

**Manuscript No.: bg-2022-225**

**Response to RC2**

Dear Reviewer #2,

We thank the reviewer for providing valuable comments for this manuscript. We have carefully considered the comments and incorporated most of the changes made by the reviewer. Please see below for a point-by-point response to the reviewer's comments and concerns.

**Reviewer's comments to the authors:**

**Reviewer #2**

The manuscript "Dissolved organic matter composition regulates microbial degradation and carbon dioxide production in pristine subarctic rivers" by Saarela et al. provides a nice comparison between DOM and CO<sub>2</sub> production in the a clear water vs brown water systems in the high latitude watershed. Studies linking DOM and CO<sub>2</sub> has become very crucial in recent years with increasing amount of greenhouse gas emission from inland waters, and this manuscript provides valuable findings on the topic. The manuscript includes a number of advanced techniques including CO<sub>2</sub> measurement, FT-ICR-MS, and qPCR, which combined with appropriate statistical analysis seems adequate to support the major findings. The manuscript will be valuable addition in the field of aquatic biogeochemistry and will benefit the readers of Biogeosciences. I have a few suggestions for the authors to consider before the final publication of the manuscript.

Author response: We thank the reviewer for the suggestions to improve this manuscript.

**1. In lines 108-109, the author mention about adding an inoculum from the surface sediment. Since river water samples are usually added as inoculum for incubation experiments, please provide a brief explanation for adding inoculum from the sediment.**

Author response: We thank the reviewer for this comment concerning the use of an inoculum. Surface sediment was collected for the use as an inoculum because we considered the sediment to be more suitable to accelerate the microbial activity in the incubation experiment.

**2. Also, a previous meta-analysis study on BDOC measurement method (Vonk et al. 2015) reported no significant difference between the BDOC measured with or without inoculum when a 0.7  $\mu\text{m}$  filter like GFF is used. The author could have avoided adding inoculum since enough microbes pass through the filter required for microbial degradation.**

Author response: We thank the reviewer for pointing out the interesting results by Vonk et al. (2015). While the use of microbial inoculum is a common way of conducting incubation experiments, this important issue should be considered in future experimental designs.

**3. The incubation experiment for measurement of CO<sub>2</sub> is quite interesting. A little more details on the calculations methods or showing the actual data on a SI table would be helpful for the readers. Also, why did the author use a one-point calibration when at least two point is more usual for calibration.**

Author response: We thank the reviewer for pointing out these issues concerning the measurements of CO<sub>2</sub>. We have clarified the details and equations of calculations concerning CO<sub>2</sub> production to Supplementary methods (see revised Supplementary material page 6). The data of the CO<sub>2</sub> production, as well as other data presented in manuscript figures and tables, will be published and made freely available for the readers via public data repository according to journal's submission guidelines.

In this study, we conducted a one-point calibration with a standard gas (2.02 ppm of CH<sub>4</sub>, 398 ppm of CO<sub>2</sub> and 0.836 ppm of N<sub>2</sub>O) due to relatively low concentrations of CO<sub>2</sub>. The linearity of the calibration with a larger amount of standard gas concentrations have been regularly verified for this GC-MS analyzer.

**4. Please add a relevant reference for the compound classes assignment criteria (line 181-184).**

Author response: We thank the reviewer for this comment. A reference for the compound class assignment criteria has been added (page 6 line 188).

**5. In the results, I see a lot of statistical analysis results (i.e., p values); however, I miss seeing the actual values of the major parameters particularly in comparison between seasons or water types. Including some actual values for DOC, CO<sub>2</sub> etc. in the result section would improve the readability of the manuscript, whereas adding the statistical results in the figure would also be helpful.**

Author response: We thank the reviewer for this important suggestion to improve the readability of this manuscript. The average values of the statistically significant major parameters have now been added to the manuscript section "3.1 Microbial degradability of DOM". In addition, statistically significant differences between the rivers (i.e., p values indicated by asterisks) have been added to Figures 1 and 2.

**6. The discussion is well supported with references, but you may add the following reference to support the findings on lability of molecular composition (example: line 344-349). Begum et al. 2022 (<https://doi.org/10.1016/j.watres.2022.119362>).**

Author response: We thank the reviewer for suggesting this interesting review study by Begum et al. (2022) as a reference to support our findings. This paper is highly relevant to our study, and we have now included it as a reference in our manuscript.