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Biogeosciences Discussions

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.

Vanessa Joglar et al.

vanjoglar@gmail.com

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#Referee 2 comments

AC/ We are very grateful for the reviewer's comments, which have contributed to improve the manuscript.

RC/ The role that the availability of B-vitamins,speciïňĄcally 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 ïňĄndings are poorly communi-

cated and overstated in this manuscript. Most of the discussion is highly speculative and is insufiňĄciently referenced.

AC/ We have made our best to refer adequately all the relevant studies and eliminating some speculative statements.

RC/ The authors have gone "all-in" on the poorly justiïňĄed 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 physiological 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.

AC/ We are aware that sampling only at one endpoint (after 72 h incubation in our case) does not allow to discuss in detail the dynamics during each experiment, however we were particularly interested in extensively exploring the temporal and spatial variability of the response to vitamin enrichment. The experimental design, involved 36 experiments, with 8 triplicate treatments (24 experimental units per experiment). Even sampling only at the beginning and at the end we collected 972 samples for chlorophyll-a, and 972 for bacterial biomass. Initial and endpoint sampling is a common practice in enrichment microcosm experiments (e. g. Mills et al., 2004; Moore et al., 2006; Gobler et al., 2007; Bonnet et al., 2008; Koch et al., 2011), and allows the estimation of net growth rates using the following formula: ln (endpoint biomass/initial biomass)/incubation time. A previous work by Barber-Lluch et al (2019) in the same sampling area allowed us to conclude that sampling at 72 h was adequate to explore the effect of vitamins on both phytoplankton and bacterial biomass. As we agree

with the referee that the dynamics of phytoplankton and bacteria during the experiments are of interest, and following also the advice of referee 1, we now include in the manuscript two new figures where the initial and endpoint value of chlorophyll-a and bacterial biomass is represented. The response ratio figure is now included in the supplementary information. We accordingly now describe the dynamics of both planktonic components in the different experiments. We nevertheless decided to keep the response ratio as a measure of the magnitude of the effect (see below), which is very useful for the sake of comparison. We used here the response ratio as the quotient between the measured quantity of a response variable in experimental and control experimental units. Previous studies dealing with the effects of nutrients additions on microbial communities have noted the importance of expressing the change in the treatment relative to the control (Downing et al., 1999; Hedges et al., 1999; Elser et al., 2007, among others). We find that this variable is particularly adequate as a measure of the experimental effect because it quantifies the proportionate change that results from an experimental manipulation. This metric is widely use in marine ecology, and particularly in nutrient amendment experiments (e.g. Martínez-García et al., 2010; Teira et al., 2013; Barber-Lluch et al., 2019). The use of the response ratio calculated from endpoint biomass data provides the same information as the comparison of growth rates between treatments. Below we plot, as an example, the relationship between the response ratio from biomass (endpoint biomass in treatment divided by endpoint biomass in control) and the difference between growth rates (growth rate in treatment minus growth rate in the control) using data from two of our experiments (represented with different colours). It can be appreciated that the information provided by the response ratios follows exactly the same pattern as that provided by comparing growth rates. Moreover, the range of variation is higher for the response ratio, which allows to statistically detecting more subtle changes.

RC/ 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, unspeciïňĄc measures of community structure, that can be impacted by a myr-

iad 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.

AC/ B vitamins are essential growth factors for all microorganisms; therefore, the ultimate effect of a vitamin deficiency will be an impairment of growth, which is typically evaluated from changes in biomass. It is true that we only measure the effect on bulk phytoplankton and bacteria, and thus we have toned down all the conclusions about the effect on microbial community structure. We do not think that is unfortunate to have chosen phytoplankton and bacterial biomass as response variable, considering that most previous studies evaluating the role of B vitamins were based on biomass measurements (Sañudo-Wilhelmy et al., 2006; Gobler et al., 2007; Koch et al., 2011, 2012; Browning et al., 2018; Barber-Lluch et al., 2019). We are aware of many variables that could affect bacterial and phytoplankton biomass, for this reason, we compared the response of B vitamin treatments with their corresponding controls. B12, B1 and B12+B1 treatments were compared to the unamended control, while I+B12, I+B1, I+B12+B1 were compared with the I treatment.

RC/ 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.

