribution and hydrological conditions” by M. Girault et al.***

**Anonymous Referee #1**

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The authors present a useful set of hydrographic and bacterial abundance data obtained in a little-explored oceanic region, which are used to investigate possible connections between driving variables and microbial distributions. While the topic and the material is certainly of interest for the readers of Biogeosciences, the analysis has a number of limitations and should be significantly improved before publication. Of particular concern (as detailed below) is the way in which nutrient availability is assessed, lack of consideration of vertically integrated variables, use of bacterial abundance only and not bacterial biomass, lack of consideration of vertical mixing, and lack of focus on

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the underlying mechanisms that link hydrography with bacterial distributions.

A large part of the manuscript deals with the potential role of nutrients in explaining the variability in bacterial group distribution, but I am not convinced that the authors have chosen the best approach to assess nutrient availability. Only nutrient concentrations, rather than nutrient supply rates, are considered. But in the surface layer of the tropical ocean, fast microbial consumption often result in a disconnect between nutrient concentration and nutrient supply rates. Changes in the latter may lead to changes in microbial abundance and diversity – without necessarily being reflected in changes of nutrient concentrations. This was shown by Gasol et al. (2009, *Aq Microb Ecol* 56:1-12): nutrient concentration in the upper mixed layer of the central tropical Atlantic is very low and relatively constant but diffusive fluxes change by more than 4 orders of magnitude, and had an impact on bacterial activity. The present manuscript requires a better characterization of nutrient supply if the relationship between potential nutrient limitation and bacterial group distribution is to be ascertained. One possibility is to compute vertical gradients in nutrient concentration and apply diffusivity values obtained from the literature (or use parametrizations based on measurements conducted during the cruise, e.g. vertical density gradients, wind speed, etc) to obtain estimates of vertical diffusive nutrient fluxes (see Gasol et al. 2009). Another approach, less accurate but also useful, would be to use the nutricline depth as a proxy for nutrient supply (Malone et al. 1993 *Deep-Sea Res I* 40:903-924; Cermeno et al. 2008 *PNAS* 105(51) 20344-20349).

The analysis, based on a multivariate approach to relate different environmental and biological variables to bacterial abundance, suggests a rather ‘static’ view of the connection between environmental forcing and microbial distributions. No consideration is given to the role of vertical mixing and turbulence, but microbial populations are subject to vertical displacements, whose magnitude is likely to change significantly along the transect, and the authors could explore this by calculating parameters such as the Brunt Vaaisala frequency.

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Given the sampling stations are far apart, and considering that the authors have conducted a relatively high-resolution sampling along the vertical, it could be useful to calculate vertically integrated abundance and or biomass for each group, and plot them against environmental variables such as degree of stratification, mixing, nutricline depth, estimated vertical diffusive flux, etc. This approach is complementary to the multivariate analysis and well-suited to pursue a 'hypothesis-driven' analysis of the data.

The vertical distribution of temperature and nutrient concentration should be shown, even though it has been presumably included in a previous article (Girault et al 2013b). However, these data are essential for the discussion of bacterial distribution and to make the present manuscript stand on its own.

The authors use only abundance data but from a biogeochemical standpoint biomass can be more relevant. The flow cytometry data should allow calculation of cell biovolume and then an estimate of cellular biomass. Several studies that report on bacterial distribution over large spatial scales have used bacterial biomass as the key variable (e.g. articles by Zuvkov, Gasol, Moran and others). Microbial cell size is itself sensitive to both temperature and nutrient availability – therefore including cell size as a variable of study could provide additional insight.

Most of the Discussion is focused on the relationship between environmental or 'potentially driving' variables and the abundance of the different bacterial groups but there is little consideration of the underlying mechanisms. For instance, when the authors write "i) the LNA distribution is mainly explained by temperature and salinity and ii) HNA distribution is mainly explained by an association of variables (temperature, salinity, Chl a and silicic acid) rather than a single environmental factor" they are essentially re-stating the results of the multivariate analysis. But the question is: How are temperature and salinity driving the distribution of LNA bacteria? Is there a physiologically or ecologically plausible mechanism that links directly salinity with LNA abundance? Or maybe is it the case that salinity is just acting as a marker for other properties which are themselves driving the variability in LNA abundance? Beyond highlighting corre-

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lation between variables, the Discussion would benefit from a deeper consideration of the ultimate mechanisms that govern microbial distribution.

#### Specific points

The last section of the Introduction is missing a set of specific hypothesis which are to be tested. Previously the section has discussed possible relationships between environmental variables such as degree of oligotrophy and relative abundance of different bacterial groups, but no specific prediction is made as to what was to be expected along the transect.

Abstract, Line 12. The phrase 'associated with temperature and salinity' is not informative. It should be specified whether the association is with high/low temperature and/or salinity.

The different stations are grouped into different areas according to temperature and salinity (page 15801, line 8), but the detailed criteria used in the partition are not indicated.

Mixed layer depth is calculated but seems not to be included in the multivariate analysis. Why is this? Vertical mixing can have a strong impact on important processes such as nutrient input and exposure to high irradiance, among others.

Pages 15805-15806 There is a long discussion on the role of silicic acid which is quite speculative. If no previous evidence is available to show that silica is limiting for phytoplankton in the region, the mechanistic linkage between silicic acid concentration and bacterial distribution is rather weak.

Pages 15807-15808. The whole section on the role of climatic events such as El Nino/La Nina should be deleted as there is no data available to substantiate any claims on the topic.

Page 15808, lines 26-28. If a high nucleic acid content is indicative of more active metabolism and faster growth, how do you explain that HNA bacteria are more abun-

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dant under more nutrient-depleted conditions?

Page 15812, line 7. Specify if the latitudinal increase in the HNA/LNA ratio is equatorward or northward.

The countouring in Fig. 4 gives too much weight to the horizontal axis, resulting in features which are not really supported by the data. Considering the long distance between stations, the countouring should emphasize the vertical variability without presenting horizontal features which are not based on actual measurements but are just extrapolations from the countouring software.

Section 4.3 of Discussion is quite long and speculative. Considering the (inevitably for a long transect such as this one) poor horizontal resolution of the survey, not much can be said confidently about the role of mesoscale features on microbial distributions. This section should be shortened, and the related conclusions toned-down and perhaps omitted from the Abstract.

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