

## RC1: Anonymous Referee #1, 05 Apr 2024

Dear editor and reviewer,

We would like to thank reviewer #1 for their constructive comments and suggestions. Please find our response to each comment (in blue) below. 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.

Overall General:

- Please introduce research questions and hypothesis that guide through the text  
We agree with the reviewer that the research questions and hypotheses should be made clearer throughout the text and will focus on this when revising the manuscript.

Abstract

- Title is missing a preposition in the second line connecting the southern Greenland Ice Sheet  
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 a comment from reviewer #3 regarding the use of the term ‘exported pools’.
- L 18: Make sure readers not acquainted with glacial dynamics can follow your text, you could specify that the ablation season is connected to the boreal summer months  
This will be made clearer in the revised manuscript.
- L 18: Are bacteria only more active during that time or are they growing and colonising, the statement you give is ambiguous  
It has been shown that they are metabolically active and are growing (increasing in number and spatial coverage) during the ablation season - but data from other seasons are currently lacking. This sentence will be rephrased in the revised manuscript.
- L19: “the DOM may be exported”, was there any evidence that DOM is actively exported from the supraglacial ice, if so then you should use a stronger verb  
Yes, there is evidence in the literature that supraglacial DOM is exported, and this sentence will be revised accordingly.
- L24: What is meant here by supraglacial dark ice: biologically darkened ice? You should help readers not acquainted with all processes and particularities by taking them by the hand, especially for the abstract.

We indeed intended this to refer to biologically darkened ice but agree that this should be specified upon first mention. We will ensure that jargon is explained more thoroughly throughout the revised manuscript.

## Introduction

- General point: I am missing a general research question and hypothesis deduced here. Please try to make a clearer statement on your scientific goal. It would be nice to also have this question(s) mentioned in the abstract

We agree that the guiding research question and hypothesis got somewhat diluted during the internal revision stages. In the revised manuscript, we will make the general research question and hypotheses more explicit in the abstract and introduction and will refer to them directly in the conclusion.

- L59: Since aromatic composition DOM was found in glacial run-off, not all of the degradation of allochthonous aromatic DOM can happen during the transport to glacier surfaces.

Agreed - this will be rephrased in the revised manuscript.

- General Comment Introduction: Indeed, glacial runoffs are known to show a “dual” source with both aromatic allochthonous and biolabile autochthonous DOM sources. Potentially the aromatic allochthonous fraction would be expected to be more susceptible to photodegradation, while the autochthonous fraction more susceptible to biodegradation. Did you find any evidence of production of Carboxyl-Rich Alicyclic Molecules (CRAM) from algal DOM in any other studies of glacial ecosystems (14-C young aromatics then), this could help to decipher that parts of the allochthonous aromatic DOM could in fact be autochthonous as well. Did you investigate 14-C ages in your samples? If not you can have a specific look on the polycondensed aromatic fraction in your FT samples to gain insight if a high number of black carbon-like, likely allochthonous molecular formulae exist in your samples, if so they could likely be highly susceptible to photodegradation.

We have not assessed CRAM in our data - and did not specifically evaluate black-carbon-like formulae, but report condensed aromatic %RA. In depth evaluation of supraglacial black-carbon material is currently lacking and is the topic of ongoing and future research. Supraglacial radiocarbon data are limited to supraglacial streams and range from -350 to 23 ‰, and to our knowledge radiocarbon data associated with supraglacial algae or algal DOM do not yet exist. We will include a sentence on the susceptibility of autochthonous OM to photodegradation in the revised manuscript to address this.

- L70: Upon draining of DOM to the glacial bed where any studies performed that investigated how supraglacial DOM was transported in the glacial bed, were there any interactions and exchanges? I guess it is unlikely that supraglacial DOM behaves inert when passing englacial and subglacial systems. You could also review these sources.

To our knowledge there are no studies investigating the transformations of DOM en route from the glacier surface to the glacier bed or to the glacier terminus, likely due to the difficulties in accessing the englacial and subglacial environment. Yet, given the previously reported differences between supraglacial stream DOM and glacial runoff DOM, it is reasonable to assume that subglacial processes change the DOM composition through additional inputs and degradation. This will be made clearer in the revised manuscript.

