

Manuscript ID: egusphere-2025-5257 – Bioreactivity of dissolved organic carbon in ponds of the ice-wedge polygonal tundra

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Reply to Referee #1

I want to thank the co-authors for their careful proofreading, their detailed and systematic feedback, and their helpful corrections. In my view, the manuscript is ready for publication after correcting the last classic French typographic mistakes (e.g., "exemple" for "example" or "autochtonous" for "autochthonous").

RESPONSE: We carefully revised the article to correct the remaining typographic mistakes.

Reply to Referee #2

One of the questions raised during the review process was regarding the length of time before analysis of DOC concentration. The authors explained in the original version of the manuscript that samples were acidified. I am concerned about flocculation since DOC can flocculate under acidic conditions, which would result in a lower reported DOC concentration than would be due to microbial degradation alone.

In the response letter, the authors have now explained that samples were analysed 2 to 6 months after acidification due to technical problems with the TOC analyser. They explain that some flocculation was observed in some samples but not all. The manuscript has been edited to clearly state that the samples were acidified and analysed after 2 to 6 months (Section 2.4, lines 152-154). However, I am unsure how reliable the data are, as our experience with acidifying samples is that the data become highly unreliable due to flocculation. We are unsure how to proceed with the review process. We think this is a major concern. We ask the authors to explain how they can be sure that the change in DOC concentration is due to biological reactivity and not flocculation.

It would be good to at the very least include a section in the discussion about the limitations of the study from a methodological standpoint. It may be that authors have a way to test and confirm if flocculation was a concern and to what extent. This is potentially a major point impacting the reliability of the DOC concentration data.

RESPONSE: We appreciate the reviewer's concern regarding DOC preservation and potential flocculation. We clarify an important distinction: no flocculation was observed in the DOC preservation tubes (stored at 4°C in the dark following acidification to pH 2). The flocculation mentioned was only observed in the incubation bottles during the experiment itself, not during sample storage.

Acidification to pH 2 with HCl or H₂SO₄ followed by cold storage (4°C) in the dark is a widely used standard method for freshwater DOC samples (e.g., Berggren et al., 2020; Fouché et al., 2017; Guillemette and del Giorgio, 2012). This approach effectively inhibits microbial activity, which is a primary concern for DOC preservation. We are not aware of literature characterizing the timing and extent of flocculation after acidification, or how it depends on alkalinity, salinity, specific ion concentrations, and other water quality parameters. This lack of characterization limits discussion of this potential artifact. We cannot make direct comparison with the reviewer's experience, as we do not know the type of samples or analytical procedures involved.

We did observe some flocculation in three incubation bottles, but our experimental design did not allow us to quantify this loss separately (insufficient samples volume). However, the DOC loss patterns across all samples show consistent trends, and the three affected samples do not deviate systematically from these relationships. This suggests that flocculation in these specific samples (or overall) did not substantially bias our conclusions about DOC bioavailability across pond types.

To address the reviewer's concern constructively, we have added a brief statement in the Discussion (lines 502 to 508) acknowledging that potential flocculation during the experiment in a subset of samples and during acidification storage represent methodological limitations that could overestimate actual DOC bioavailability. We emphasize that our conclusions about relative differences among pond types remain robust despite this limitation.

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