Author's response to reviews

Manuscript title: Contrasting patterns of carbon cycling and DOM processing in two phytoplankton-bacteria communities

Dear editor,

please find embedded in referee comments our point-by-point responses. We have tried to address each comment to the best of our abilities, but please let us know if something still needs to be improved. Below the referee comments we have added a section called "Other corrections" where we have listed other minor improvements which we made in the manuscript.

Best regards
Samu Elovaara

Referee 1:

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, but we could not investigate this in the incubation mix containing radioisotopes. This is explained on lines 167-177, now 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. The DOM consumption experiment is the primary experiment for studying changes in the DOM pool.

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. 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. Therefore, we prefer 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. 8 (previously 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. Now the abstract already states 'two complementary experiments' (line 10) to prepare the reader for comparing the results of both experiments.

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. We changed the text a bit and omitted the word "phases" to avoid confusion (line 12). 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 was added in the text (lines 113-114).

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 as well as possible (lines 207-210, 418-420, 651-667 and 673-677). 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. We expanded this reasoning in the text (lines 345-349).

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: (Fig. 2 is now Fig. 3.) 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 350-352). This will likely cause some bias. The effect of growth phase differences on interpretation is discussed on lines 522-532 and 583-591, and we have improved this discussion. We concluded that it should be safe to compare the species despite the differences in the growth phases.

Fig.2b: (Fig. 2 is now Fig. 3.) Cannot tell which is white, which is grey, left bar is grey but too small to show?
Authors: Words “left” and “right” were added to the figure captions for all boxplots.

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 added this explanation in the methods with an appropriate reference (lines 250-251).

L388: This result is from which table or figure?
Authors: References to appropriate figures were added. We also considered this paragraph to be a bit misplaced, as some of the related results were not presented yet. We therefore moved the paragraph into section 3.4 (lines 481-486). The paragraph was also modified a bit to make it clearer.

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 a class anymore 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 throughout section 4.2 (lines 601-667).

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: (Fig. 7 is now Fig. 8.) 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: Corrected (lines 497-498).

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. This has been corrected and the whole paragraph improved (lines 522-532).

L503: less labile DOM is revealed from peak C here?
Authors: Yes. We specified which signals we meant (line 548).

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 551 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 8b-

c, g-I (previously Fig. 7)) and from cultures without added bacteria (Fig. A1 (previously 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. 8a (previously Fig. 7)).

L579: Why? What is Bacteroidia related to? They tend to degrade less active DOM?
Authors: This paragraph was poorly organized, and the message was not delivered clearly. We fully revised the paragraph (lines 622-632), and it should make more sense now. The relationship between Bacteroidia and DOM is explained better. The last two sentences of the paragraph were moved later in the section (lines 665-667) as they now felt misplaced in this paragraph.

L607: But final bacterial communities are different between two phytoplankton culture in Fig.5. What do you mean here?
Authors: (Fig. 5 is now Fig. 6.) 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*. This is now corrected (lines 652-653).

Referee 2:

The manuscript determined the carbon cycling and subsequent effect on bacterial community composition using an experimental approach based on monoculture of two common phytoplankton species in the Baltic Sea, A. malmogiense and R. marina. The manuscript found the clear differences in carbon cycling including the DOM composition and degradability which cause predominance of different bacterial species. The conclusion was derived from comprehensive experimental data, i.e., carbon cycling with 14C tracer experiments, DOM degradability with its composition, bacterial community composition etc. This manuscript is novel and contains some very exciting results that I think will be of interest to readers of Biogeosciences. However, I have some major comments which will improve the manuscript.
(1) The discussion, conclusion, and implication to natural environments are generally well written based on the experimental results. However, I do have one concern about this point. The authors added natural bacterial inoculum to phytoplankton incubations. However, the major bacteria in the DOM consumption experiments was derived from non-axenic phytoplankton cultures but not from natural seawater inoculum. In this case, what is the source of bacteria in non-axenic phytoplankton cultures? Furthermore, is the production and degradation of DOM by these bacteria comparable to the bacterial community in natural seawater? The authors should describe/discuss these issues in the revised manuscript.
Authors: The bacteria in the cultures originate from sea water from when the cultures were first established (lines 97-100). These bacteria end up dominating the bacterial communities even after adding the inoculum of sea water bacteria, and so they are likely responsible for most of the DOM processing we observed. The small contribution of the added bacteria to the bacterial processing of DOM is acknowledged and discussed at lines 207-210, 418-420, 651-667 and 673-677.

(2) The current manuscript, in particular the method section, is hard to follow. One of the reasons is likely that some information is only available in figure caption (e.g., Fig. A1). It is recommended that the authors move all information about the methods described in the figure captions to the methods section of the text. The other reason is probably that the manuscript contains many experimental lines having diverse chemical and biological analyses. I guess it's better to combine Fig. 1 and Table A1 to overcome this issue.

Authors: We tried to make the methods section easier to follow. Major and minor improvements were done throughout the methods section. Everything from Appendix A was moved into the methods section. Figure depicting 'production line' of the DOM release experiment (Fig. 2, previously Fig. A1) is now adjacent to the text where the production line is described. However, we decided to keep most of the information presented in the caption of Fig. 2 only in the figure caption. As the figure captions should anyway be comprehensive, it would have been too repetitive to have the same information also in the main text. Now all the information related to the production line are close to each other, so we considered it sufficient to present the details of the production line only in Fig. 2. Also Table A1 (now Table 1) was moved to the end of section 2.1. We considered combining Table 1 with Fig. 1, but Fig. 1 already contains quite a lot of information and adding everything from Table 1 to it would make it too cluttered. Fig. 1 caption already contains brief list of the measured variables at each stage and a reference to Table 1. Now with updated organization it should be easier to use Fig. 1 and Table 1 together to understand the experimental design.