## Materials and Methods

- L83 please define “small” by estimate or accurate metrics  
Dimensions will be added in the revised manuscript.
- L85 was the auger hole freshly produced? How long did it take for auger holes to refill?  
The auger hole was drilled in at 07:00 local time on July 28, 2021. Refill rates varied throughout the day, ranging from 24 seconds to 117 minutes.
- L90 times given are local time? Why was not timestamp given for the sampling of Q  
Correct, these were in local time, which will be made clearer in the revised manuscript. Sampling of Q also took place at 14:00 - we understand that phrasing was unclear, and this will be updated in the revised manuscript.
- L90 it should already be clearer what this Q is without having to inspect Figure 1  
Agreed - this will be made clearer in the revised manuscript.
- L93 last comma replaced by “and”  
This sentence will be edited for clarity in the revised manuscript.
- L94 I wonder that SPE samples were stored in simple PC bottles, while DOC aliquots were stored in PTFE / glass bottles. The SPE samples will be considered more delicate than the DOC aliquot, could you elaborate on this. Did you conduct process blanks for SPE samples with FT analysis (also including the GF filtering procedure)?  
We stored the samples for DOM extraction in acid-cleaned polycarbonate bottles as these have been shown to produce minimal DOC contamination ([S0043135498004072](#)) and are lighter and less fragile than glass bottles. Given the limited sample volume available and the amount of sample required for DOM extractions, we could not collect the DOM samples in duplicates and therefore it was essential to prevent breakage of sample containers. As DOC analysis requires much less volume than DOM extraction (in the case of glacial samples), we were able to collect duplicate samples to minimize potential sample loss as a result of glass breaking in transit from the ice camp to the laboratory in Germany. We used MilliQ water to conduct a process blank for DOM, including filtering and extraction, and formulae with a relative abundance >0.1% in the procedural field blank were removed from the dataset (see L182 in section 2.8).
- L96 replace “home” by something more clear (where)

This will be updated to the specific laboratory (GFZ Potsdam).

- L96 Which analysis did filter retentates (surface debris) undergo, it came out of the blue in the M&Ms and results should be mentioned in the abstract and POM should be discussed in the introduction

POM is not included in the introduction as we did not perform analysis on the POM. We only used the surface debris to generate a cold-water extract to represent the portion of DOM that might feasibly be released from the surface debris on the ice sheet surface, where it is in contact with cold water. We will make this clearer in the abstract and introduction of the revised manuscript.

- Figure 1: Minimap (A) has weak contrast, the box study area is not well readable, northing information is missing. Statement on the used basemap is missing; Map B is fairly too zoomed in, it doesn't become clear what is shown (valley, slope, hill crest) maybe use the drone image underlain by additional geomorphologic information that make the sampling information clearer; The "field site" was not found by me on any of the maps; The categorisation of ice types shown in the legend does refer to illustration F only (?), how can you suspect this illustration to be true, is it clear that the depth of layers has roughly these dimensions- was ground trothing carried out by digging a snow / ice profile? Dimensions in panes C,D,E and F are missing.

The color of the map will be changed in the revised manuscript. The field site is marked in panel A by a black square and coordinates are provided in section 2.1 as the use of additional information in Figure 1 would limit readability, but we will add additional information to the figure caption for clarity. A digital surface model to show 'valley, slope, hill crest' was not included for clarity and conciseness of the figure, but it may be added in the revised manuscript or revised supplementary information. Scale bars will be added to panel C-E. Panel F is an exemplar schematic which intentionally does not include scales – the depths of the layers are variable and were not confirmed (although water table height is included in the results and hydrological modeling). No ice or snow pits, note that this is the snow free ablation zone, were dug. Panel F simply presents a conceptual model to help the reader visualize the supraglacial hydrology discussed in the manuscript.

