RC3: Anonymous Referee #3, 18 Apr 2024

The authors of “Molecular level characterization of supraglacial dissolved organic matter sources and exported pools the southern Greenland Ice Sheet” present a research project on DOM composition from various sources and hydrologic flow of material to the coast of southern Greenland. The goal of the work relies on molecular composition comparisons of samples collected in the field and leached in the lab. The work describes themes of DOM transformations on the Greenland ice sheet and during downstream transport but is organized in a way that is confusing. Some of those points are noted as important (for example in the introduction) and language focuses on assessing potential transformations of DOM from sample collections, but some connections between results and assessing these transformations of DOM are confusing throughout the text, and some parts may be missing (for example % biolabile values are included in the abstract, but not reported on in the main text). Maybe the introduction can be reorganized to narrow the scope/set the foundation for what’s to come a bit better, creating a clearer start, and the rest of the manuscript text follows with more clarifying text so that the messages are clear and continuous throughout. The language describing the importance of microbial transformations and photochemical transformations in the introduction is not paired well with the lack of microbial and photochemical data for this project. It reads like something is missing. The first two paragraphs of the introduction create a wide scope of the work, which is not supported by the rest of the text. Connecting those ideas across the introduction and results sections will be greatly strengthened and reduce confusion. All recommendations for revision can be achieved. The following comments are divided into two groups, major and minor revisions.

Dear editor and reviewer,

We would like to thank the reviewer for their constructive comments and suggestions. Overall, we agree that the manuscript needs to be streamlined to make the research questions, hypotheses and major findings clearer and easier to follow. Please find our responses to each individual comment below (in blue). We appreciate the time and effort from the editor and all three reviewers, and hope that our responses will be sufficient to be allowed to submit a revised manuscript for publication in Biogeosciences

Major Revisions

1. The fluctuating use of the following words, compounds, molecular formulae, and composition, needs to be corrected for consistency and clarity. Parts of the introduction and results section use these terms interchangeably, yet they each have different meanings and may point to different measurements. It is not clear if the introduction is discussing composition measurements of DOM from various instruments or are they all FTICRMS? Are some of these studies measuring aliphatic compounds directly?
   We will correct terminology throughout the manuscript for consistency and clarity and will make methods by which DOM in other studies was characterized explicit in the introduction.

2. Introduction: Confusing themes, see some examples already stated in the first paragraph of this review. The first two paragraphs of the introduction seem like they should be the second and third paragraphs of the section and an opening paragraph should be added that sets the stage. The first sentence of the introduction focuses the reader on microbial blooms. Is that the most important thing to start with? Why? The same type of comment is true for the beginning of the second paragraph.
   We agree with the reviewer that the scope of the introduction is too broad and should be edited to more explicitly introduce the most important themes, the main research question and the main hypotheses. We will improve this in the revised manuscript.

3. Some text in the abstract, introduction, and conclusion states more than what experiments were conducted and what was measured. Please clarify the language to be more specific and reduce confusion. State where you are speculating. Some text reads as though you monitored the transformations of DOM during downstream transport. This is a major source of confusion. Please clarify. Example in Line 74, “as it is transported” suggests that you followed a parcel of water and made collections during transport. Is that true? Were they grab samples along a gradient?
   In the revised manuscript, we will rephrase statements to remove ambiguity and focus on the main findings of the dataset. We feel that the edits suggestions in point 2. will help set up the manuscript so that this will be easier to achieve.

4. FTICRMS details in the methods are missing. What ionization technique and mode were used? If one type of ionization and mode were selected, why? Were your ions singly charged? If so, how did you confirm that? This is especially important when going from m/z values to masses in Da. What was the signal/noise threshold? How were molecular formulae assigned? These are important details to include in the main text. The supplementary section doesn’t provide enough details either and repeats some of what is included in the main text. This work would also benefit from reporting on their instrument performance from their DOM optimization standards, see Hawkes et al., 2020, especially since they are reporting on AImod, m/z, H/C, and O/C metrics from their samples. Are there instrument biases?
   Reference: Hawkes, J.A. et al. 2020, An international laboratory comparison of dissolved organic matter composition by high resolution mass spectrometry: Are we getting the same answer? Limnology and Oceanography Methods, 2020, 18: 235-258.
   We will revise and update the methods and supplementary methods section in line with reviewer suggestions. See below for a more elaborate 21T method description, which will be further edited and elaborated in the revised supplementary information.

