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.

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#Referee1 comments

AC/ We very much appreciate the useful and constructive comments made by the reviewer, which surely contribute to improve the manuscript quality. We have considered all the suggestions and made the requested modifications, as detailed below.

RC/ My main initial request is to include figures for the actual bacterial and phytoplankton biomass changes in the experiments, rather than simply ratios, including the values for the initial conditions. I believe this should be in the main manuscript, not just the

Supporting Information. These data can be displayed as a mean with error bars representing the spread across the three treatment replicates. I believe this will give a better indication of how the community responded in the experiments. The ratio figures can be included too for discussion/interpretation purposes. Please also label the treatments below each bar in each case – I found treatment identification a little difficult in the current figures.

AC/ We have now included in the main manuscript the requested figure (new figure 5 and 6). We present the response of phytoplankton and that of bacteria separately. The former figure 5 with the response ratios is now included as supplementary information.

RC/ Secondly I think the manuscript should also note how trace metal contamination could have biased the results. This is currently not discussed at all, but could have had an important influence. For instance, if contaminating iron had been inadvertently included in the treatments. Contamination would likely originate from the metal CTD-rosette, the rosette bottles, during bottle sampling, from the incubation bags, from the nutrient additions etc. Where certain procedures were carried out to reduce this, these should be described. This is significant, as this microbes in this region could be experiencing primary iron limitation – see Blain et al. (2004). Blain, S., Guieu, C., Claustre, H., Leblanc, K., Moutin, T., Quéguiner, B., Ras, J. and Sarthou, G., 2004. Availability of iron and major nutrients for phytoplankton in the northeast Atlantic Ocean. Limnology and Oceanography, 49(6), pp.2095-2104.

AC/ We did not use trace metal clean techniques for sampling. We used standard stainless CTD-rosette and Niskin metal-free bottles. We did not have a trace metal clean lab on board, so, even though samples were carefully manipulated contamination by trace metals could have eventually occurred. 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.

Specific comments

RC/ In the abstract I would recommend making reference to the study region (i.e. 'North east Atlantic', or 'off the northwest coast of Spain') AC/ The study area has been specified in the abstract (L15)

RC/ Figure 1b and c: please indicate when experiments were sampled for (i.e. which day? day 0?) AC/ Sampling day for each experiment has been indicated in the graphs (Fig. 1b and Fig. 1c)

RC/ I would recommend noting the microbial responses to major nutrient supply, in addition to B12/B1, in the abstract. AC/ The response to inorganic nutrient additions has been considered in the abstract (L16-L17)

RC/ Line 15–16: rephrase 'was not of great concern' AC/ This has been rewritten (L18)

RC/ I would recommend stating the number of the 36 experiments where bacteria/ phytoplankton responded positively/negatively to vitamin supply in the abstract. AC/ This information has been included in the abstract (L19-L21)

RC/ Line 21 'Growth stimulation by B1 addition was more frequent on bacteria' – relative to phytoplankton? AC/ This has been clarified (L25)

RC/ Lines 35–36 and elsewhere: I would recommend seeing the more recent studies of Browning et al., 2017 and Browning et al., 2018, which also perform trace-metal-clean B12 addition bioassay experiments in upwelling/coastal/offshore regions. Browning, T.J., Achterberg, E.P., Rapp, I., Engel, A., Bertrand, E.M., Tagliabue, A. and Moore, C.M., 2017. Nutrient co-limitation at the boundary of an oceanic gyre. Nature, 551(7679), p.242. Browning, T.J., Rapp, I., Schlosser, C., Gledhill, M., Achterberg, E.P., Bracher, A. and Le Moigne, F.A., 2018. Influence of iron, cobalt, and vitamin B12 supply on phytoplankton growth in the tropical East Pacific during the 2015 El Niño. Geophysical Research Letters, 45(12), pp.6150-6159. AC/ Both studies have been

C3

cited in the revised version (L40)

RC/ Line 39: synthesized by prokaryotes and archaea? AC/ Archaea is included within the prokaryote organisms. This has been clarified in the manuscript (L43)

RC/ Line 42: Have not defined 'cobalamin' (In general I recommend choosing B12 or cobalamin and sticking to it throughout) AC/ This has been corrected (L47)

RC/ Line 79: Perhaps mention here succinctly what Gobler et al. (2007) found? AC/ This has been explained in the revised text (L85-L86)

RC/ Line 79: the reference Barber-Lluch et al. (2019) does not appear in the reference list âËŸA. AC/ This citation has been included in the reference list (L621-L623)

RC/ Lines 114–115: How was this water sampled? From the regular stainless CTD? If so, trace element contamination should be acknowledged. Also see general comment. AC/ This has been acknowledged in the revised manuscript (L122; L136-L138)

RC/ Line 125: Was there any treatment of the whirl-pak bags (e.g. acid and deionized water rinses) to remove contamination? Also see general comment. AC/ We used these bags mainly because they 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.

