

Biogeosciences Discuss., author comment AC2
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Reply on RC2

Flora Mazoyer et al.

Author comment on "The dominant role of sunlight in degrading winter dissolved organic matter from a thermokarst lake in a subarctic peatland" by Flora Mazoyer et al.,
Biogeosciences Discuss., <https://doi.org/10.5194/bg-2022-26-AC2>, 2022

Answer to comments from Reviewer 2

We thank the reviewer for his (or her) time evaluating the paper and for his (or her) detailed report. Please, see our answers to your well-argued comments below.

Time between sample collection and initiation of the incubation. Over four months elapsed between initial sample collection and the beginning of the incubation study. While the authors point out that the sample was stored in the dark at 4 °C, this still represents a dramatic shift from the sampling environment (0.5 °C just below the ice). Even under refrigerated conditions, four months is a long time for aquatic microbes to make use of the most labile portions of the aquatic DOM. It is likely that comparatively low microbial activity observed during the incubation may be at least partially due to the fact that this sample essentially underwent a four month microbial incubation prior to the beginning of the official experiment. This needs to be explored and results need to be considered within this context. On a related note: it appears from Matveev et al., 2019 that the sample from SAS2A was collected on 19 March while the present paper states it was collected on 24 March. Please verify that the reported sampling date is correct.

REPLY: The goal of this study was to investigate the fate of winter DOC pool during spring time, when oxygenation, sunlight and higher temperatures are coming back. As replied to reviewer 1, we agree that this delay, due to logistical reasons, was not ideal. However, we consider that the prolongation of these winter-like conditions is of 2 months rather than 4, because the ice-break naturally happens mid-May (Matveev et al., 2019). Yet, it is true that bacteria continued to transform DOM during 2 supplementary months, as already underlined in the first version of the manuscript (lines 102-106). Therefore, the changes in DOM properties during the delay (now described and justified more explicitly; lines 108-118) were potentially stronger than what naturally occurs in situ until spring melt.

We also agree that the difference in temperature under the ice cover (between 0.5 and 2°C) and the environmental chamber where the container was kept (4°C) is not negligible and could have accelerated the bacterial transformation of DOM and potentially changed the community composition. This is now pointed out in the revised manuscript (lines 519-524). However, Adams et al. (2010) for example found that only 4 OTUs out of 79

were unique to the temperature of 2°C in the bacterial community from an Arctic lake. We assumed that all bacterial species were still present in the container water, but potentially in different proportions as in the lake at ice melt. This is always the case for container water kept to make experiments, probably already after a few days; this is not unique to water kept for months.

We agree that the experimental biodegradation could be underestimated because of this longer delay, and we now have addressed this more extensively in the manuscript (lines 519-534). However, we think that the absence of DOC consumption during this delay (in fact there was an increase in DOC by 15%) supports the claim of recalcitrance. Other DOM changes happened (in SUVA₂₅₄, CDOM and FDOM), but we expect these changes to be similar to changes occurring under the ice in dark and cold conditions, but simply extended in length. We cannot tell if they resulted from the action of the bacterial community, from flocculation, or from a combination of both.

About the date issue: samples used for the Matveev et al. (2019) study were indeed collected from the 19th March, but the water brought back in the carboy for our specific study was collected separately, at the end of the sampling campaign, on the 24th.

Incoming Solar Energy: *If the Teflon bottles are light diffusers, then solar radiation measured outside the bottles is not representative of the energy experienced by the samples. You may be able to apply a simple correction by placing a pendant or pyranometer inside a Teflon bottle (or cut a bottle to make a Teflon cover) – this will be a more useful measure of the solar radiation that reached the sample.*

REPLY: As replied to reviewer 1 above, we added some justification about the use of Teflon, which is transparent to wavelengths of interest (lines 157-159). We hope it will satisfy this request.

Challenges with sample filtration. *(lines 120-131) It is unclear why different filtration schemes were used when preparing water for the experiment (e.g., 0.2µm Tuffryn vs a two-step process with a 0.7 µm glass fiber filter and 0.2 µm cellulose acetate filter.) Please explain the sample preparation more clearly, perhaps with a flow chart.*

REPLY: We understand your comment and tried to clarify this further (lines 140-144). We had to use both techniques to obtain a 0.2-µm filtrate for pure logistical reasons (shortage of filtering capsules after the ones planned started to clog). We describe honestly the steps, but do not want to distract the reader with these details into a flow chart.

Closing the DOC mass balance. *The authors observed a large mismatch (58 to 1214%) between DOC lost and the concomitant gain in DIC and bacterial production. They attribute this to DOM flocculation which was noted, but unfortunately not quantified. In the absence of supporting measurements, the authors probably don't need to spend so much time discussing flocculation and simply admit they don't know.*

Additional considerations could include: DIC outgassing to CO₂ during the incubation. It is possible that some DIC was out of solution in the form of an air bubble in the bottle. This DIC would not have been accounted for. Was this observed or checked? CO₂ loss during the two-month period of sample storage between sampling and analysis. While sample storage in exetainer vials has generally been reported as stable, it has been demonstrated that CO₂ concentrations were up to 14% lower than expected in vials that had been stored for 84 days.