AC/ We agree that some statistical methods needed further clarification and we recognize that some analyses were somehow redundant and have been excluded from this revised version. Specifically, we have eliminated the Pearson correlation between response ratios and the clr (centered-log-ratio) abundance of taxonomic groups (reported in former table 1) as was redundant with the dbRDA. Regarding the RELATE analysis to explore the relationship between the responses to B vitamin treatments (response ratios of phytoplankton and bacteria) and (1) the environmental variables (including nutrients, temperature, salinity, B12, chla and BB), (2) the prokaryotic, or (3) eukaryotic community structure, we believe that nicely shows that the responses are only significantly related to the prokaryotic community structure. We have clarified how we constructed the resemblance matrices (L272-L280). It is important to note, that as we are aware of the statistical limitations when working with relative abundance of sequences, prior to statistical analyses, ASV abundances were transformed using the centered log ratio (Fernandes et al., 2014; Gloor et al., 2017).

RC/ 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 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.

AC/ We now provided all the requested details about the vitamin B12 quantification method and included all the references (L207-L217).

RC/ 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.

AC/ We did not use strict trace metal clean techniques for sampling. It is important to note that the water for the experiments was pooled into a 20 l acid-cleaned carboy before filling the bags, thus all the bags would have the same incidental input of trace metals. We are aware that microbes could be limited by other trace elements or nutrients not considered in our treatments, such as iron or other B vitamins. For this reason, we based the discussion on the response ratios at the end of the experiments. The whirl-pak® bags are made of low density polyethylene, are sterile, non-toxic and transparent to the whole solar spectrum, thus avoiding UVR absorption of most other materials, and have been frequently used for experimentation with plankton communities (Gonzalez et al., 1990; Davidson and van der Heijden, 2000; Pakulski et al., 2007; Teixeira et al., 2018). The bags were not additionally treated as were used only once.

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Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2019-306/bg-2019-306-AC3-supplement.zip

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Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2019-306, 2019.

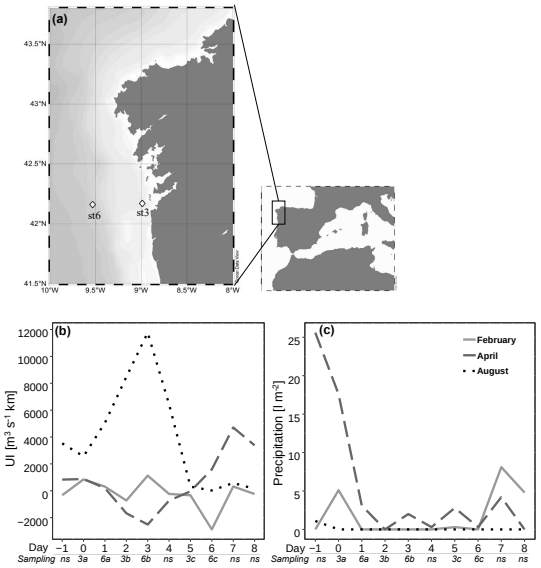

Fig. 1. (a) The NW Iberian margin and locations of the stations that were sampled, (b) distribution of daily coastal upwelling index (Iw)and (c) registered precipitations.