RC/ Line 127 and on: What were the chemical stocks of the nutrients (e.g. brand and purity). Again, if these nutrients were not pre-treated to remove trace element contamination, this should be acknowledged. Also see general comment. AC/ All the reagents were from Sigma of highest purity. Stocks were prepared with autoclaved Milli-Q water. No additional treatment for trace element contamination was applied.

RC/ Line 137: Were the tanks screened, or open to the air? AC/ Tanks were screened to attenuate light intensity (L151-L152)

C4

RC/ Line 147: Was any time given for the fixative to act on cells before flash freezing in liquid nitrogen? AC/ Samples were incubated 20 min for fixative to act (L162-L163)

RC/ Section 2.5: If known, what was the recovery percent of the B12 preconcentration/ extraction? (i.e. via use of a standard) AC/ Average B12 recovery percentage was 93% (L215-L217)

RC/ Line 271: How was the upwelling index calculated (cannot see this in methods). AC/ Upwelling index was calculated by calculating the Ekman transport from surface winds at fix-station (st3) located at $42°$ N and $8.88°$ W. This information has been included in the revised text (L131-L134)

RC/ Figure 5: It is not clear that the value being displayed is the RR Chla OR RR BB and not the ratio of these. AC/ This has been clarified (Fig. S2 in the supplement)

RC/ As the Figure 5 has signs indicating statistical significance, the error in the spread across the treatment replicates must have been prorogated somehow? Can this error be included as error bars in the figure? AC/ The error bars representing the standard error of the three replicates have been included in the new figure 5 (now Figure 5 and 6).

RC/ 337–338: Specifically which experiments showed serial limitation by B vitamins? AC/ This has been specified in the revised manuscript (L385-L388)

RC/ Line 402: 'clarify the paper of vitamins'? AC/ This fragments has been corrected for clarity (L444)

RC/ Lines 417–419: Please distinguish between the phytoplankton/bacteria responses in this value of 75%. AC/ Taking into account the responses of phytoplankton and bacteria separately, the percentages were 75% for phytoplankton and 50% for bacteria (L457-L548)

RC/ Line 425: No full stop (perhaps also rephrase to 'community assemblage'?) AC/ This sentence was removed.

RC/ Lines 491–495: This doesn't quite make sense – in the first sentence it states that phytoplankton responses to B1 supply were restricted, and in the second the stimulation of phytoplankton is discussed. AC/ Phytoplankton responses to B1 are overall restricted. The second sentence refers to the particular simultaneous stimulation of phytoplankton and bacteria by B1 addition found in subsurface oceanic waters in February (L533-L534)

RC/ I would advise including a table summarizing initial conditions (i.e., nutrient concentrations, temperature, chlorophyll-a, initial bacteria and so on). AC/ We have added a supplementary table including detailed information about initial conditions (Table S2 in the supplement)

AC/ In addition to the modifications suggested by the reviewer, we have made the following change. The named OTUs (operation taxonomic units) has been replaced by ASV (amplicon sequence variant) due to the sequence analysis method used DADA2. 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.
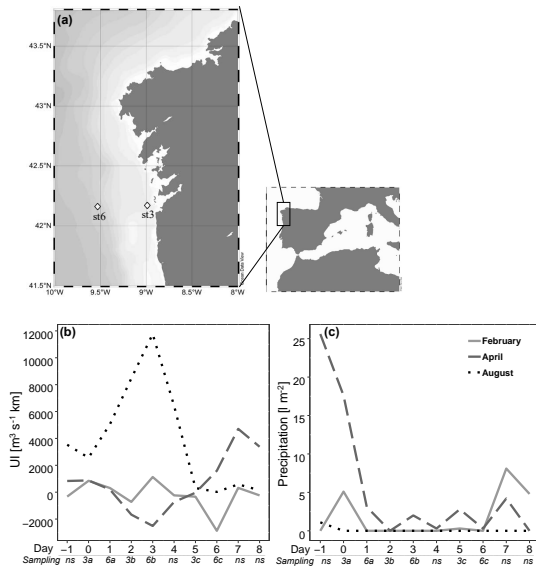
Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2019-306/bg-2019-306-AC2-supplement.zip