(3) I could not understand the reason why the authors set the DOM line mix.
Authors: The DOM line 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, but we could not investigate this in the incubation mix containing radioisotopes. This is explained on lines 167-177, now 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.

In addition, the authors seemed to determine the cell specific BP by tracer experiments of production line mix and bacterial abundance of DOM line mix. Are these two results directly comparable to determine cell specific BP?
Authors: You are correct, tracer incorporation in production line mix was divided by bacterial abundance from DOM line mix. This was a practical necessity (justified now on lines 170-172) and indeed not the most optimal solution. However, the results are only used to compare the two phytoplankton treatments. As the same procedure was followed for both species, this approach should be enough for this purpose.

(4) It is recommended that a subsection regarding with carbon cycling (e.g., points described in graphical abstract) is added to the beginning of discussion section. Some descriptions in the result section and the 4.3 subsection may be able to move this new subsection.
Authors: Good suggestion. We added a short summary of the main results at the beginning of the discussion (lines 514-520). However, we did not move text from section 4.3. because we did not want to scatter discussion of related topics.

Specific minor comments
L 131-133: Please add this information in Fig. 1.
Authors: Monitoring of phytoplankton abundance and optical properties of DOM are now explained in the caption of Fig. 1 (lines 120-121). We did not add them in the actual figure, as all the other measurements are also only mentioned in the caption and in Table 1 (previously Table A1). We removed the part about bacterial abundance measurements during the monitoring period, as these measurements are not reported.

L 679-383: I could not understand how the authors determined the incorporation of 14C-labled DOC and bacterial production by 3H-thymidine and 14C-leucine and. Please explain it with more details. Again, please move these descriptions in the method section of the text. In addition, it is recommended that the meaning of ratio of leucine to thymidine incorporation is explained here.
Authors: ^{14}C activity in DOM and in bacterial biomass was determined using ^{14}C-$NaHCO_3$, which was given to phytoplankton, who then converted it to DOM. This is explained on lines 236-244, now in more detail. We also added a reminder of this in the caption of Table 2 (previously Table 1) (line 411). Incorporation rates of ^{3}H-thymidine and ^{14}C-leucine were only measured as indicators of bacterial production. The meaning of leucine:thymidine incorporation ratio was added to the methods (lines 250-251).

Figure 2 and other similar figures: In Figure 2 and other similar figures, the results of A. maim. located left side in the time series (Fig. 2a) but that located right side in the box and wisher plot (Fig. 2b). I was sometimes confused by this difference. I think it is better to place the A. main. data on the right (or left) side of every panel in all figures.
Authors: The positions of the species in the boxplots in Fig. 3, 4, 5, 8 and A3 (previously Fig. 2, 3, 4, 7 and B3, respectively) have been swapped. Now *A. malmogiense* is always on the left side and *R. marina* on the right side in every figure.

Figure 5: I assume that the bacterial community composition of DOM release experiment and that of Day1 in DOM consumption experiment should be similar, because the major fraction of bacteria in these experiments was derived from the same non-axenic phytoplankton culture. However, bacterial community compositions seem to be largely different between two treatments in Fig. 5. Why?
Authors: (Fig. 5 is now Fig. 6.) In the DOM release experiment all the bacteria were present but in the DOM consumption experiment we tried to remove most of the bacteria by filtering (lines 179-181). Filtering likely removed larger cells more efficiently, which caused the differences in the initial conditions of the DOM consumption experiment. This is acknowledged for actinobacteria on line 641-642. (We also corrected an error in that sentence as we had given a wrong filter pore size.)

L443-445: It is recommended that the DOC data is added in Fig. B1.
Authors: Was added to Fig. A1 (previously Fig. B1).

L482-484: I don't agree with this conclusion. The qualitative parameters of DOM, namely S275-295, HIX, BIX etc, changed significantly with incubation time and differed among KPI3 1-3 (Fig. 7B).
Authors: (Fig. 7 is now Fig. 8.) 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. This has been corrected and the whole paragraph improved (lines 522-532).

L495-496: "are be able to". Please rephrase it.
Authors: Corrected (lines 540-541).

Other corrections:

Added "cell specific" on line 12.

Minor rewording of methods to improve clarity (lines 100 and 112).

Simplified Fig. 2 (previously Fig. A1).

Added reference to Table 1 on line 177.

Added “in dark” on line 216.

Removed “bacterial production” from title of section 2.2.2.

Minor rewording on lines 310-311 to improve clarity.

Moved this sentence from the third paragraph of section 4.2 to the end of the section, to improve the clarity of the discussion: “The difference between control treatments and experimental replicates of the DOM consumption experiment. suggests that phytoplankton-derived DOM, not the growth medium, is the main driver for bacterial community and DOM processing dynamics.”

Moved this sentence from the third paragraph of section 4.2. to the fifth paragraph of the same section, to improve the clarity of the discussion: “The observed pattern in the bacterial community composition supports the interpretation that DOM was more labile in *R. marina* treatments than in *A. malmogiense*.” The sentence was also improved a bit.

Added bacterial production measurement results to Appendices (Fig. A2 (previously Fig. B2) and Table A1 (previously Table B2)) and reference to them on lines 371 and 375. These results are not necessary for understanding the main conclusions, as we presented results for thymidine and leucine incorporation, from which bacterial production is derived, in the main text. However, bacterial production is referred to in the discussion, so we included the data.

Added a missing reference (Gargas 1975).