   Instrumentation: ESI Source.
   Sample solution was infused via a micro electrospray source[1] (50 µm i.d. fused silica emitter) at 500 nL/min by a syringe pump. Typical conditions for negative ion formation were: emitter voltage, -2.8-3.2 kV; S-lens RF level: 40% ; and heated metal capillary temperature, 350 ° C.

Instrumentation: 21 T FT-ICR MS.

DOM extracts were analyzed with a custom-built hybrid linear ion trap FT-ICR mass spectrometer equipped with a 21 T superconducting solenoid magnet.[2,3] Ions were initially accumulated in an external multipole ion guide (1-5 ms) and released m/z-dependently by decrease of an auxiliary radio frequency potential between the multipole rods and the end-cap electrode.[4] Ions were excited to m/z-dependent radius to maximize the dynamic range and number of observed mass spectral peaks (32-64%),[4] and excitation and detection were performed on the same pair of electrodes.[5] The dynamically harmonized ICR cell in the 21 T FT-ICR is operated with 6 V trapping potential.[4, 6] 100 individual Time-domain transients with AGC ion target of 2E6 charges per scan[AM1] of 3.1 seconds were conditionally co-added and acquired with the Predator data station that handled excitation and detection only, initiated by a TTL trigger from the commercial Thermo data station, with 100 time-domain acquisitions averaged for all experiments.[7] Mass spectra were phase-corrected [8] and internally calibrated with 10-15 highly abundant homologous series that span the entire molecular weight distribution based on the “walking” calibration method.[9] Experimentally measured masses were converted from the International Union of Pure and Applied Chemistry (IUPAC) mass scale to the Kendrick mass scale[10] for rapid identification of homologous series for each heteroatom class (i.e., species with the same CcHhNnOoSs content, differing only be degree of alkylation).[11] For each elemental composition, CcHhNnOoSs, the heteroatom class, type (double bond equivalents, DBE = number of rings plus double bonds to carbon, DBE = C –h/2 + n/2 +1)[12] and carbon number, c, were tabulated for subsequent generation of heteroatom class relative abundance distributions and graphical relative-abundance weighted images and van Krevelen diagrams.[13] Peaks with signal magnitude greater than 6 times the baseline root-mean-square (rms) noise at m/z 400 were exported to peak lists, and molecular formula assignments and data visualization were performed with PetroOrg © software.[14, 15] Molecular formula assignments with an error >0.5 parts-per-million were discarded, and only chemical classes with a combined relative abundance of ≥0.15% of the total were considered.

References

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Minor Revisions

Title: Typo. There is a word missing in between “pools” and “the”. Is the word “sources” necessary? Is “exported pools” a bit farfetched for this work? The work describes a supraglacial microcatchment, so perhaps “exported pools” is too wide a phrase.

It seems that the word “on” disappeared from the preprint .pdf file, while it does show up in the online version of the preprint. In the revised version, the title will be updated to “Molecular level characterization of supraglacial dissolved organic matter in a hydrologically connected Greenland Ice Sheet micro-catchment” or similar - in line with changes during manuscript revision and following this comment.

Abstract: There is one sentence of results in this abstract and then the following sentence states how these findings have implications and importance for future work. Not enough information about the results of the work and why it is important is included in the abstract.

We will revise the abstract to include more results and will elaborate on consequences of those results.

Introduction:

Lines 43-44: Not true. This has been characterized in Antarctica. Please be more specific.

To our knowledge, this has been characterized on snow in Antarctica, but not on glacier surface ice, as is stated in the introduction. In the revised manuscript, we will state this more clearly.

Line 49: Define DOC.

We will define DOC on first mention in the revised manuscript.

Line 57: The use of the word “compounds”. Were compounds directly measured?
Thank you for spotting this - this should be aliphatic formulae, which will be corrected in the revised manuscript.

Line 61: Please provide definitions of allochthonous and autochthonous DOM pertaining to ice sheets.
We will specify this in the revised manuscript.

Methods:
Line 82: Add a comma after “collection”
Thank you for spotting this - we will correct this in the revised manuscript.

Line 94: Add the word “concentration” after DOC
Thank you for spotting this - we will correct this in the revised manuscript.

Line 95: What is PC?
Polycarbonate - this should have been defined at first mention and will be corrected in the revised manuscript.

Line 96: What is “back in the home laboratory”?
This will be updated to the specific laboratory (GFZ Potsdam).

Lines 83-96: Provide more information about the site names/sample names that correspond with the figure. See next comment about the confusion of the figure and caption for examples. Samples for FTICRMS were only collected at D and Q?
This will be clarified in the revised manuscript.