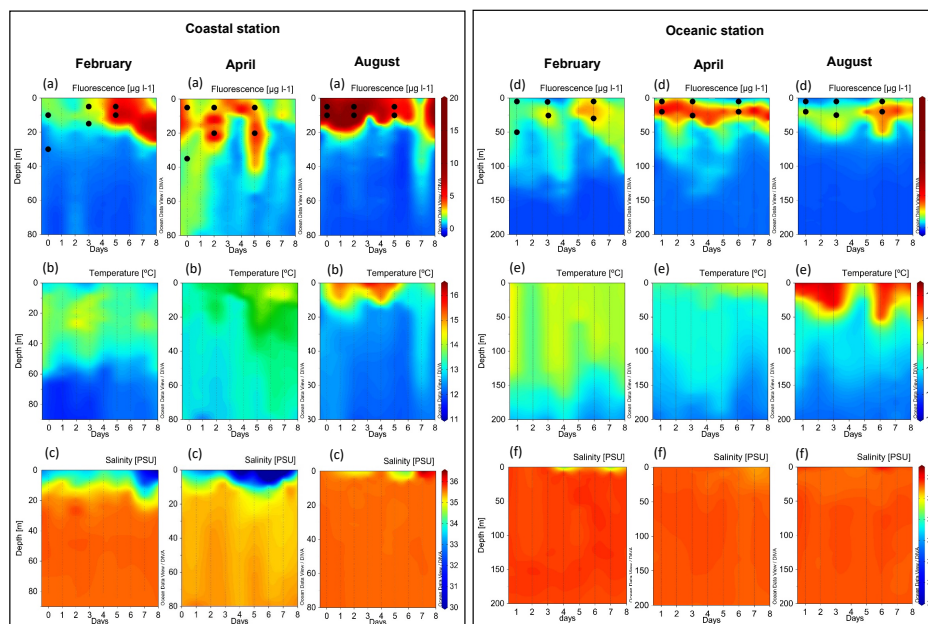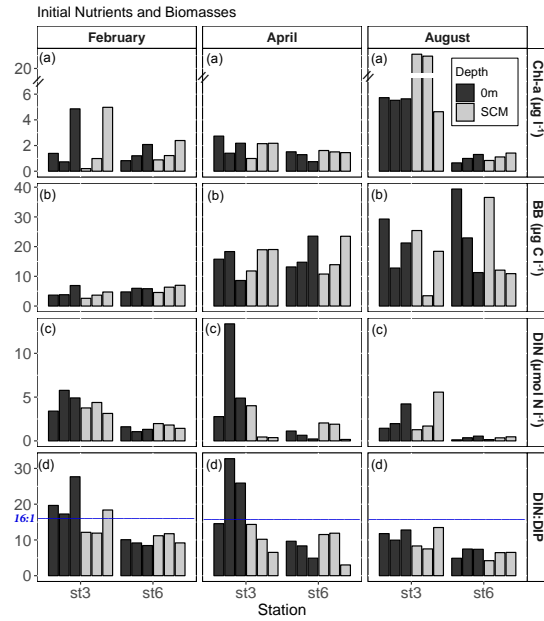**Fig. 1.** (a) The NW Iberian margin and locations of the stations that were sampled, (b) distribution of daily coastal upwelling index (Iw) and (c) registered precipitations.
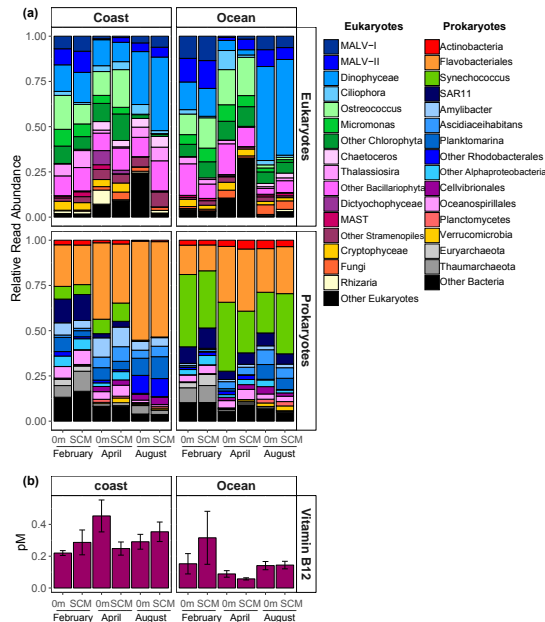
C7