REPLY: We acknowledge that we have no direct measurements of flocculation, but we would not be the first to rely on indirect observations to discuss results! We really think that this paragraph deserves to remain in the discussion. First, because we have been

systematically observing flocs in filtered water originating from SAS peatland lakes, under different circumstances of sample preservation. Second, we think that it is relevant to raise awareness of the readers on measuring flocculation when investigating degradation of DOM in waters rich in aromatic components. Flocculation is an overlooked process that may be challenging to measure on small volumes, but we think it really deserves attention.

Nonetheless, we toned down the subtitle (line 464) and added to the revised version of the manuscript these two other potential factors that could have contributed to the DOC-DIC gap (lines 481-485). Notably, the air bubble in the incubation bottle at sampling was quite small (1-2 mm in diameter), so we suspect this was a negligible loss. We thank the reviewer for underlining this paper on CO₂ loss during storage in Exetainers, which will be relevant to researchers using them.

Line 8 (and elsewhere): *suggest rewording "retroaction loop" with "positive feedback loop".*

REPLY: This was changed accordingly and throughout the manuscript.

Line 18: *"full mineralization to CO₂" implies that the entire DOC pool has mineralized; this is not consistent with your data.*

REPLY: With the word -full- we meant the complete chemical conversion from the original DOM composition to CO₂, which applied to a portion of DOC. Full was not intended to mean that the full amount of DOM would have been mineralized to CO₂ but instead that a fraction of the DOM pool was completely mineralized to CO₂, because CO₂ increased in the light treatment (and from the beginning of the incubation when bacterial regrowth was minimal). A second fraction of the DOM pool underwent photodegradation that did not lead to mineralization, a third fraction putatively flocculated, and one last fraction was probably not sensitive to photodegradation at all. We removed the term "full" to avoid any confusion (also raised by Dr L. Shirokova) and the sentence now reads: "We demonstrate that sunlight was clearly driving the transformation of the DOM pool, part of which went through a complete mineralization into CO₂."

Line 22: *replace "undirect" with "indirect".*

REPLY: Changed accordingly.

Line 23: *"outstanding boosting factor" is awkward wording, please find alternative wording.*

REPLY: We replaced this expression by "considerably stimulated" (now line 20).

Line 84: *you refer to Fig. 7 of Vincent et al. (2017); perhaps you can include this figure in the supplemental information.*

REPLY: We would prefer not adding it and just keep it mentioned as it is, because it is not directly related to the experiment in itself. It is a reference for readers who would like to obtain more information on the study site.

Line 86: *Field sampling – just refer to the date the sample was actually collected (was it 19 March or 24 March?).*

REPLY: Actually, both dates are correct. The field data collection was carried out on the 19th and 24th (see table S1), while the water for the experiment was collected on the 24th (it is specified at the end of the paragraph). That is why we introduced the paragraph with

that full period.

Lines 144-145: *provide a reference for the light-filtering properties of Teflon.*

REPLY: We added this information, as mentioned above (lines 157-159).

Lines 211-213: *Why include the unpublished data in the PARAFAC analysis?*

REPLY: An extra dataset involving another experiment at the same site was added to our dataset in order to develop and validate a PARAFAC model more easily, but this other experiment is not yet published. According to Stedmon and Bro (2008), having a minimum of 100 samples indeed generally makes the component extraction more efficient during the validation steps. We clarified that in lines 226-228.

Line 248: *replace "unfrozen" with "thawed".*

REPLY: The comment refers to a sentence talking about water samples that were melted at room temperature. We replaced unfrozen by melted in the revised manuscript. It is probably better to keep thawed for solid material.

Lines 294-303: *Please provide more supporting references in your discussion of the fluorescence results.*

REPLY: We added some examples of supporting studies in this paragraph, but all supporting references are provided in Table S2 (now explicitly specified in the manuscript, lines 310-317).

Figure 6: *Please show the DIC and Biomass as separate portions of the bar chart (stacked to show the total).*

REPLY: Since the percent into biomass is quite small it would not be seen easily as stacked bars (especially for treatments in the dark), so we instead added the percent represented by DIC over each bar.

Line 427: *"... carbon canalized to bacterial production..." I think you mean to say "... carbon allocated to bacterial production..."*

REPLY: Changed accordingly.

Lines 540-541: *These details about filter preparation and problems with cracking need to be presented in the methods section.*

REPLY: We transferred this information to the methods section, as suggested (lines 138-140).

Lines 577-580: *This material about the DNA content of cells should be excluded from this paper.*

REPLY: We do not understand the reason why the reviewer asks for this exclusion since the DNA content of cells provides indications on the structural composition of bacterial communities. This is now clarified in the manuscript (lines 623-628). We also changed Fig. S3 to better illustrate this structural change in the bacterial composition (providing the three populations in percent composition). With this new figure, we can clearly see that 0.2 μm -filtration eliminated MNA and HNA bacteria (in C and L), but when kept in the dark (C), the structural composition of the community converged to the same as in 1.5 μm -filtered samples, while the community exposed to sunlight (L) became much richer in MNA

and HNA. We would like to keep this interesting result.

Cited references

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Stedmon, C. A. and Bro, R.: Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial, *Limnol. Oceanogr. Methods*, 6, 572–579, 2008.