

## ***Interactive comment on “Spatial and temporal variability in the response of phytoplankton and bacterioplankton to B-vitamin amendments in an upwelling system” by Vanessa Joglar et al.***

### **Anonymous Referee #2**

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The role that the availability of B-vitamins, specifically vitamin B12 and B1, play in shaping the marine microbial community is very relevant. The authors of this manuscript conducted an extensive experimental campaign with the goal of providing some insight to these processes. Unfortunately, their findings are poorly communicated and overstated in this manuscript. Most of the discussion is highly speculative and is insufficiently referenced. The authors have gone “all-in” on the poorly justified concept of “response ratio”. I feel like this calculated metric is overly general and prevents an in-depth analysis of the actual data which likely contains subtle variations that could either support or undermine the authors primary conclusions. I don’t understand why the authors chose to use response ratios rather more traditional ecological and phys-

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iological metrics. While response ratios could be a useful part of the discussion, they should be just that, a part of the discussion. Additionally, the authors ignore the rates of community growth and dynamics and only assess the response at the end time point relative to the initial point. While it is not possible at this point to change the experimental design, the authors need to change their interpretation of the data to acknowledge the limits of their data.

It is unfortunate that the only measures of biomass performed by these authors during their experiments were bacterial abundance and chlorophyll A. These are very broad, unspecific measures of community structure, that can be impacted by a myriad of environmental factors. The authors make some substantial claims about the roles that B-vitamin additions are playing on the microbial community; however, I wonder if they really have enough resolution in their measurements to make these claims. The author’s use of “response rate” to obscures the fact that they are only measuring bacterial abundance and chlorophyll concentration. There are so many variables that impact these measures, it’s not clear to me that the authors are actually looking at responses from B-vitamins.

I have some substantial concerns about the conclusions the authors make about community diversity and B-vitamins. Their exact statistical methods need to be better explained. Additionally, the authors need to fully explain the limits of their statistical methods, and not overstate or be overly speculative about the observed correlations between abiotic/biotic factors, B-vitamins, and the amplicon data.

The manuscript needs substantial copy editing/English language editing. All sections need to be streamlined. The interpretation of results tends to be far too speculative. The authors need to only make claims that their data can support.

The B12 analytical method appears to be derived from previously published methods. Specifically, those published by Heal et al. 2014, Sañudo et al. 2012, and Suffridge et al. 2017. It is troubling to me that the authors do not cite any of these papers in

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the methods section, despite the fact that the described method is a nearly an exact match of those described in the above papers. Additionally, SPE extraction efficiency and limits of detection need to be included.

How were the whirl-pak bags prepared? Were they prepared to be trace clean? Were they sterile? What sort of plastic are they made out of? Trace metal or trace organic (B-vitamin) contamination is a real concern in experiments like these, especially when the authors want to make conclusions about the impact of a trace-component. Many plastics contain trace contamination from the factory, and if the bags were not properly prepared, this variability could interfere with all results.

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