

Biogeosciences Discuss., author comment AC1
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Reply on RC1

Allanah Joy Paul et al.

Author comment on "Upwelled plankton community modulates surface bloom succession and nutrient availability in a natural plankton assemblage" by Allanah Joy Paul et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2022-44-AC1>, 2022

We thank the Reviewer for their constructive comments and suggestions and respond to these point-by-point below. Our comments are presented in *italics*.

General comments

The manuscript shows a nice experiment planned to demonstrate the influence of the chemistry and biology of upwelled water on the development and later progress of phytoplankton blooms in coastal upwelling systems. In my opinion, the design of the experiment is correct, in which the simulation of the dilution caused by an upwelling episode stands out. However, upwelling most likely does not cause a full 1:1 mixing of upwelling water with surface water. Usually, upwelling pushes up, compresses the surface layer, and so supplies nutrients (and plankton) by diffusion and turbulent mixing at different intensities.

The conclusions are correct, although expected. The first conclusion is the best known. That is, nutrient supply to surface water with low nutrient concentrations induces phytoplankton blooms, mainly diatoms. The second is somewhat new, but not strange. It is reasonable to expect that plankton populations reaching the surface with upwelled waters modulate the bloom and its later evolution. This experiment clearly demonstrates that this happens. However, a better characterization of the species and/or genera of phytoplankton involved is lacking. The flow cytometer has only allowed the characterization of *Synechococcus*. For the rest of the community there was only a proxy of its size with very low detection of microphytoplankton. In addition, chlorophyll was not fractionated. This information is especially important in the post-bloom, when divergence between treatments and the variability within treatments is more evident. However, there is also variability among treatments during the bloom, as inferred from the differences in chlorophyll concentration on day 4 (Fig. 3A) and in the different abundances of nanoplankton (Fig. 5D, E, F). On the other hand, the results reporting significant silicate drawdown in the HN biology treatment point to the importance of diatoms, which could be different from those found in other treatments, including the LN biology treatment. Microphytoplankton (mainly diatoms) are likely causing the divergence observed in both bloom and post-bloom.

Although the introduction and the discussion read well, this is not the case for the results. In my opinion, this section, of great importance to support the conclusions, is written in a cumbersome way. It is necessary to read it several times and with enough attention to catch the information. Figures are not always properly cited, nor is supplementary material. There are tables in the supplementary material that are not cited in the text.

In my opinion, this results section could be improved to remove weaknesses and make the manuscript more attractive to potential readers. The manuscript will probably improve by focusing the description of results on those relevant to the conclusions and ignoring those with low contribution to the two main conclusions.

Despite the lack of information on phytoplankton species composition, which in my opinion represents the greatest weakness of the manuscript, the design of the experiment and the difficulty of its execution, lead me to recommend the publication of the manuscript with major revisions.

Author response: We thank the reviewer for their constructive comments and acknowledge the feedback on the cumbersome results section. We would revise the methods section to improve clarity, focus more on the key results presented in the discussion, ensure accurate citation of figures and that all supplementary materials are appropriately cited in the manuscript. A number of comments from Reviewer #2 will also help in revising these sections (please also see our responses to these comments).

We also agree with the reviewer that a better characterisation of the phytoplankton community would be preferable, this is also a point raised by Reviewer #2, and we acknowledge this as a limitation of our study. We made this decision based on practical reasons and limitations in the experiment set-up, in particular, the sampling volumes necessary and the space available for incubations. While the information in flow cytometric analysis does not enable characterisation of species, it does provide functional information such as size, fluorescence, etc that can be useful in interpreting observations with the benefit of requiring much smaller sample volumes. This was a particular advantage for our experimental set-up that did mean we sacrificed some more detailed information on the plankton community. Fractionating chlorophyll would be another way to determine the size structure of the phytoplankton community but again requires quite large volumes compared to flow cytometry to achieve this.

Not only Synechococcus was characterised in this study, but indeed certain key groups by size/fluorescence (not at the species/genera level). Groups, where a treatment effect was clearly observed, were highlighted to focus the manuscript and the key conclusions. Future experiments would certainly benefit from this knowledge of significant responses in this study to understand which analyses should be incorporated to better understand the biological drivers of phytoplankton bloom initiation and succession.

Specific comments

Introduction

Line 29. ...is considered the most productive... from where? Maybe ..."the most productive upwelling region" or something similar

Author response: *We would use the suggested "... the most productive upwelling region in terms of fish yield..." for a revised manuscript.*

Lines 66-68. The Peruvian productivity paradox is a common paradox to all upwelling systems. With strong upwelling, chlorophyll concentration is low because surface water is recently upwelled water with high nutrient concentrations. Chlorophyll concentration increases when upwelling relaxes. This time lag also has translation into spatial heterogeneity. Chlorophyll concentration is low (few phytoplankton) in the upwelling center where there is deep water recently upwelled. High chlorophyll levels can be found in the surroundings.

