

Biogeosciences Discuss., author comment AC2
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Reply on RC2

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-AC2>, 2022

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

General comments

This ms describes the time evolution of nutrients, chlorophyll, flow cytometric groups, chromophoric dissolved organic matter in experimental 15L bags during 10 days after mixing surface sea water with an equivalent volume of 'deep' water under different conditions (nutrient rich, nutrient poor, filtered (with natural organic matter and nutrients but no organisms) or not (with natural nutrients, organic matter and deep microorganisms). A nutrient control was set by mixing surface sea water with filtered surface sea water and adding nitrate and phosphate. The design of the experiment was set to explore not only the effect of nutrients on the sea surface nutrient consumption and phytoplankton dynamics and composition under the influence of upwelling episodes, but also that of nutrients + organic matter, and that of nutrients + organic matter + natural deep communities upwelled from the 'deep waters'.

If the objectives of the experiment are clear, a huge work and many parameters shown, an interesting introduction and discussion, the design and the results are very complicated to follow. I suggest to the authors to simplify denominations/definitions of the different combinations in the bags and to rewrite the m&M and the result section to give it clearer. In addition, I was left a bit frustrated as:

For a biogeochemist point of view, we have no information on ammonium concentrations. Regenerations sources could have been high in the bags, particularly considering the high concentrations of nitrate added: around 8 and 3 μM in HN and LN, respectively. This information should have been pertinent to state nitrogen limitation status and examine N/P ratios (and for this reason, you should use the term NO_x instead of DIN for the whole text, as it refers only to the sum nitrite + nitrate)

For a microbiologist point of view, again, we have only partial information:

First on the microphytoplankton response: There is discussion on a diatom response, but we have not any information on the taxonomic composition of diatom communities, even

using proxies that could be have been given for instance by size fractionation of chlorophyll. Indeed, flow cytometry analysis only allows small size class to be counted. There was only few information given on a "chain group", on a "small microphytoplankton group", and on a "large phytoplankton group" which was not statistically represented in the flow cytometry analysis (Fig. S2), and furthermore the time evolution of abundances in these groups is not plotted (just initial conditions, table S1) or statistics (table 2).

Second, there is no information on heterotrophs. There is discussion on the potential role of heterotrophic bacteria on the degradation of organic matter, and on the the phytoplankton regulating process during its decay period. But not any data is available on heterotrophic bacterial abundances and/or on the grazer community composition, or virus abundances.

The choice of deep samples at 90-105 m at station A for HN experiment, and 40-45m at station B for LN experiment is crucial to compare experiments. Clearly the vertical distributions of physical properties, nutrients, abundances of flow cytometric groups and organic matter properties at these 2 stations, could have been helpful for the readers, particularly those not familiar with the biogeochemical context in this area, to situate these "deep" water masses in the frame of depths of nutriclines, OMZ, deep chlorophyll maxima, euphotic zone depth etc... Were these depths taken out of the euphotic zone? out of the OMZ?, Were they constituted only by heterotrophs or were there also some phytoplankton?

And Lastly, why taking waters from the mesocosm experiment instead to in situ surface waters close to the mesocosm? Why day 20?

For all these reasons, I would recommend the publication of the ms with major revisions.

Author response: Both reviewers have commented that the Materials and Methods and Results sections would need some revising to improve clarity of this complicated set up. We would provide significant modifications to the text in these sections and work on simplifying the treatment definitions and simplifying the text. We would also change "DIN" in the text to "NOx" as suggested. The depth range of 40-45m should read 40-55m and was an unfortunate typing error for Station B, which we will correct during manuscript revisions.

During the deepwater collection, we went out along the Callao transect during sampling by Instituto del Mar del Perú (IMARPE). This transect extends off the coast along 12°S and is regularly sampled (see e.g. Graco et al. (2017) or also the Graco et al. (2019) as cited in line 97 for background hydrographic data and biogeochemical context). We took a CTD profile at each station and looked at the oxygen, pH and hydrogen sulphide (H₂S) profiles to indicate in which depth range water was still oxygenated and therefore should still contain some nutrients, in particular nitrate, and no H₂S as this is toxic for plankton. We then selected depths above any anoxic layer and as deep as possible to ensure high(er) nitrate concentrations, which was a central factor for the design of our study.