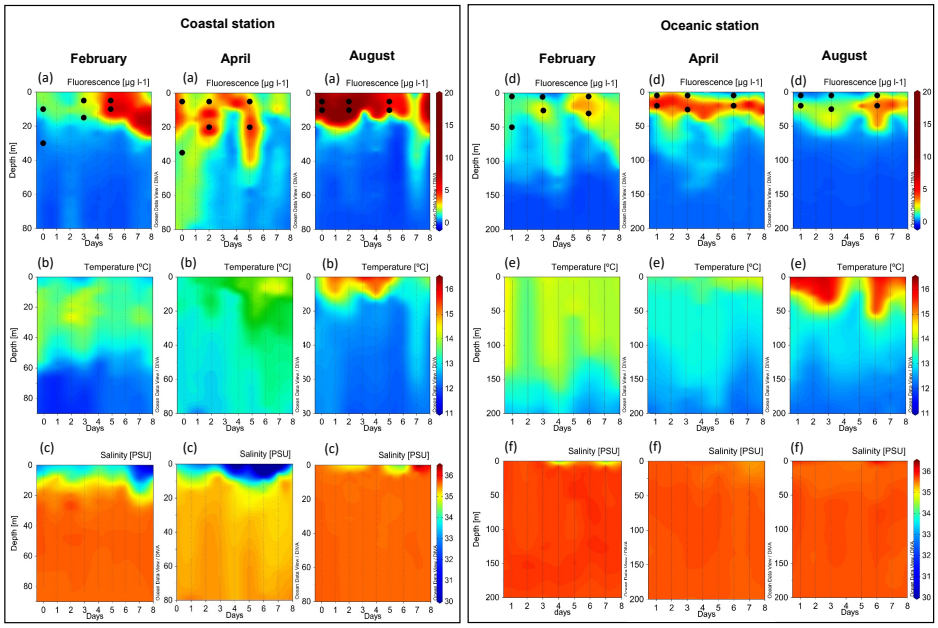

Fig. 2. Vertical distribution in the coastal and oceanic station of fluorescence (μg l-1), temperature (°C) and salinity (PSU) over time for February, April and August

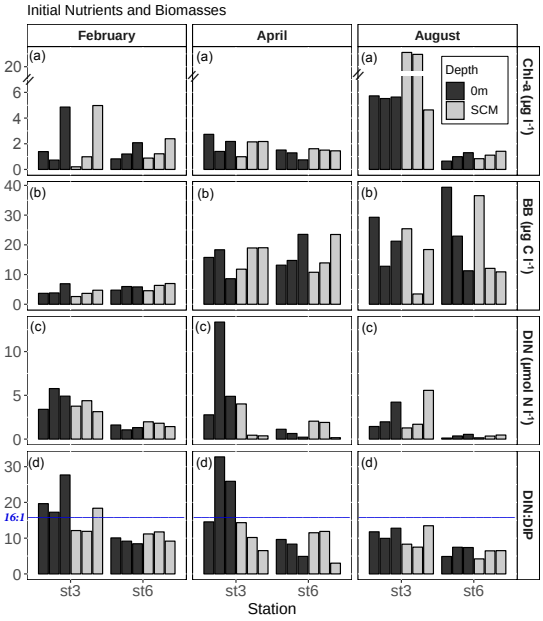

Fig. 3. Initial biological conditions and abiotic factors at the coastal (st3) and oceanic (st6) sampling stations.

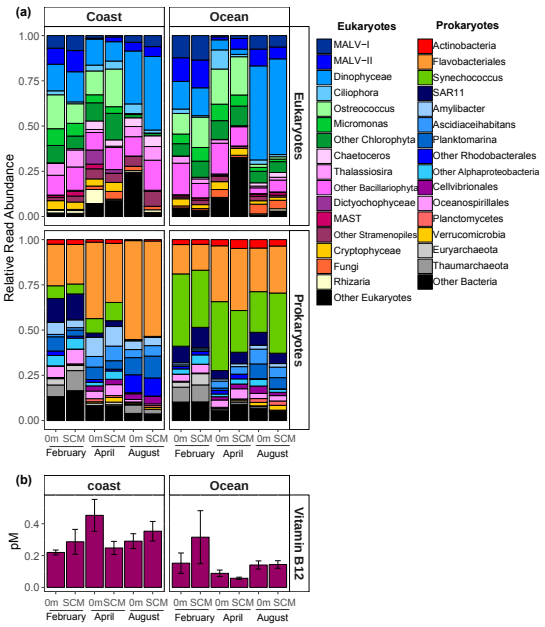

Fig. 4. (a) Averaged relative contribution of reads to the major taxonomic groups of eukaryotes and prokaryotes. (b) Averaged B12 concentration (pM).

