

Biogeosciences Discuss., referee comment RC2
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Comment on bg-2022-44

Anonymous Referee #4

Referee 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-RC2>, 2022

Review Paul et al « Upwelled plankton community modulates surface bloom succession and nutrient availability..... » submitted to Biogeosciences

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.

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?

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

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 .

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

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

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?

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.

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,

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?

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

Paragraph 2.3 Is there any information on the distribution of heterotrophs? heterotrophic bacteria? Heterotrophic nanoflagellates? ciliates? the filtration on < 64 μm could have a

cascading high effect on ciliates, which becomes the top predator in the bags.

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"

Line 256. refer also to Table S1

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

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.

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

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?

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"

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

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

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

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.

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

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

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?

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).

Line 324. Refer to fig 5E

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

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%.

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

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.

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?

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.

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

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.

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 .

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

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?

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.

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.

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.