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Comment on bg-2022-225

Anonymous Referee #1

Referee comment on "Dissolved organic matter composition regulates microbial degradation and carbon dioxide production in pristine subarctic rivers" by Taija Saarela et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2022-225-RC1, 2022

In "Dissolved organic matter composition regulates microbial degradation and carbon dioxide production in pristine subarctic rivers," Saarela et al. examine differences in DOM composition and CO2 production between clearwater and brownwater rivers in subarctic Finish Lapland. They find clearwater river DOM more biolabile, with interesting implications for how future increases in terrestrial DOM inputs may not increase CO2 fluxes. The manuscript covers an understudied region and could be a useful contribution to our processed based understanding of CO2 production. However, there are a few major issues with the study design and methods that will need addressing and clarifying in the manuscript itself.

Incubation design: As laid out in figure S3, the overall incubation design causes me some concern and I do not think its potential implications are addressed in the paper. While I understand the desire to use the incubation (C in the Figure S3) without added inoculum for FT-ICR MS so as to avoid the influence of the inoculum on molecular formulae, I am worried about the direct comparison between CO2 analyses conducted in a set of bottles with an inoculum added and ICR metrics for a final time point derived from a completely different set of bottles with no inoculum added. To me it seems very much like comparing apples and oranges. I understand that in lines 120-122, the authors explain that there was no difference in potential CO2 production between bottles with the inoculum and those without, though I admit I do not understand how this was analyzed since Figure S3 shows that treatment 2 (C, the one with no inoculum) never had its CO2 measured, so I am not sure where that potential CO2 production measurement comes from. Please explain this in the manuscript. But CO2 production is not the only change that the presence of microbes can cause—they can change the composition of DOM through partial consumption, and it is possible the different size classes of microbes present in the filtered (treatment 2) and inoculum (treatment 1) bottles would do different things to DOM composition.

Thus, I do not think it is valid to compare CO2 production to ICR composition at the end of the incubation period, after the processing both sets of bottles have undergone has diverged. Comparing it to the composition at the beginning of the experiment makes sense, since those were the molecular starting conditions that led to the CO2 production.

It would greatly strengthen the manuscript if you removed reference to the final timepoint for FT-ICR MS (or at least the comparison of that time point to CO2 production), and focused on the relationship between the starting FT-ICR MS data.

Contaminant peaks: The extremely high relative abundance peaks in the top left corner of all your van Krevlen diagrams that you attribute to the aliphatics class look to me like classic surfactant peaks that are often added to FT-ICR MS analysis through the SPE process. They are often of the O_3S_1 class, and are several series of homologous peaks (separated by a CH2 unit). If they are these common contaminants, they should be removed from the analysis. This will change the % relative abundances of your compound classes, since these currently (and inaccurately, I believe) dominate.

At this point it isn't clear where your study system is situated compared to the systems you say are highly and less studied; the difference between "boreal catchments" (line 44) and "northern high-latitude streams" (line 46) is not clear—boreal catchments certainly are one type of northern high-latitude stream. In line 71 you say "subarctic rivers," which might be a good description to use in line 46 to contrast with boreal catchments. Further, right now you cite five sources for boreal catchments and five for high-latitude streams, completely contradicting the point you are making in the text that one is more studied than the other. Perhaps you don't need to set it up as an either/or scenario, but simply explain why it is good to study subarctic rivers.

Section 2.6: I have a few concerns about the FT-ICR MS methods you describe, and it would be helpful if you could clarify these points in this methods section. Why was only m/z range 150-500 analyzed? Normally DOM masses extend well into m/z 800-1000. Please add a short (even half a line long) explanation. Further, it sounds as if formulae were assigned one by one rather than in homologous series (the standard and far more powerful way of assigning formulae). If that is true, please justify it, or add some acknowledgement of how this may impact the results. If anyone is undertaking ultrahigh resolution mass spectrometry, they should work with the best software possible to ensure that their assignments are correct, and homologous series assignments are far more accurate than single formulae assignments. If, however, for some reason that is not what was done in this study, it at least needs to be acknowledged explicity in the paper with a small explanation.

Section 2.7: I believe this is not the normal way of counting bacterial abundance, and flow

cytometry would have been far more accurate. Please add some explanation of why this method was chosen to assess abundance.

Lines 155-157: Please include whether the CO2/DOC ratio is using the DOC concentration at the beginning or end of the measurement period.

Lines 244-251: Please specify whether you are talking about the mean or mean weighted average m/z, O/C, and H/C in this section. In Table 1, you say mean, but it's not clear how that is calculated. Did you add up all the m/zs for each sample and divide by the number of samples? That's what mean implies. If you mean mean weighted average, as in the mean m/z weighted by the relative abundances, please specify that. Same for O/C and H/C. At this point it's unclear what this metric is.

Lines 252-253: Do you mean the percent of formulae based on number of formulae, or based on percent relative abundance? They can mean very different things. Please clarify.

Lines 254-257: Relative peak intensities by definition add up to 100% for a single spectrum, so I do not understand how they could be higher in one river type than another. Do you mean the average relative peak intensity?

Lines 257-259: same as above—do you mean weighted average AImod? You need to specify. There is not just one AImod value for each sample, which is what this sounds like.

Lines 264-779 (and Figure 4): The percentages that are in this section appear to be percent of total molecular formulae in each compound class. Normally in this field folks refer to percent total relative abundances. These two concepts convey different things, and which you want to use depends on what you're looking at (number of specific/rare formulae versus the contribution that compound class makes to the overall DOM signal). You might want to think about switching to percent relative abundance if you want other papers to be able to cite this for comparison, since most folks work in %relative abundance. A caveat to this is in my next comment—the massive signal peaks you are assigning as aliphatics that are probably surfactant contaminants (O3S1 class, etc) found in most FT-ICR MS spectra that use SPE. These peaks should be removed before calculating %relative abundance.

Lines 346-347: Unlike what you are stating in this line, Figure 5 looks to me like the two rivers do not have significant differences in bacterial abundance (just very different ranges). Could you add in some statistic earlier to show they are significantly different, if they are?

Figure 3: First, the font is too small to read, and the dots of color indicating compound category in the legend are too small to see. Please make the figure and legend legible. Of more concern are the high relative abundance peaks at high H/C low O/C ratio (top left corner)—these look a lot like the surfactant contaminants that are common in FT-ICR MS analysis, and belong to the O_3S_1 or O_4S_1 classes of homologous series. Please see if these peaks are O_3S_1, and remove the contamination series before analysis.