Regeneration of nutrients, in particular nitrogen, is very likely in this N-limited system but unfortunately no ammonium concentrations could be measured in this set-up due to the high risk of sample contamination. We respond to this specific comment in more detail below in responses to specific comments, including how we would address it when revising the manuscript.

Size fractionated Chl a would have been one way to analyse the size structure of the phytoplankton community. Indeed, flow cytometry analyses can give a proxy for cell size that can be even more highly resolved than the standard <2µm, >20 µm fractionation carried out in filtrations, although as correctly mentioned, this is limited by the size range

the flow cytometer can detect. We were also quite limited in sample volume for this 10-day long study, hence Chl a samples were only taken every 2nd sampling day, whereas flow cytometry only requires ~2mL and could be taken more frequently. Hence, we were interested in the temporal dynamics and placed priority on this as we were not sure how rapidly the plankton community would respond to the nutrient addition in this particular set-up. Please also see our response to a similar comment by Reviewer #1.

It is an oversight that the heterotrophic community was not analysed in greater depth as this would give better insight into regenerative production and phytoplankton bloom dynamics. Virus abundances are notoriously difficult to analyse and we did not have the means to do this in this particular study, but of course would be an interesting dynamic to probe in future studies.

We wanted to use mesocosm water rather than surface water because the water masses inside the mesocosms are comparatively well characterised and experience less mixing and no tidal movements, unlike outside the mesocosms. The initial aim was to link the results of this incubation study to the mesocosm responses, but this was more challenging in hindsight, nevertheless determined the experimental design initially. Day 20 was selected because we had ship time to go out and collect the deepwater in the days prior, because the initial collection did not work. This is perhaps a benefit as around Day 20 the nutrients were depleted but primary producers were still relatively abundant so that our treatments could better control the nutrient concentrations.

Specific comments

Line 109. Specify even if you refer to Bach et al what was the status of phytoplankton on the day 20 of the mesocosm study: steady state? exponentially growing? decaying?

Author response: *We would add the following description to provide information on the phytoplankton status in the collected surface water to Line 112: "The mesocosm plankton community was in a post-bloom phase where inorganic nitrogen was low and a sub-surface Chl maximum had developed between 5-15m depth."*

Line 119. The authors should moderate this sentence "... no TM or DOM measurements were made... these were assumed to be different..."

Author response: *It is unclear what specific part the reviewer suggests moderation of in this sentence. As some treatment differences were detected between the inorganic and the organic treatments for the HN deepwater, but not for the LN deepwater (see e.g. Table S5c), we believe that this corroborates this statement. As this is presented in the results section and supplementary material, we do not consider it relevant to add this information to this early part of the material and methods.*

Figure 2. station A and station B should be removed in the lines "inorganic", somewhere it should be drawn that nitrates and phosphate were added. In the legend for memory it should be reminded that "unfiltered" is in fact < 64 µm and the "filtered" is < 0.1 µm.

Author response: *We can see that it is difficult to distinguish the two separate filtration steps used, as both reviewers have highlighted this point. In a modified manuscript we would suggest 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. We will clearly describe this in the manuscript and consistently use this terminology throughout. We would also revise Table 2 (please see our response to this aspect in "General Comments" above).

Line 135 for the "inorganic" treatment why not also adding Silicates to get similar changes for N/Si ratios?

Author response: *This study was intended as an auxiliary experiment to the larger mesocosm study (see description in Bach et al. 2020, BG), where deepwater with two different N:P ratios were added. Hence, we selected the nutrient treatments based on N and P only also for this study. Adding an additional N:Si treatment would have significantly increased the number of incubation bottles needed and would not have been manageable workload within the framework of this study or possible within the limitations of the experiment set-up in Peru. Nevertheless N:Si ratios would definitely be an additional interesting factor to investigate in future experiments, as it is also likely important in selecting for particular phytoplankton groups.*

Line 138, 140. Be more precise and everywhere add the porosity of the filtration.

Author response: *Please see our response to the reviewer comment on Figure 2 above.*

Line 172. As the samples were not fixed, how were stored samples between sampling and analysis? was there a long delay between the first and the last sample analyzed?