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.
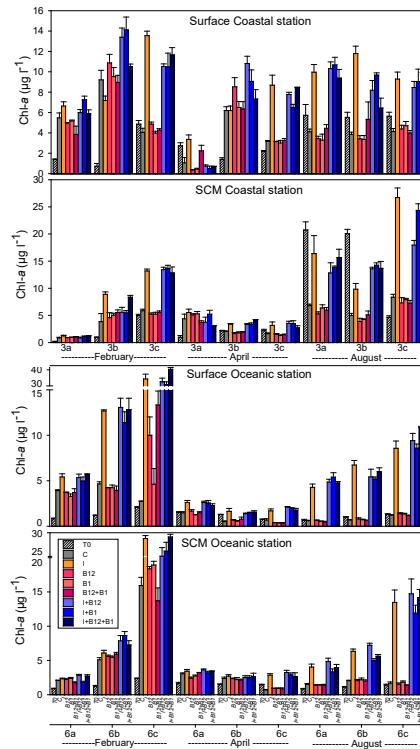
C8

Fig. 3. nitial biological conditions and abiotic factors at the coastal and oceanic sampling stations.
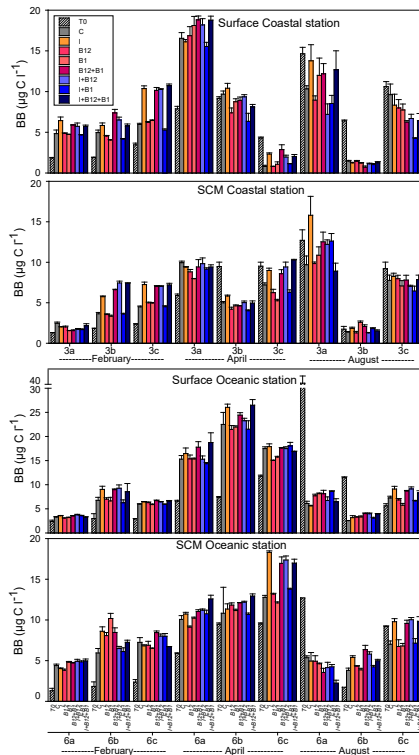
Fig. 4. (a) Averaged relative contribution of reads to the major taxonomic groups of eukaryotes and prokaryotes. (b) Averaged B12 concentration (pM)
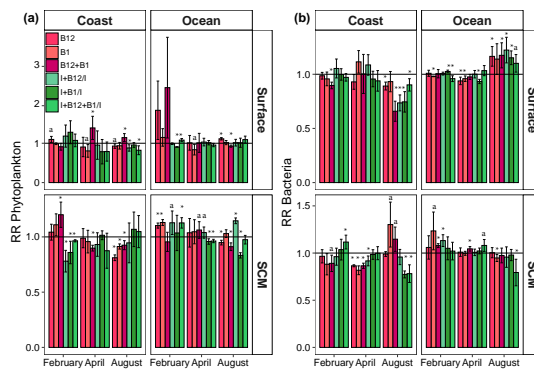
Fig. 5. Phytoplankton biomass (estimated as Chl-a concentration) (μg l-1) in the time-zero of each experiment (striped bars) and in the final-time of each treatment (colored bars)

C11

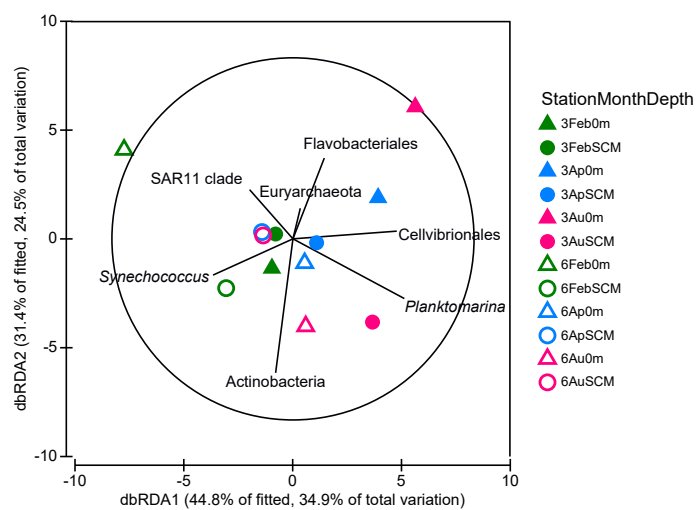**Fig. 6.** Bacterial biomass (μgC l-1) in the time-zero of each experiment (striped bars) and in the final-time of each treatment (colored bars) in the experiments

C12

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.

C13

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

C14