Biogeosciences Discussions

Biogeosciences Discuss., author comment AC1
https://doi.org/10.5194/bg-2021-220-AC1, 2021

Reply on RC1

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Author comment on "Contrasting patterns of carbon cycling and DOM processing in two phytoplankton-bacteria communities" by Samu Markku Elovaara et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-220-AC1, 2021

Dear referee

Thank you for your kind and constructive comments! Please find embedded in your comment our initial suggestions for improving the issues pointed out and answers to your questions.

This study compared carbon cycling and microbial communities in two phytoplankton-bacteria cultures and found while primary production and respiration were higher in the dinoflagellate A. malmogiense culture, DOC produced by cryptophyte R. marina was more labile and supported more bacterial production. It also showed that two phytoplankton also supported different bacterial community structures. This work generates a variety dataset in two model systems and has implications on how different phytoplankton species may shape carbon transfer among trophic levels and interactions between microorganisms. But there are certain aspects need to be addressed to convey the study more clearly. First, the description of incubation design is somewhat confusing. The DOM consumption experiment is to see how bacteria respond to dissolved pool excreted by phytoplankton. But the DOM release experiment DOM line mix is also inoculating bacteria to phytoplankton exudate in dissolved phase, what is the difference between this and DOM consumption experiment? Just different incubation time length, or using phytoplankton exudate harvested at different growth stage? Looks like DOM consumption experiment use phytoplankton exudate in later stage when cell density is high, but KPI 2,3 is also at high cell density. Or is the DOM line just used as a control comparison to the production line? The purpose of the experimental design need to be better explained here.

Authors: The DOM line indeed served mainly as a control for the production line. We wanted to investigate if any changes would happen in bacterial abundance and optical DOM properties already within the 12 h key point incubation. This is explained in lines 153-154 but will be explained in more detail. As there were no changes in the DOM line variables, we pooled all the time points of DOM line. The role of the DOM line was bigger in the design of the experiment than it turned out to be. We still wanted to describe the experiment how it really happened, even though some of it might seem redundant now. The measurement of nutrient concentrations before and after the exclusion of phytoplankton also allowed us to measure nutrient concentrations in phytoplankton cells.

Second, in a lot of figures and results, the DOM release experiment and DOM consumption

experiment are all mixed up, it is hard to follow when talking these two back and forth. To me, DOM release experiment may be focusing on the production of DOM and the interaction between phytoplankton and bacteria at different phytoplankton growth phase, while DOM consumption experiment is focusing on how bacteria respond to this produced DOM. If these two parts can be more separated in a clear logical way in the results, this can improve the structure to be more explicit.

Authors: Throughout the manuscript we discuss the two experiments in tandem because we consider them to be complementary experiments studying the same system/phenomena. Especially in the discussion we go from topic to topic (e.g. DOM, bacterial community) and discuss what we can infer from both experiment. In an earlier version of this manuscript we had the results section presented so that the DOM release experiment was presented first and the DOM consumption experiment later. During a review of that version the reviewers considered that structure to be too complex. We then decided to also organize the results section so that it better supports the discussion. We would very much like to hold on to the current structure. Now all the information related to one topic of discussion can be found in a single figure instead of two figures. E.g. Fig. 7 contains the DOM related results from both experiment. Also, the variables are presented so that the panels presenting the same variable from both experiments are always on top of each other.

Specific comments:

L12: What two later phases? Stationary and decay phase?

Authors: The two phytoplankton cultures are not in the same phase during the two later incubations, as discussed later in the manuscript. Therefore we simply used "two later phases" in the abstract to save space. The possible consequences of this lack of synchrony in sampling on the interpretation of the results are discussed in the manuscript.

L113: Is 4C winter Baltic Sea temperature?

Authors: Yes. This will be added in the text.

L131: As the phytoplankton culture is not axenic, how will the co-cultured original bacteria community affect the result? Any no-TFF inoculum treatment as a control to compare?

Authors: The purpose of the added bacteria was to challenge the co-cultured bacteria. We wanted to see if there were bacteria in the added natural community which could utilize the phytoplankton derived DOM better than the cultured bacterial community. The effect of the added bacteria turned out to be minimal, so the original community is responsible for most of the observed processes. We have tried to acknowledge this in the discussion as well as possible. We did not include a control without added bacteria. Bacterial addition was considered an integral feature of the experiment with the goal of better relating the experiment to the natural environment, not a treatment that should be specifically controlled. The experiment was also conducted at the maximum logistical capacity so, unfortunately, extra controls were not possible.

L176: How much particle-attached bacteria will retain on 0.8um filter?

Authors: We did not measure this. We used flow cytometry for counting bacteria, which also cannot accurately count particle attached bacteria. So all the bacterial abundance measurements consist practically of free-living bacteria. This is an inherent issue of the methods we used. However, bacterial abundance is only used to compare the two phytoplankton species. We do not discuss the absolute counts in relation to natural bacterial abundances. Thus the rough estimate of bacterial abundance counting only the

free-living bacteria should be sufficient for this experiment.

L183: You mean at beginning, the inoculated bacteria only account for a small fraction in the original phytoplankton-bacteria culture?

Authors: Yes, you are correct. This is the biggest setback of the experiment. Concentration of bacteria using tangential flow filtration is a widely used method for concentrating bacteria and it worked fine during the pilot phase of this experiment. We cannot really say why it performed so poorly during the actual experiment. Thus most bacteria consist of the bacteria already present in the cultures. We hope we have taken this into account in our interpretation of the results as well as possible.

L319: intracellular phosphate storing in phytoplankton? And then will be released?