Author response: *Sampling was rapid and on-shore so samples were taken to the lab within 1 hour of sampling and measured within 6 hours of sampling from the incubators. Samples for flow cytometry were stored cooled in the dark in the cool box until analysis.*

Line 171-180. The authors should add more details on the set up of the flow cytometer (and/or on the legend of Figure S2): debit and duration of the analysis, i.e. the volume analyzed), also did you add beads to set limits between the different size classes and how this differentiation was done. Add information on wavelengths of the different windows (FL3A, FL2H, FL4H) that could be indicated on the legend of the Figure S2. In this figure some cytograms were excluding some populations and other not? explain more in the legend. As demo, a plot FL2 / FL3 would have been useful too.

Author response: *We will add additional information to the methods section to better describe the flow cytometer set-up and analysis in accordance with the Reviewer suggestions here. Each samples was measured over ~10 minutes per sample on fast flow rate (~66 µL per minute) to measure a total volume of 650 mL. No beads were added, instead sizes were determined via sequential size fractionations with polycarbonate filters of different poresize as described in Veldhuis and Kraay (2000). We used the wavelengths excitation/emission of FL3 = 488/670, FL2 = 488/585, and FL4 = 640/670. We considered all populations for the quantitative analysis but of course not all populations are necessarily shown on all plots. For gating, some identification was needed on specific fluorescence channels (e.g. Synechococcus on FL2) and these are then excluded from the other plot (e.g. FL3 vs. FSC) to avoid overlap with the other populations. This is a*

standard gating procedure. We will also add a plot of FL2/FL3 to the Supplementary Material, as is provided already for the size-fluorescence cytograms in Fig. S2.

Could you really consider counts of micro-II as significant? we just have initial conditions in terms of percentage on table S1, but no idea of any abundance,

Author response: The abundances of the micro II (large microphytoplankton) were much smaller than for other groups. These ranged between 0 and 11 counts per sample (analysed volume = 100 μ L) but were included due to their larger size and contribution to chlorophyll fluorescence signal. We focussed our attention in the discussion however on more dominant groups where we also considered the underlying data to be more robust.

For a paper dealing on the potential effect of seeding microorganisms with upwelled waters, I find rather strange to get so much groups counted with the cytometer and having finally only a plot of the evolution of *Synechococcus* and nanophytoplankton.

Were *Prochlorococcus* abundances detectable?

*Author response: In the manuscript we focused on the key outcomes and main effects observed, hence the focus on *Synechococcus* and nanophytoplankton. As there was little impact of treatment on the other groups gated, we highlighted this outcome, even though we of course measured and gated other groups. We will add these figures to the Supplementary Material to show how these groups varied by time and treatment.*

*Prochlorococcus was not detectable in this study. From experiences in other incubation and mesocosm studies, *Prochlorococcus* usually does not survive in the bottles or other enclosed spaces, even if they are present in the initial community.*

Line 190. Were all the FV/Fm measurements done on the same time of the day?

Author response: Yes, they were carried out at the same time. After sampling, the samples were adapted to dark conditions during 20 mins prior to measuring F_0 , F_m and F_v .

Paragraph 2.3 Is there any information on the distribution of heterotrophs? heterotrophic bacteria? Heterotrophic nanoflagellates? ciliates? the filtration on < 64 μ m could have a cascading high effect on ciliates, which becomes the top predator in the bags.

Author response: We have presented all data collected during the study and unfortunately there is no further information on heterotrophic organisms. This is definitely a component of the food web that would be interesting to probe in more depth in future experiments, as rightly mentioned, these smaller grazers (<64 μ m) could have profited from the relief of grazing pressure on themselves and may also display mixotrophic behaviour.

Line 229. Please explain better for non-specialists: "the contrast matrix... the organic treatment was used as the control for the linear mixed model analysis"

Author response: The following sentence was meant to explain the importance of this contrast matrix and would be modified to make this clearer e.g.: "This contrast matrix hence means that reported model significance refers to the difference between organic vs. biology and organic vs. inorganic to distinguish the biological treatment effect and the organic/trace metal effect."

Line 256. refer also to Table S1

Author response: We would add this to the reference to Fig. 3 as follows: "... (Fig. 3, Table S1) ..."