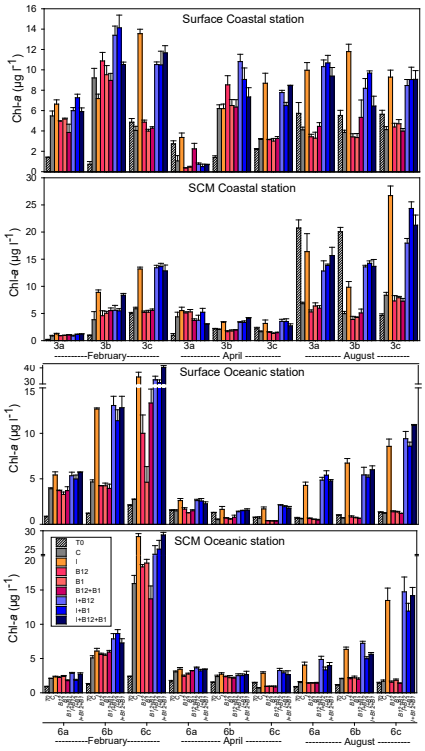

Fig. 5. Phytoplankton biomass (estimated as Chl-a concentration) (μg l-1) in the time-zero of each experiment (striped bars) and in the final-time of each treatment (colored bars) in the experiments.

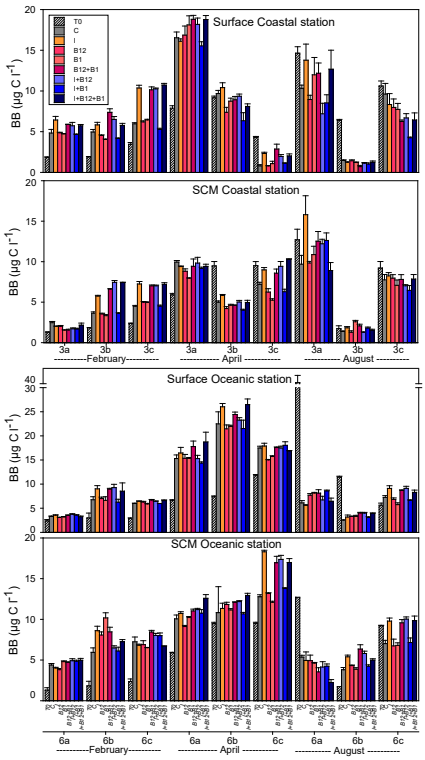

Fig. 6. Bacterial biomass (μgC l-1) in the time-zero of each experiment (striped bars) and in the final-time of each treatment (colored bars) in the experiments.

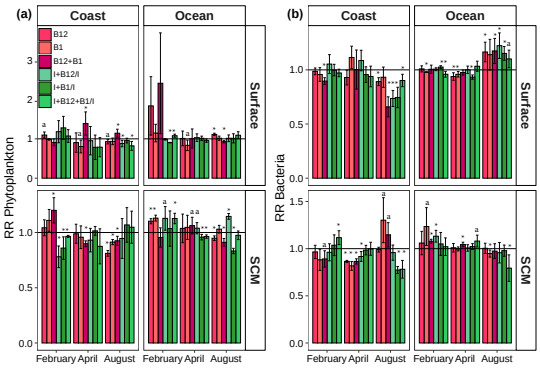

Fig. 7. Monthly averaged response ratio (RR) of (a) total phytoplankton community and of (b) bacterial community at surface and SCM in the coastal and oceanic station.

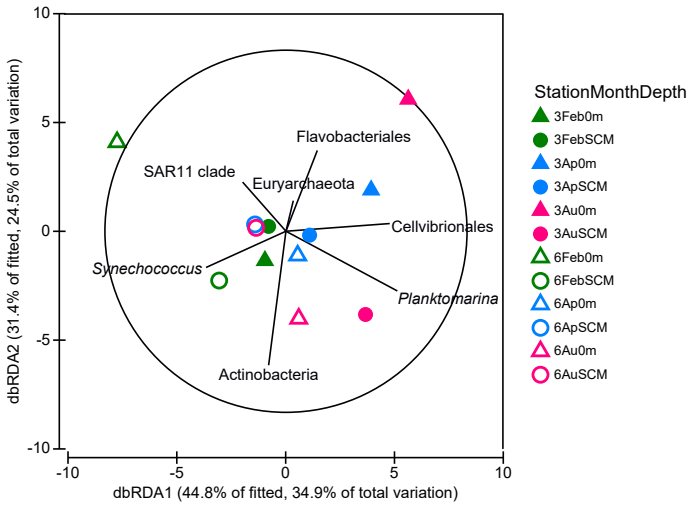

Fig. 8. Distance based redundancy analysis (dbRDA) of B vitamin responses by microbial plankton based on Bray-Curtis similarity.