Figure 1: The lettering in panel B shows up as purple online and is very difficult to discern from the background. When the page is printed in black and white, it’s nearly impossible to read. Please change the color scheme to improve clarity. Is site Q on the other side of the stream? Is Q the stream itself? What is SI? The lettering scheme of panel B combined with the lettering scheme of the figure is confusing. My recommendation is to keep the panel lettering scheme and change the panel B lettering scheme of the sites to lowercase letters, numbers, or numerals. The letter “X” is shown in the legend for the sample site, but not used in the figure. What’s the difference between field site and sample site?
We will clarify Figure 1 and improve the color scheme in the revised manuscript.

Line 107: Were these odd numbered hours? Please state that. Odd hours is not clear. How many time points were there between 7:00 and 21:00? n=?
Thank you for spotting this! We will clarify this in the revised manuscript.

Line 111: A full description of what?
This was meant to refer to the hydraulic conductivity and water table calculations. This paragraph will be edited for clarity and in accordance with comments from reviewer #1 in the revised manuscript.

Line 125: Please add the word “concentration” after “DOC”
This will be corrected in the revised manuscript.

Line 138 and throughout the text: Please check/correct nitrogen and carbon and N and C for consistency.
This will be corrected in the revised manuscript.

Line 141: What is “back in the home laboratory”?
This will be updated to the specific laboratory (GFZ Potsdam).

Line 151: What is “Predator data station”? If it is a software system, please provide that information.
The methods will be edited and clarified in the revised manuscript to address this and earlier comments regarding details in the methods.

Line 165: Good use of the word “compositions”. Check the manuscript to reduce confusing when fluctuating among uses of “compounds”, “composition”, and “molecular formulae”.
This will be checked for consistency in the revised manuscript.

Lines 171-173: This seems important, especially when the use of the term “biolabile” is used often and reported on in the abstract. Was this metric calculated? In Line 179, it states that formulae classifications are informative for this study, but not all mentioned were reported on. Please clarify.
Yes, we used this metric to assess DOM composition but will make this more explicit throughout the revised manuscript for clarity. We will ensure that all mentioned metrics will be reported on in the revised manuscript.

Line 173: Delete the word “above”.
This will be corrected in the revised manuscript.

Line 174: Either use “Notably,” or start the sentence with “These boundaries…”. Delete “It has to be noted that”
This will be corrected in the revised manuscript.

Results:
Figure 2: The purple font is difficult to read in the inset. Is the black triangle the north direction indicator? Is there a significance of the symbol used for Site D in the main figure? That box with the criss-cross in it? Can that be a circular data point instead? Consider using a black outline for

the yellow starting point arrows. They cannot be read in the figure, in the caption, online, and in a printed copy. What is the elevation difference between Site D and the supraglacial stream?
This figure will be clarified with a) a color change, b) removal of the superfluous north arrow, and c) clarification about the location of hole D. The elevation difference is not included as it is not relevant (see response to R1 regarding this figure) but is less than the length of the transit pathway (see scale bar).

Line 200: Add a comma after “TC”
This will be corrected in the revised manuscript.

Lines 200-205: Please report on the results from the leachate DOC concentrations.
We did not report these as the concentrations do not represent anything meaningful in an environmental context. However, for completion we will report them at minimum in the supplementary information and in the revised manuscript we will address this consideration.

Line 207: Provide a definition of what unique molecular formulae are and/or include it as assessment criteria in the methods section.
This will be updated in the revised manuscript.

Lines 207-209: What does “no significant difference between sample types” mean? They all had the same what? And this suggests they were all the same in terms of number of formula assigned? What does that mean? How is it relevant? You can absolutely have the same number of formulae in a bundle of samples from the sample place or in different locations but completely different chemistry/chemistries. Please explain.
We agree that samples can have the sample number of formulae but very different compositions and did not intend to suggest otherwise in this paragraph. We will rephrase this in the revised manuscript.

Line 215: The use of the word “compounds” is confusing. Did you measure compounds or composition, etc. (see major revision comment)? This also shows up in Lines 227. 230, 239, 258, 260, 261, 264, 323, 325, 328, and 382.
This should be formulae / composition throughout and will be corrected in the revised manuscript.

Line 231: This is a good example of the limitations of composition analysis but seems like a random point to make here. Why is this stated here? Discussion section instead? Make the ties to introduction sections that point to microbial and algal blooms?
This section will be rephrased or moved to the discussion in the revised manuscript - it was included in the results section to avoid the presentation of new data in the discussion without presenting them in the results section.