Line 259. refer a254 with Figure 4e, and on line 262 E2:E3 ratio with fig 4f

Author response: We would add this information as suggested by the reviewer to line 259 to read: "... CDOM measurements (a_{253} , Fig. 4E) ..." and to line 262 to read "... DOM molecular weight ($E_2:E_3$, Fig. 4F)."

Line 264. "surface water.... nitrogen depletion". Initial concentrations in NO_x in "inorganic" (2.07) versus those in organic LN (2.49 and 3.17 (organic LN) were not so different and where all evolving the same way considering nitrate (Figure 3b) or LAP activity (Figure 3F)

Author response: We are not entirely sure what would need modification here. As the reviewer points out, the NO_x concentrations (inorganic) were very similar between the treatments. This was deliberate so that any contribution of the organic nutrient component added via the deepwater treatments could be distinguished, if this was a significant effect. Lack of significant difference in LAP activity also indicates that organic nutrients (nitrogen here) did not play such an important role in fuelling production in the nitrogen limited plankton community here.

Line 266. please explain in the Material and method in 2.3 section clearly how is calculated the "relative contribution of each group to chlorophyll a fluorescence in flow cytometric analysis" Also if it is this parameter used on table S1 when reporting percentages of the different flow cytometric groups, and not simply relative abundances, it should be explained in the legend.

Author response: We would add this information to the Materials and Methods section in the revised manuscript and ensure this is explained in the Table S1 caption, as this data does underlie the percentages reported in Table S1.

Line 268. Change formulation "R statistic" here and in Table 2 Not the software, but the type of test should be indicated.

Author response: The "R statistic" here is not referring to the software but to the output from the statistical tests (ANOSIM + SIMPER) that is described in the methods section in Section 2.6.

Line 273. It is not visible in Fig 3B plot, were the limits of detection for nitrate (0.123 μm as stated in the methods) reached in all experiments?

Author response: *Yes, all replicate bottles had NO_x concentrations below the detection limit on Day 4 with some bottles below detection on Day 3. This data is now openly accessible on the PANGAEA database: <https://doi.org/10.1594/PANGAEA.941138>*

Line 282. Is there an error here? I would rather write this sentence like this: "DIP is more consumed relative to DIN in LN treatments compared to HN treatments"

Author response: *Yes, this is correct and we thank the reviewer for bringing this up. We would modify this sentence accordingly in the revised manuscript.*

Line 283-284. Is there an error here? rather it should be 9.82 for HN and 6.25 for LN?

Author response: *Yes, this is correct and we thank the reviewer for bringing this up this error. We would modify this sentence accordingly in the revised manuscript.*

Line 285. "... where initial N was lowest". No, initial N was the lowest in the inorganic treatment.

Author response: *We can see that the treatment naming is difficult to follow and we will work on this in a revised manuscript. To clarify this specific result, here we were referring to the nutrient status (high vs. low nitrate) without any reference to any of the three treatments (inorganic, organic, biology).*

Line 289. "higher recycling... ". Ammonium concentrations were not available? Probably like DIP it was produced by regeneration between days 6 and 8, see lines 325-326

Author response: *Ammonium concentrations were not measured in the nutrient analyses. Regeneration of nutrients is likely, as we suggest in lines 325-326, as the detection limits for DIP are much lower than for NO_x . Also, the incubations were limited in NO_x , rather than DIP, any regenerated N would have been rapidly assimilated and hence would not accumulate and remain undetectable in any of the NO_x pools, or in ammonium if it had been analysed.*

Line 289. " .. or highest N utilization efficiency under low nitrate" There is another hypothesis, a higher top-down control of phytoplankton by grazers under high nitrate.

Author response: *We address this more in depth in our response to the Reviewer's feedback on line 372 (see below).*

Lines 291-292. This sentence on initial nitrate nitrite concentrations should be cited at the beginning of section 3.1

Author response: *The initial NO_x (nitrate + nitrite) concentrations are already mentioned at the beginning of Section 3.1 (line 256-257).*

Figure 3. Why plotting silicate drawdown when absolute concentrations are plotted for Chl, DIN and DIP? For plots based on 'deltas' like figure 3D and 3E the authors should explain in the legend if the difference is always made with T1 concentrations

Author response: *Silicate was never depleted and the point of the figure was to show the differences in silicate consumption, despite nitrate/NO_x depletion. We would add information to the legend to indicate the silicate drawdown was calculated from the T1 concentrations.*

Figure 4A. For the legend, indicate how DIN was calculated. Was it DIN at T1 minus DIN at the time of max chlorophyll, i.e. T4 for all samples except T3 for LN organic?