- L 108: Calculations based on Stevens 2018: please elucidate more on this  
These calculations are described only briefly in this manuscript as they are a) already published, b) described in the supplementary information and c) would add considerable length to the manuscript detracting from its main research story. We believe that suitable reference is provided for interested readers to further explore the detail, nuance, and evaluation of the point hydrological methods used - an approach which was also applied by Stevens in the following papers, where it was deemed acceptable: [s43247-022-00609-0](#) and [s41467-021-24040-9](#). We will add a clearer reference to the supplementary information, which is currently located at the end of this paragraph, in the revised manuscript.

- L118: Can you elucidate from the literature how Milli-Q extracts might influence the DOM yield compared to other extraction techniques  
 Some of the co-authors are currently working on POM extractions that include other extractants. However, we did not include a discussion on this in the manuscript as we, for this dataset, are only interested in the DOM that might be ‘extracted’ from surface debris by supraglacial meltwater (cold water) alone, rather than in a full characterization of all DOM that might be extracted from surface debris using more elaborate chemical methods that do not represent environmental conditions.
- L 120: “wwPTFE” = PTFE |Typo?  
 This refers to water-wettable PTFE filters as stated on the filter packaging. We will change it to hydrophilic-PTFE to in the revised manuscript to avoid confusion (most PTFE is hydrophobic).
- L123: Usually such products are called water extracted organic matter (WEOM)  
 “laboratory leachates” could also be column leachates asf.  
 We will change this to water soluble organic matter (WSOM) in the revised manuscript.
- L128: The high temperature combustion technique doesn’t need to be described with this detail, it appears to be a standard  
 This section will be condensed in the revised manuscript.
- L 136 – 137 see comment on L128  
 This section will be condensed in the revised manuscript.
- L 141: see comment L 96  
 This will be updated to the specific laboratory (GFZ Potsdam).
- L 149: please indicate what these supplementary methods are roughly about  
 This will be elaborated on in the revised manuscript.
- L160 and L163: Since Equation 1 and 2 are standard and published, citations will suffice instead of formulae  
 This section will be condensed in the revised manuscript.
- L 165 f.: I highly recommend changing the naming of your “composition groups” to a less ambiguous naming. The current naming implies structure which cannot be determined by mass spectrometry. A less ambiguous nomenclature is presented by Merder et al. 2020 (<https://dx.doi.org/10.1021/acs.analchem.9b05659>) Table 2 in the Supplements, which is also co-authored by some of the Co-Authors here.  
 Most studies presenting glacial DOM data relevant to our manuscript use these classifications, enabling some degree of comparison between our data. The current naming is not intended to imply structure (e.g. peptide-like) and is broadly considered

conservative. We will make it clearer that we refer to '-like' formulae in the revised manuscript as to avoid ambiguity regarding structural information.

- L 171: This sentence can be deleted; I expect you wouldn't act in the opposite way. If this is true on the other hand, is not proven by your sentence, you would need to argue this in the introduction justifying your selection of metrics by citations. Further metrics could be tested such as IDEG (Flerus et al., 2012) or ITERR (Medeiros et al., 2016) and the investigation of pcARO could also function as metric/  
This section will be updated to reduce ambiguity and include the relevant citations.
- L 176/177: Can you present any insight into the actual biolability of purpurogallin as an empiric measure for your discussion. It doesn't become clear to me what you imply here  
Example: An essential amino acid like tyrosine contains an aromatic ring structure and is widely considered biolabile: If the classification of biolability is closely connected to refractory characteristics of structures, this discussion capsizes when conditions of biological decomposition are not made clear or no empirical landmark on actual degradability is given.  
This section will be updated to reduce ambiguity and include relevant citations, including to Perini et al 2023 ([s00248-022-02033-5](#)) who report the biodegradation (removal of the sugar moiety) of the purpurogallin pigment in fungal incubations.
- L 180 2.8 Statistics: Besides homoscedasticity did you test for normal distribution and were the sample sizes evenly distributed. With heteroscedasticity, non-normality and uneven samples sizes (which is the common case in geosciences) metric testing becomes less and less trustworthy. Please add normality testing, histograms and sample sizes to your supplements. In case of multiple violations of prerequisites for metric testing consider either multiple non-parametric testing e.g. with package "multcomp" (Bretz et al. 2011 ISBN 9781584885740) or Box-Cox transformation of data prior to ANOVA  
Normality testing, histograms and sample sizes will be added to the supplements of the revised manuscript as suggested by the reviewer.
- Figure 2 and section 3.1: Figure please add DEM data to the figure, here the viewer can just see blurry white with a large pixel size. I also wonder, what is the margin of error for your 9 days of travel time. The resolution of the orthophoto indicates that there are several potential travel passes that might occur for a single particle with certain likeliness  
This figure demonstrates velocity of water flow in the weathering crust. A DEM represents the bare earth surface only, not natural features (such as an ice sheet) and would not add relevant information to Figure 2. Adding a DSM would also be redundant here as a combination of the water table and hydraulic conductivity of the weathering crust determines water flow paths along the hydraulic gradient, and this is is not necessarily the same as surface slope which would be shown by a DSM. We can include a DSM and water table map in the supplementary information if needed, but in our opinion these should not be in the main text as they are data that contribute to the