Authors: We only measured that phosphate concentration was high before filtration and low after filtration and interpreted this so that the phosphate must be stored in cells which do not pass through the 0.8 µm filter, i.e. phytoplankton. We do not have measurements of the release of P from phytoplankton, but we assumed that P anyway circulates among phytoplankton, DOM, and bacteria and that the system is therefore not P-limited.

Fig.2a: KPI for A.malm is at early exponential, late exponential and stationary phase, while KPI for R.marina is at early exponential and two decline phase, will this cause the comparison between two biased?

Authors: The initiation of the KPIs was dictated not only by the growth phase of the culture, but also by progression of the season, as we wanted to use only pre-spring bloom bacterial communities in the bacterial inoculum (lines 321-323). This will likely cause some bias. The effect of growth phase differences on interpretation is discussed in lines 484-490 and 541-546. We concluded that it should be safe to compare the species despite the differences in the growth phases. We will try to make the justifications for this decision clearer in the text.

Fig.2b: Cannot tell which is white, which is grey, left bar is grey but too small to show?

Authors: Words "left" and "right" will be added to the figure captions, where necessary.

L344: Why do you measure both leucine and thymidine incorporation? One indicate protein synthesis while the other indicate DNA, what does their ratio indicate? Introduce it either in method or in result.

Authors: We measured both because they give slightly different, yet complementary, estimates of the bacterial production and they can safely be measured in same samples. Their ratio has been used as an indicator of the metabolic status mainly to tell if the community is in balanced growth. We will explain this in the methods and add appropriate references.

L388: This result is from which table or figure?

Authors: References to appropriate figures will be added.

L405: Community of KPI 2 and 3 are more similar than KPI 1, because these two sampling time more close to each other? Is this corresponding to the growth curve?

Authors: Yes, that is a likely reason for similarity of community between KPI 2 and 3. In *A. malmogiense* treatments the measurement time between KPIs 2 and 3 was not as short as in *R. marina* treatments, but the bacterial community still changed little between KPIs 2

and 3. Likely the bacterial community reached a stable composition already at the late exponential phase in *A. malmogiense* treatments.

L424: This is confusing, Betaproteobacteriales is in the class of Gammaproteobacteria?

Authors: Silva database, which is widely used in taxonomical classification, uses also phylogenomic information (Parks et al. 2018) and thus Betaproteobacteria is not anymore an own class but an order under Gammaproteobacteria. Reference: Parks, D., Chuvochina, M., Waite, D. et al. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol 36, 996–1004 (2018). https://doi.org/10.1038/nbt.4229

L430: This tight grouping is interesting, consistent with the less labile DOM in A.malm, may add some discussion on this point to connect bacteria community shift with DOM lability

Authors: From the perspective of the analysis, the tight coupling is likely caused by the small changes in the bacterial community composition and DOM composition in the *A. malmogiense* treatments when compared to *R. marina* treatments. This grouping was mentioned here mainly to acknowledge the possible limitations of the analysis. The connection between bacterial community and DOM lability is discussed on lines 568-599.

Fig.7: Why don't start with same DOC concentration at the beginning of DOM consumption experiment? This will avoid dose effect when comparing between two phytoplankton-derived DOM.

Authors: The initiation of the incubation was dictated by the progression of season, as explained in an earlier comment. We also did not want to disturb the cultures by doing additional dilution/concentration procedures. They would have also affected the bacteria, not only DOM. We also did not have the capacity for real-time DOC measurements so some estimation, and therefore variation, was inevitable. We consider the current DOC concentrations at day 1 to be close enough for comparisons.

L464: "comparable **between two phytoplankton** and higher than in the control"

Authors: Will be corrected.

L482: Need clarification here. What does trend similar mean? All showing increasing trend of DOC from KPI 1 to KPI 3, so DOC is produced instead of consumed here?

Authors: This statement was poorly formulated, we apologize. We meant to say, that the trend was similar between the two phytoplankton species. I.e. if a variable was rising in one phytoplankton treatment from KPI 1 to 3 it did so also in the other treatment. We did not mean to say that the KPIs were similar. Will be corrected.

L503: less labile DOM is revealed from peak C here?

Authors: Yes. Will be specified in text.

L507: excessive production of protein-like DOM by phytoplankton will explain the increase of peak T, but how to explain increase of peak C? phytoplankton production and bacterial consumption both occur? And optical characteristics is measured before the start of KPI, so this is from original co-existing bacteria in phytoplankton culture? And production is larger than consumption so overall DOC is increasing as mentioned in L482?

Authors: On line 506 we gave two references for increasing humic-like fluorescence

because of bacterial activity, but this has been connected to decrease in protein-like fluorescence. We cannot explicitly say why both increase in this case, so we discussed some possible mechanisms. Optical characteristics for the DOM release experiment are given from two sources, DOM line mix (Fig 7b-c, g-i) and from cultures without added bacteria (Fig. B1). There are slight differences in values, likely because of sea water CDOM brought in along the bacterial inoculum in the DOM line mix, but the patterns are the same. Therefore both measurements support the same conclusions, and they are not discussed separately. Yes, production of DOC is larger than consumption in the DOM release experiment (Fig. 7a).

L579: Why? What is Bacteroidia related to? They tend to degrade less active DOM?

Authors: They have been shown to process less labile DOM, but the first sentence of the paragraph is indeed confusing, and our message does not come out clearly. We will improve it.

L607: But final bacterial communities are different between two phytoplankton culture in Fig.5. What do you mean here?

Authors: We meant to say that the community around *R. marina* at the end of the DOM release experiment resembled the community at the end of the DOM consumption experiment, and vice versa for *A. malmogiense*. Will be corrected.

Please let us know, if you would prefer some issue to be solved in a different way or if you think that any of your questions were not answered properly. We are happy to discuss further.