Author response: *We will add a reference to Eqn. 1 in the legend to indicate how this was calculated. This ratio was calculated for each replicate individually (see line 247) and the day of nutrient depletion (DIN_{min}) and Chl a_(max) may therefore differ.*

Figure 4B. Again it is unclear if the difference is made as concentrations at day 6 minus concentrations at day 10 of these box plots are simply the means of the data presented figure 3E for the period T6 to T10. Be clearer in the legend. It seems that here are presented the distribution of the 20 data (T6 to T10 time points x quadruplicate bags).

Author response: *We will modify the figure legend to clarify this as the mean of daily calculated values between Days 6-10 to read as follows (new text underlined): " ...B) mean relative drawdown in nitrate to phosphate during the post-bloom period (mean of daily calculated Δ DIN: Δ DIP on Days 6-10), ..."*

Line 324. Refer to fig 5E

Author response: *We will add this figure reference accordingly in a revised manuscript.*

Line 330. The last sentence with infos on initial conditions should be cited in section 3.1

Author response: *We will shift this information to Section 3.1 in a revised manuscript.*

Table 2. Modify "R statistic", indicate in the legend what were the bloom and post bloom periods considered for the tests. For the relative contribution, in percentages, I don't understand to what they refer, as the sum of contribution of each group does not make 100%.

Author response: *We will add information to the legend on the definition of the bloom and post-bloom periods.*

As described in our response above, the "R statistic" here is not referring to the software but to the output from the statistical tests (ANOSIM + SIMPER) that is described in the methods section in Section 2.6 and is correct to report as an R statistic. The contribution reported in the table is the contribution to the detected treatment differences in the SIMPER Posthoc test. This is described in the legend for Table 2.

Line 369. It is up to 12 µg/l as seen from the figure 3A

Author response: *The maximum Chl a concentration was 12 µg L⁻¹, as the reviewer correctly points out. However, here we refer to the high variability observed in maximum Chl a concentrations between the three treatments (organic, inorganic and biology) for the high nitrate incubations. Hence the 6 µg L⁻¹ difference between the treatments we refer to in Line 369 is in our opinion correct here.*

Line 372. "... than any impact of grazing": But some grazers were present in the surface water taken in the minicosms. Furthermore, this surface water was filtered through 64 µm, and consequently with no top predators, all microzooplankton (heterotrophic ciliates) could have been rapidly growing in response to the increase of pico nano and small microphytoplankton. Note also that his sentence lines 370-372 has no verb.

Author response: *We agree that other groups that could graze on phytoplankton in different size classes may have been growing rapidly in the incubations and can see that this statement is a little unclear and would remove the reference to grazing impact. Hence, we would revise this section to read as follows: "Sharper bloom biomass development in the filtered high nitrate organic and high nitrate inorganic treatments suggests a primarily bottom-up driven food web response to nutrient addition. Bloom development in high nitrate biology was more muted as nutrient competition within the plankton community (e.g. with heterotrophic bacteria) was likely higher, due to the lack of organism dilution compared to the filtered organic/inorganic nutrient treatments. Alternatively, this muted biomass development could suggest an increase in grazing pressure via potential introduction of microzooplankton grazers (<64µm) in the addition of unfiltered deep water. Hence, higher retention of Chl a post-bloom in this treatment, suggests potentially longer sustained periods of productive biomass when deep water plankton are added concurrently with upwelled nutrients. The precise mechanism(s) underlying this response requires however further detailed elucidation. "*

Line 372-374. Rather, I would imagine than heterotrophic bacteria would find more favorable growth conditions with surface water mixed with deep filtered sea water, as the surface heterotrophic bacteria are diluted in deep water by a factor 2 as well as their grazers, and thus have less predatory control, together with more access to nutrients and DOM provided by the deep waters.

Is there any information on abundances of heterotrophs? heterotrophic bacteria? flagellates? ciliates?