Line 234: Same point for aglycone degradation product as the comment in line 231.
See response to the previous comment.

Line 236: What does “Aromaticity in dark ice was high” mean? Those values look low for aromatic nature (thresholds of AI are 0.5 and 0.67) but are greater than what was reported for the supraglacial stream. Please clarify and consider using more specific terms like “greater” instead of “high”. The word “high” is confusing. If it is helpful, put these thresholds in the methods section or further define them here.
This will be corrected in the revised manuscript.

Line 239: Molecular diversity is not the same as number of formulae. Please clarify.
We will use number of formulae throughout in the revised manuscript.

Table 1: Add “(DOC)” after “carbon” and consider adding the identifier “determined by FTICRMS analysis” after “composition” in Line 247. Based on the text, it seems like the biolabile information is missing from the table. Are these results all from Sites D and Q? Define all acronyms in the Table caption, not just RA and #, or point to the methods section for that information.
This will be corrected in the revised manuscript.

Line 267: Add a comma after the word “aromatic”
This will be corrected in the revised manuscript.

Line 266: What’s the definition of “significantly different” DOM compositions? Is this based on calculations or chemical characterization?
This was intended to refer to the significant differences reported in Table 1 and the clustering observed in Figure 3A, but we realize that the statement is ambiguous and should be rephrased in the revised manuscript.

Figure 3: Define “PC” in the caption. Point to the methods section for the acronyms and their definitions or provide them here. Are all these data from Sites D and Q?
This will be corrected in the revised manuscript. The data presented here were from sites D, Q and SI - this will be made clearer in the revised manuscript.

Figure 4: Is panel A really all molecular formulae assigned in all samples in the dataset? This looks wrong. Consider moving the word “aliphatic” in panel A to a different location, maybe near the dotted line at H/C = 1.5 on the right-hand side? The chemical character information “aliphatic, HUP, etc.” should be included in the caption. Typo for panel E in the caption, change that symbol, which likely was an automatic revision, to (E). The note about the two CHONS formulae may confuse readers that these might be outliers? Are they? Were they a part of a homologous series?
Panel A is the molecular formulae that were assigned in all samples – i.e. only molecular formulae that were assigned in every sample in the dataset are included in this plot, not all molecular formulae assigned across the dataset. The figure will be updated with the suggested edits in the revised manuscript.

Line 287: Be cautious here. The molecular backbone of DOM is CHO containing formulae, not CH, especially since this work doesn't consider hydrocarbon molecular formulae. Classifying CHO as a heteroatom group is incorrect and confusing. Correct the text in the methods section to match.
This will be corrected in the revised manuscript.

Line 293: Molecular diversity is not exclusive to number of formulae. Please correct.
We will use number of formulae throughout in the revised manuscript.

Discussion:
Lines 303-305: This sentence is repeated from the methods. Is it needed here? Please include any discussion of the comparisons across samples.
This will be corrected in the revised manuscript.

Line 308: Edit "DOC" to "DOM"
This will be corrected in the revised manuscript.

Line 310: Edit "concentrations or DOM composition" to "concentrations and/or DOM composition."
This will be corrected in the revised manuscript.

Line 329-330: What does "preferentially leached" mean?
With 'preferentially' here we mean DOM that has already leached from the surface debris and that will therefore not leach from the material again when preparing the water extract. We will rephrase this for clarity in the revised manuscript.

Line 341: Is this statement saying that one molecular formula accounts for 5.7-8.8 %RA of the dark ice DOM samples? How?
Correct – this will be elaborated on to clarify in the revised manuscript.

Line 360: Consider revising "…DOM pools, are hydrologically connected" to "DOM pools, may be hydrologically connected." Measurements of hydrologic connectivity provide results to support what is written, but it is only speculated that that information may come from molecular composition information.
Great point - we will revise accordingly.

Lines 380-382: Confusing and contradictory to what was stated in the previous sentences. What is the main message here? What is the definition of upstream contributions? Just DOM? Debris? Microbes? Processes?
Here 'upstream contributions' was intended to refer to snow melt or surface melt generated at locations above the sampling site. We will rephrase to make this clearer in the revised manuscript.

Line 382: Typo. Delete the extra period, ".", after the parenthesis after "…0.05 %RA)

This will be corrected in the revised manuscript.

Conclusions:
Line 390: The part about microbial communities is confusing/incorrect as there were no biology measurements on microbial communities. Please revise.
This will be corrected in the revised manuscript.