final processed product presented in Figure 2. Regarding uncertainty, the hydrological modeling approach used combined with unrepeatable point measurements means that we are not able to provide a meaningful uncertainty estimate, and these data should be viewed in that context.

- L 230: I like how you follow these single formulae through your dataset, I imagine it might be interesting to produce a figure from this finding and also underlie it with some of your matching metrics. Since purpurogallin should absorb light, maybe you could also add some UV absorbance values if you still have some back-up sample to analyse- these might match. The current way of pure text and numbers presentation is making it hard to follow these exciting insights  
This is a great suggestion, and we will aim to include a figure to summarize these findings in the revised manuscript. Unfortunately, we do not have any sample available for UV absorbance analysis, but we agree that this would be a great addition to future work.
- L 239: There are dozens of definitions on diversity, please specify which diversity you refer to  
In the revised manuscript we will avoid the use of 'diversity' and will stick to 'number of formulae' to avoid confusion.
- L 249: Lettering indication should be self-explanatory please delete starting from "where values that have...."  
This will be updated in the revised manuscript.
- Figure 3: There are  $n = 17$  samples and  $n =$  explanatory variables. The low ratio of sample to variable (1.21) suggests that the PCA model is not as selective as it could be. Please check your variables for collinearity and make sure to remove collinear variables. I am missing the Eigenvalue {Variance} / Component documentation, please add this to your supplementary data.  
We will double-check this and be sure to include details, including Eigenvalue {Variance} / Component documentation in the supplementary information of the revised manuscript.
- Figure 4: The pane lettering in A-F is necessary but it would help the readership to also name the sample type above the pane. Since van Krev. plots are always prone to overplotting please consider scaling the point size to %RA. You could also specify in the plot what is % RA of the shown formulae versus the excluded formulae to specify not only diversity but also intensity  
We will update the pane titles to be clearer. However, in our experience the scaling of point size to %RA causes overplotting to be worse instead of better and was therefore not used in presenting this dataset.
- Figure 4 vs Table 1: how do values in Table 1 correspond to Figure 4: Since you conducted a perfectly interesting subtraction technique for Figure 4 it would be nice to

append a table 2 with the respective metrics (as indicated van Krev. are overplotted and usually not as insightful)

We will update Table 1 accordingly in the revised manuscript.

- L 276 there is a € instead of (E)

Thank you for spotting this - this will be corrected in the revised manuscript.

## Discussion

- General: In the discussion you present a large number of metric data (x +- y % ) asf. Please try to limit these numbers to an absolute minimum and rather state the trends and significant differences from your results by rephrasing them in words. This will make it easier to follow the arguments. Also please try to discuss one thought in one paragraph only. It might be a good idea to enter subheadings above paragraphs to make clear which idea is discussed at the moment

We agree that the number of metric data in the text should be reduced, and will streamline the general structure of the discussion in the revised manuscript.

- 4 Discussion: Instead of descriptive heading for 4.1 and 4.2 I would be very happy if you could include your research questions into the headings
- This will be incorporated in the revised manuscript.

- L 309: The whole section about the problem of lysis is too prominent in my eyes. You use very much space to discuss a potential artefact that you then rule out in the end did probably not happen at all or have no effect. Please shorten here. The readership should learn more about what the data tell you about potential processes here

This section will be condensed in the revised manuscript.