Author response: *We agree with the physical mixing processes described by Reviewer #1 that would dilute the biomass (Chlorophyll concentrations) in the upwelling centres where the nutrient concentrations were highest.*

Our understanding of the Peruvian productivity paradox and the reason why it was mentioned here, seems to be a little different to the understanding of Reviewer #1. Figure 4 in Messie and Chavez (2015) shows how the potential new production, based on nutrient inputs via upwelling, are out of phase seasonally with estimates of primary production in the Peruvian Upwelling system. This seasonal mismatch is not indicated for any of the other three main Eastern Boundary Upwelling Systems (California, NW Africa, Benguela). We would suggest the following modification to line 67 to clarify this and to read (new text underlined) "... out of phase seasonally ...".

Materials and methods

Line 105. According to Figure 1, the range of 15 m was only at station A, at station B it was 5 m.

Author response: *We thank the reviewer for picking up on this typing error. This should be 40 – 55m and would be modified accordingly in the revised figure.*

Line 109. ...collected from the mesocosms (*M* in Fig.1)

Author response: *Thank you for this suggestion. This would be modified accordingly in the revised manuscript.*

Lines 129-131. The last sentence reads, "Both the surface (mesocosms) and treatment water (deep water) were filtered... However, the deep water added to the two biology treatments was unfiltered.

Author response: *We can see that it is difficult to distinguish the two separate filtration steps used. In a modified manuscript we would use "screened" to refer to the gauze filtration to remove larger predators and "filtered" to refer to the 0.1µm filtration used to remove microbes for both the inorganic and the organic treatments.*

Line 135 ...were set to the same *two levels* as in the organic...

Author response: *Thank you for this suggestion. This would be modified accordingly in the revised manuscript.*

Line 173. (picoeukaryotes, *nanophytoplankton*, small microphytoplankton, large microphytoplankton). It may be appropriate to add a few words here to inform that microphytoplankton is not well estimated by this technique, although it is recognized in the legend of figure S2.

Author response: *It is correct that the distinction between the two microphytoplankton groups based on size and FL3 fluorescence in the cytogram has its limitations and was sometimes difficult to gate precisely. We suggest the following modification to acknowledge this in the revised manuscript: "Gating of the microphytoplankton groups based on size (small, large), was modified to the best fit for each sample, however, there is a source of uncertainty associated with this approach due to overlap in some samples between the groups (see Fig. S2 for two cytograms with identified groups)."*

Results

Initial conditions (Day 1)

Lines 256-257. If referring to all nutrients, Fig. 3B and C and Table S1 should be cited. If only nitrate is referred to, Fig. 3B should be cited.

Author response: *Thank you for this suggestion. This would be modified accordingly in the revised manuscript from "nutrient" to "nitrate", citing just Fig. 3B in the first sentence.*

Lines 262-265. Fig. 4E should be cited when discussing a254. For E2:E3 it should be Fig 4F. Add Table S1 to Fig 3F when LAP activity is discussed; the slightly higher activity is better seen in the table than in the figure.

Author response: *Thank you for picking up on these inconsistencies. We would modify these as suggested in a revised manuscript and thoroughly check all figure citations to ensure these are correct in the revised version.*

Lines 266-271. Table S1 should be mentioned when commenting about the phytoplankton community. The same table can be mentioned for Fv/Fm, Fig. 4D is the figure.

Author response: *We would add a reference to Table S1 and include the figure reference in a revised manuscript. See also our response to the previous comment.*

Line 274. ...between Day 3 and 5. Better between Day 3 and 6 (Fig. 3D).

It is difficult to follow the chlorophyll in this paragraph, it would be better to specify something else, for example: Peak Chl a concentrations of up to 12 $\mu\text{g L}^{-1}$ (HN organic)

and $\sim 6 \mu\text{g L}^{-1}$ (LN inorganic and biology). According to figure 4A, there are differences between various treatments on this day 4.

Author response: *We would add the specific reference to the treatments where the peak Chl a concentrations were observed as suggested by the reviewer to clarify where treatment differences were observed. This would then read "Peak Chl a concentrations of up to $12 \mu\text{g L}^{-1}$ (HN) and $\sim 6 \mu\text{g L}^{-1}$ (LN) were observed on Day 4 (Fig. 2A). A significant treatment effect of nutrient concentration (HN - LN) was detected in the organic and inorganic treatments, and a significant treatment effect of biology (biology - organic) was detected in the LN treatment (Table S2a, b)."*

Line 285. It is difficult to follow this about the ratio DIN drawdown to maximum Chl a accumulation. This ratio was higher in LN only for the case of organic treatment (Fig. 4A). I think the next paragraph about higher recycling of N or highest N utilization efficiency under low nitrate needs further explanation. How this higher N recycling or N utilization efficiency deduces from a lower DIN ratio drawdown to Chl a accumulation? It seems too risky to attribute these differences in the ratio only to N. Variations in the ratio may also be due to different cell concentrations of chlorophyll. Mixotrophic behavior can also affect this ratio. The ratio changes through changes in N, changes in chlorophyll, or in both. Here phytoplankton composition could provide additional information.

