

## ***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 #1**

Received and published: 4 September 2019

The question asked by Joglar and co-authors is an important one (‘how does vitamin B1/12 availability influence coastal and oceanic microbial communities?’), which they have addressed using a large number of detailed bioassay experiments. The research question fits within the scope of Biogeosciences and I suggest that ultimately the results should be published in this journal. However in its current form the manuscript suffers a bit from a lack of clarity and succinct conclusions, making it hard to understand what the take-home messages of this work are. Given the very large amount of work this study has involved, this is a shame. Below I make some recommendations for improvement. The manuscript would also benefit from checking by a native English speaker.

C1

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.

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.

Specific comments

In the abstract I would recommend making reference to the study region (i.e. ‘North east Atlantic’, or ‘off the northwest coast of Spain’)

Figure 1b and c: please indicate when experiments were sampled for (i.e. which day? day 0?)

Line 15–16: rephrase ‘was not of great concern’

C2

I would recommend noting the microbial responses to major nutrient supply, in addition to B12/B1, in the abstract.

I would recommend stating the number of the 36 experiments where bacteria/phytoplankton responded positively/negatively to vitamin supply in the abstract.

Line 21 'Growth stimulation by B1 addition was more frequent on bacteria' – relative to phytoplankton?

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.

Line 39: synthesized by prokaryotes and archaea?

Line 42: Have not defined 'cobalamin' (In general I recommend choosing B12 or cobalamin and sticking to it throughout)

Line 79: Perhaps mention here succinctly what Gobler et al. (2007) found?

Line 79: the reference Barber-Lluch et al. (2019) does not appear in the reference list

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.

### C3

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.

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.

Line 137: Were the tanks screened, or open to the air?

Line 147: Was any time given for the fixative to act on cells before flash freezing in liquid nitrogen?

Section 2.5: If known, what was the recovery percent of the B12 pre-concentration/extraction? (i.e. via use of a standard)

Line 271: How was the upwelling index calculated (cannot see this in methods)

Figure 5: It is not clear that the value being displayed is the RR Chla OR RR BB and not the ratio of these.

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?

337–338: Specifically which experiments showed serial limitation by B vitamins?

Line 402: 'clarify the paper of vitamins'?

Lines 417–419: Please distinguish between the phytoplankton/bacteria responses in this value of 75%

Line 425: No full stop (perhaps also rephrase to 'community assemblage'?)

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.

### C4

I would advise including a table summarizing initial conditions (i.e., nutrient concentrations, temperature, chlorophyll-a, initial bacteria and so on)

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