- L338 how impactful can the two described degradation pathways of viral infection and fungal attack be. Undoubtly, they will have effect on the composition, but I would expect the effect to be much smaller. Also if I understand correctly, you identified the sample by the algal pigmentation visible as dark ice? So there was no tremendous viral induced loss of pigmentation

We suggest that fungal infection can result in the loss of pigment from algal cells (note that there is no current evidence of viruses attacking glacier ice algal cells and we do not suggest this as a mechanism). Work by Fiolka et al (2021, [s41598-021-01211-8#Sec22](#)) investigated a biologically darkened ice surface and found that approximately 25% of all cells in the sample collected from this biologically darkened site were infected with parasitic fungus. Hence, we argue that it is reasonable to expect a contribution of pigment leaching to the surface ice DOM pool. We will rephrase this argument for clarity in the revised manuscript.

- L340: The NOSC metric could be used to maybe hint into the same direction.

This will be considered in the revised manuscript.



- L344: A similar study was following DOM from source to sink in a closed alpine system. there is clear indication of photodeg Part 4.3 <https://doi.org/10.5194/bg-20-3011-2023>  
Interesting! We will include this reference in the revised manuscript.
- L345: Especially dark ice could also shield lower lying aromatics from sunlight and subsequent photodegradation by the low albedo of overlying aromatics. This is also shown for ocean darkening by various indicators  
<https://doi.org/10.3389/fmars.2020.547829>  
Yes, shielding is likely to also play a role in the supraglacial ecosystem.
- L 350 I would also suggest shielding of underlying aromatics  
This will be included in the revised manuscript.
- L362 here it would of course have been nice if you had carried out some photodegradation experiments with your samples to track this pathway  
Agreed! We hope to include photodegradation experiments in future studies to improve our process understanding of biogeochemical cycling during supraglacial transit.
- L365 here also lysis products might accumulate  
Correct - this will be included in the revised manuscript.
- L369 treat EPS as plural pls.  
Thank you for spotting this - this will be updated in the revised manuscript.
- L 373, that is an important recommendation, but it is very big in the light what a single paper can achieve, that why it would be better to ask this in the form of questions and to mark knowledge gaps more precisely  
Great point - we will make the specific questions to be addressed more explicit in the revised manuscript.
- L382 but the, if the stream is not sharing a large amount of DOM composition and not showing mixing, how can you attribute it to be a connector of pools as happened in 3.3, then the outcome must be that pools are distinct and not connected by continuous flow, which can make sense with low flow velocities  
This is incorrect. The stream does share a large number of formulae (see Figure 4). The 12 formulae mentioned in L382 are the only formulae that were assigned in all stream samples, but not in any other samples (i.e. uniquely assigned).
- L382 double “.”  
Thank you for spotting this - we will fix this in the revised version.
- L 377 f., the last paragraph comes without any reference to other scientific works, please try and also discuss these findings in the light of existing literature

The discussion needs some restructuring to focus on one argument per paragraph as mentioned in a RC above. We will align the discussion with the research question and hypothesis more explicitly in the revised manuscript.

- General question: how do you assess the different contributions of bio- and photodegradation in your sample set. I would like to see a clearer statement on which pools are to what extent affected by what  
We will make this distinction clearer and more explicit in the revised manuscript.

#### Conclusion

- General: I would prefer to also see a connection to research questions in this chapter  
We aim to make the research question and hypotheses more explicit in the abstract and introduction and refer back to them directly in the conclusion.
- L 390 what exactly do you mean by “microbial communities” this implies that micros where somewhat investigated more closely then presented here  
We will rephrase this to be in line with what is presented in this dataset for the revised manuscript.
- L 392: You state distinct composition differences; this is where you should say what exactly you found instead of suggesting more research  
Agreed - we will update this in the revised manuscript.
- L 394 and 395 The citations of Niwano and Müller,Keeler could also be moved to the introduction, this rather seems to be a motivation for your study than something relevant to the conclusions.  
Agreed - we will move these citations to the introduction in the revised manuscript.