Author response: *We agree that the surface heterotrophic bacteria would also benefit from the dilution in the sterile filtered deepwater but as we didn't measure their abundances,*

this is difficult to quantify. In line 374, we were referring to the increased competition in the unfiltered biology treatment where this dilution, and potential increase in relative inorganic/organic nutrient concentration, did not occur.

Line 385. The noticeable net increase of DIP in experiments between T6 and T8 suggests that ammonia could have been also regenerated through grazing processes during that period. This increase of DIP about 0.1 to 0.3 $\mu\text{mole/l}$, based on a N/P ratio of 16, could signify that as much as 1.6 to 4.8 μM of ammonia could have been regenerated, even based on a $\Delta\text{DIN}/\Delta\text{DIP}$ of about 6, this give up to about 2 $\mu\text{mole/l}$ ammonia regenerated.

Author response: *Yes, we agree that ammonia regeneration for example due to grazing by smaller grazers still present in the experiment is also likely but it would have been rapidly assimilated in the N-limited plankton community and hence remain undetected in the dissolved nutrient pools. Nevertheless, this may have been visible in the Chl a biomass as we suggest in line 384. This regeneration of N via ammonia could have helped to sustain Chl a biomass at higher levels during the post-bloom period. Unfortunately, limitations on the size of the incubations meant we could not carry out grazing assays that require large volumes (>5L).*

Line 393. The authors should cite the initial DIN/DIP ratios here, and write that DIP was never depleted in the experiments.

Author response: *We would modify the sentence here to read (new text is underlined): "Our results also indicate that up to over 50% more phosphate was consumed per mol of DIN in the low nitrate treatment (mean initial DIN:DIP = 1.21 \pm 0.24) with higher initial excess phosphate, than with high nitrate (mean initial DIN:DIP = 3.92 \pm 0.32). Phosphate was never depleted in this study. "*

Line 396. Without ammonia measurements, it is difficult to speculate on N regeneration. However, if abundances of heterotrophic prokaryotes are available, I suggest to calculate per cell LAP activities.

Author response: *Unfortunately, heterotrophic prokaryotes were not distinguished in this study and so we cannot calculate per cell LAP activity.*

Line 401 "LAP was higher...". Before comparison with other studies the concentration of leu-AMC used by other authors should be verified as it influences rates. The concentration added here (500 μM) is high. Mabmig et al used 200 μM .

Author response: *Yes, this is correct, and we suggest the following modification of this section to include this (new text underlined): "The LAP activity was one to two orders of magnitude higher than most literature values. For example, in a study from the same region but further from shore, the LAP activity was 20-65 nmol AMC L⁻¹ h⁻¹ in natural communities (Mabmig et al., 2020). Partly, the high LAP activity in this study could be due to the high concentration of substrate we used (500 $\mu\text{mol L}^{-1}$ leu-AMC), which aimed to measure maximum hydrolysis rates. However, this cannot be the only reason for the high values. For comparison, we used only 2.5 times higher substrate concentration compared*

with Maßmig et al. (2020). The high LAP activity and close relationship with fresh, labile organic matter production suggests that LAP was produced to support bacterial production above the oxycline (Loginova et al., 2019), rather than compensating for higher N-limitation in the low nitrate treatment. "

Line 428. "Irradiance levels increased upon incubation". Were the levels of irradiance in bags higher than in the surface mesocosms?

Author response: *The blue foil used reduced the incoming irradiance to ~25% which corresponded to equivalent PAR at ~ 2-3m deep (25% light intensity) and we aimed to use representative light conditions in the incubation study. However, the water in the mesocosms was in general mixed within the upper mixed layer of ~ 5-10m, meaning that the average PAR was probably below 25% light intensity in this mixed layer. So while the light level in the incubation was in good agreement with the depth where the water was collected from, the reduction in vertical mixing upon incubation would have increased the amount of incoming irradiance and likely increased the amount of PAR available to phytoplankton.*

Line 440 "viral presence". Because the authors made a 0.1 µm filtrations the ratio viruses to their host is very high in initial conditions., could it be in the favor of viral lysis?

Author response: *The reviewer correctly notes that we filtered the deepwater added to remove as many particles such as viruses from the deepwater as possible but of course some smaller viruses may have been retained in the sterile filtered water (inorganic and organic treatments). The whole size spectrum of viruses (<64 µm) present in the unfiltered biology treatment. Viral lysis may therefore have been favoured in these filtered treatments due to the higher concentration of smaller viruses. This would be an alternative argument to the one we use but we have no data to support this.*

Line 433. "... rather than the manipulated deep water". It would have been interesting to have initial compositions of populations included in the two "deep waters" used in this experiment.