Author response: *We thank the author for bringing up this point as we had viewed this observation with just one lens and it is very true that Chlorophyll a changes may also explain this result.*

Although there are many limitations and uncertainties, we calculated the FL3 (chlorophyll) fluorescence per cell to see if any variations in cell chlorophyll content could be observed in the flow cytometry analyses, within the cell size range that is detected. This is an approximation of the chlorophyll content of chlorophyll-containing cells. Deviation between treatments did appear to emerge in the nutrient depleted period between Day 6 and 10 (see Fig. R1 below) and was likely driven by divergence in cell size between treatments that emerged around the same time (see line 293-296 and Fig. 4C in the manuscript). Highest mean chlorophyll fluorescence per cell was measured in the HN inorganic treatment and the lowest chlorophyll fluorescence per cell was measured in the LN biology treatment on Day 10 (Fig. R1).

Fig. R1: Relative cellular chlorophyll content estimated from flow cytometry data (FL3 fluorescence and cell counts) during the study (see attached pdf file).

We would incorporate the other possible explanations in the following suggested change to line 289 (new text underlined): "recycling of N or highest N utilisation efficiency under low nitrate in this treatment. Variations in the ratio may also indicate different cellular Chl a content or mixotrophic behaviour."

The description of the ANOVA output for the main effects and interaction effects, is a standard way of reporting the statistical data, however we can see this can be unclear to

readers that are not so familiar with statistics. We would suggest retaining the ANOVA output in the text as is for lines 284-287, and then adding the following sentences thereafter to describe in plain words what this output means. This could be as follows: "This means that more Chl a was accumulated in the bloom per nitrate consumed in the low nitrate treatments compared to the high nitrate treatments. There was no significant difference however detected, neither between the treatments (inorganic, organic, biology), nor a combined effect (i.e. interaction) between nitrate concentration and treatment type. "

Lines 291-292. The last sentence indicating that the initial concentration of DIN was 3 times higher in HN than in LN can be deleted. It was reported at the beginning of the results.

Author response: *This sentence would be deleted in the revised manuscript.*

Line 322-323. I understand the association between higher silicate drawdown and higher chlorophyll concentration, but not with nanoplankton abundance. There is no information on the species that are in the nanoplankton fraction. On the other hand, the increase in chlorophyll could well occur in micro diatoms. Maybe the sentence could write like this:

The highest Si(OH)₄ and phosphate drawdown, and consequently Chl a concentration was observed in one replicate. This replicate also showed highest nanophytoplankton abundances (Fig. 5B).

Author response: *This sentence would be modified as suggested by the reviewer, including the reference to Fig. 5B.*

Fig. 5. I think the symbols on the panels do not correspond to the ones on the labels, where they are all circles.

Author response: *We apologise that part of the figure legend for the symbols is missing, and thank the reviewer for picking this up. There are four different symbols (circle, square, triangle, diamond) used to distinguish the four replicates. This information would be added to the figure in the revised manuscript.*

Discussion

Line 370-372. I think this sentence about bottom-up and grazing control is missing something.

Author response: *Yes, a verb is missing. The sentence should read "... high nitrate inorganic treatments **suggest** a primarily bottom-up driven food web response ...".*

Line 420-422. Silicic acid consumption could well have occurred by micro-sized diatoms. It is difficult to conceive that all or nearly all of the nanophytoplankton were diatoms. Usually, there are many flagellates in this fraction.

Author response: *We agree with both of these points: that some silicic acid could have been consumed by larger (micro-sized) diatoms and that flagellates were likely abundant in the nanophytoplankton group. However, the divergent response in the nanoplankton size class and silicate drawdown in the one replicate, suggests it was a silicifying species that consumed a lot of silicate. This could have been a silicifying nanoflagellate but as these are usually in the micro size range >20 μm (Hernández-Becerril and Bravo-Sierra, 2001) and due to the magnitude of silicate drawdown, we considered this more likely to be a diatom species. The interesting point we find here, is that the divergent biological response had an impact on the nutrient concentrations, even if we cannot precisely attribute this to a particular species. We hope that this outcome is clearly presented and is understandable in the manuscript. We would suggest the following modification to line 421-422 as follows (new text underlined): "... likely diatoms based on the magnitude of dissolved silicic acid consumption."*

Lines 429-430. Diatoms were not analyzed and, therefore, it cannot be confirmed that the different behavior of the two treatments was due to the different response of the diatoms and the different seed population. What can be said is that the different behavior of the two treatments could be attributed to a different response of the diatoms and probably also to differences in the seed population.

Author response: *We would change "diatom community" to "silicifying phytoplankton" in line 428/9 accordingly, to more broadly refer to silicic acid consuming phytoplankton.*

Line 490-491. The highest silicate uptake only occurred in a biology treatment, in the HN biology. In the LN biology it did not occur (Fig. 3D).

Author response: *Yes this is correct, and this is acknowledged in line 491 ("within a given deepwater").*

Please also note the supplement to this comment:

<https://bg.copernicus.org/preprints/bg-2022-44/bg-2022-44-AC1-supplement.pdf>