Author response: *We took subsamples from the seawater added and analysed these using flow cytometry directly after mixing but not of the deepwaters themselves, as we were interested in the treatment differences. This drove our sampling strategy. While we agree it would have been interesting to better characterise the initial populations of the deepwaters specifically, our aim was to see if a different water source was at all an important driver of phytoplankton communities and considered characterisation of the individual treatments sufficient when carrying out the experiment. In future studies, we would strongly consider genetic sampling to better characterise the microbial communities present (i.e. 16S and 18S) and even transcriptomic analyses to understand differences in nutrient utilisation.*

Line 455. "...and higher post-bloom Chla concentrations were sustained in this treatment". Yes, but in the "inorganic" too, so the source of the variability is not only due to the variability of responses of the seeded communities, those of surface too.

Author response: Yes, we agree that there were similar mean Chl a concentrations post-bloom in both the biology and inorganic treatments (see e.g. Fig. 3A). Nevertheless, the heterogeneity, or variability, between replicates was much higher in the biology treatment, evident in the error bars in Fig. 3A, 3D and 4A. We would suggest the following changes to line 455 (new text underlined): "Over 2.4 $\mu\text{mol L}^{-1}$ more silicate was consumed after nitrate was exhausted and higher post-bloom Chl a concentrations and nanoplankton abundances were sustained in the one HN biology replicate compared to the three other replicates."

Line 480. Sentence unclear, does the term "that" refer to physical factors? If yes do you discuss about the horizontal mixing by showing the example of tidal mixing? If yes write it.

Author response: We would suggest the following revision with the modified text underlined: "In the ocean, physiological and ecological drivers (e.g. growth rates, transcriptional response times, mutualisms, symbioses, Sect. 4.2) would act in addition to other physical factors that regulate plankton biomass accumulation and succession in the surface waters following upwelling e.g. seed community abundance present in subsurface waters (Seegers et al., 2015). Such physical factors, such as dilution, mixing and horizontal transport of water masses via regular tidal transport onshore (Stauffer et al., 2020), could not be included in this experimental set-up."

References

Graco, M., Anculle, T., Aramayo, V., Bernales, A., Carhuapoma, W., Correa, D., Fernandez, J. C. E., Garcia, W., Ledesma, J., Marquina, R., Quipuzcoa, L., Romero, D., Sarmiento, M. and Solis, J.: Analysis of oceanographic and biological conditions of the coastal upwelling off Callao in contrasting periods during 2018, *Bol. Inst. del Mar del Peru*, 34(2), 519–543, 2019.

Graco, M. I., Purca, S., Dewitte, B., Castro, C. G., Morón, O., Ledesma, J., Flores, G. and Gutiérrez, D.: The OMZ and nutrient features as a signature of interannual and low-frequency variability in the Peruvian upwelling system, *Biogeosciences*, 14(20), 4601–4617, doi:10.5194/bg-14-4601-2017, 2017.

Seegers, B. N., Birch, J. M., Marin III, R., Scholin, C. A., Caron, D. A., Seubert, E. L., Howard, M. D. A., Robertson, G. L. and Jones, B. H.: Subsurface seeding of surface harmful algal blooms observed through the integration of autonomous gliders, moored environmental sample processors, and satellite remote sensing in southern California, *Limnol. Oceanogr.*, 60(3), 754–764, doi:10.1002/lno.10082, 2015.

Stauffer, B. A., Sukhatme, G. S. and Caron, D. A.: Physical and Biogeochemical Factors Driving Spatially Heterogeneous Phytoplankton Blooms in Nearshore Waters of Santa Monica Bay, USA, *Estuaries and Coasts*, 43(4), 909–926, doi:10.1007/s12237-020-00704-5, 2020.

Veldhuis, M. J. W. and Kraay, G. W.: Application of flow cytometry in marine phytoplankton research: current applications and future perspectives, *Sci. Mar.*, 64(2 SE-Articles), 121–134, doi:10.3989/scimar.2000.64n2121, 2000.