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April 2nd, 2025

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Dear Reviewer 2,

Brief Communications: Stream Microbes Preferentially Utilize Young Carbon within the Ancient Bulk Glacier Dissolved Organic Carbon Pool.

Amy D. Holt, Jason B. Fellman, Anne M. Kellerman, Eran Hood, Samantha H. Bosman, Amy M. McKenna, Jeffery P. Chanton & Robert G. M. Spencer.

We sincerely thank you for your positive feedback and helpful comments on our manuscript. Please find below a detailed response to your comments with proposed revisions. Your comments are in italic and our response in regular text. Line numbers refer to the original posted manuscript. We hope with these refinements and additions you now find the paper suitable for publication in The Cryosphere: Brief Communications.

Response:

General comments: This paper uses multiple analytical techniques to characterize the pool of dissolved organic carbon in the outflow of three Alaskan Glaciers, and in a supraglacial stream of one of those glaciers. The authors pair these measurements of glacial DOC with bioincubation experiments and measure the isotopic signature of respired CO_2*. They conclude that the young and aliphatic-rich portion of the DOM pool is most bioavailable. The paper is generally well written, and the suite of measurements provide valuable insights into glacially-sourced DOM cycling, which is of appropriate scope for The Cryosphere. However, there are questions/comments detailed below regarding conclusions drawn from this experimental design, which lacks controls, replicates, and measures of uncertainty. These issues should be addressed, and conclusions carefully articulated within the limitations of the dataset.*

We thank the Reviewer for their positive feedback, we have addressed your concerns below.

Specific Comments: Figure 1: this figure could be significantly improved so that each glacier is visible. Consider using a satellite image (from Jul, or ideally close to the field sampling period) and zoom in enough so that precise sampling locations are visible relative to the glacier terminus/proglacial lake/stream network.

We thank the Reviewer for the advice and suggestion. We would like to change the map to that below:

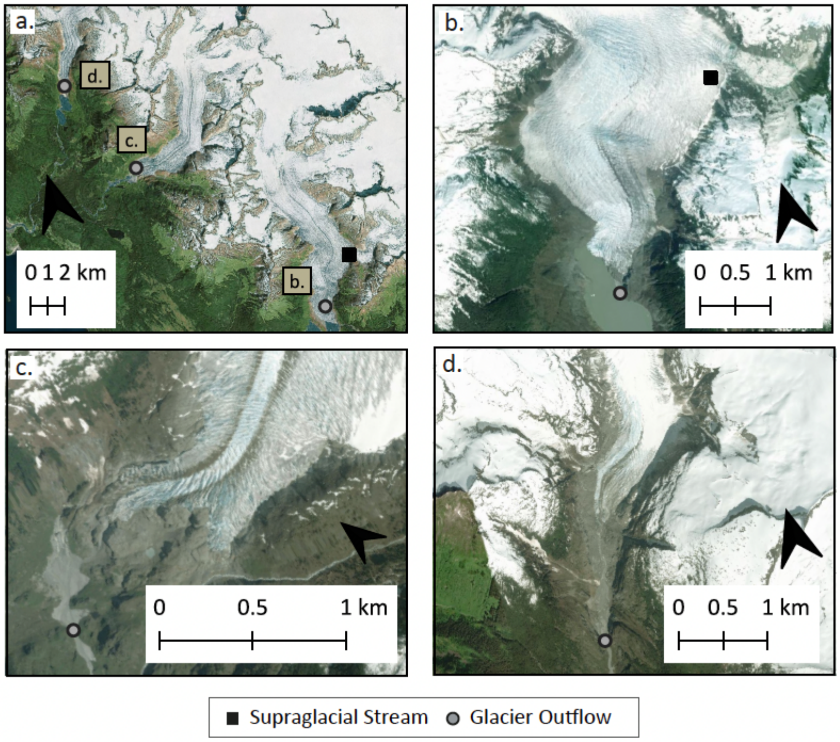

Figure 1: Location of July 2022 sample sites within (a.) the Juneau Icefield highlighting panels (b.-d.), and (b.) Mendenhall Glacier, (c.) Herbert Glacier and Eagle Glacier catchments. Map data: Bing Satellite.

Section 2.1: This section would benefit from a more detailed description of these glaciers, including comments on their potential subglacial hydrology and geology, and biogeochemical context from previous work.

Thank you for the suggestion. We propose the following edits and additions to the site description:

"Water samples were collected between the 11th and 14th of July 2022, from three glacierized watersheds in coastal southeast Alaska (Figure 1). The study area is situated within the Juneau Icefield, in the coastal temperate rainforest and has a cool (annual mean temperature 5.6°C), maritime climate, with the majority of precipitation falling in autumn and winter (Behnke et al., 2020). The geology of the upper watersheds, where the glaciers are found, is dominated by Tertiary-Cretaceous aged, foliated tonalite sill of the coast plutonic complex (Wilson et al., 2015). These glaciers are well studied and are known to have discharge regimes and biogeochemical characteristics representative of glacial systems throughout the Gulf of Alaska. These glacier rivers are highly turbid, with low summer temperatures (<5°C) and oligotrophic conditions (Hood and Scott, 2008; Fellman et al., 2014; Spencer et al., 2014). Based on past observations of conductivity, at the time of sampling, water residence times within the glaciers are short (~hours), and subglacial drainage is relatively efficient, with the vast majority of meltwater having a supraglacial origin (Spencer et al., 2014).

Water samples were collected from the surface and outflow of Mendenhall Glacier, as well as downstream (~ ≤1 km) of the terminus of both Eagle and Herbert Glaciers. Between the glacier terminus and sampling sites glacier outflow rivers flowed through recently deglaciated terrain (i.e., barren ground of cobble, gravel and glacier silt, with few colonizer plants) and thus there was limited potential for OC inputs from vascular plants and soil organic matter. At Mendenhall, outflow sampling was conducted on a rock/silt bar <100 m from the glacier outflow. Water here is extremely turbulent and flows rapidly into Mendenhall lake. As such, there is limited influence of the lake water, and sampling is representative of water exiting the glacier. The supraglacial sample was collected from a small (<1 m across) flowing supraglacial stream on the bare ice surface accessed by helicopter, ~ 3 km upslope from the glacier terminus."

Line 77: provide more details on where the supraglacial sample was collected. Improvements to Figure 1 will help, but it's worth noting here that it came from a supraglacial stream (which you mention in results). Knowing where was collected relative to the terminus/snow line has implications for interpreting DOC sources.

Thank you and in line with your previous comment, we suggest adding the following to text:

"The supraglacial sample was collected from a small (<1 m across) flowing supraglacial stream on the bare ice surface accessed by helicopter, ~ 3 km upslope from the glacier terminus."

Line 77: describe where you collected your outflow samples in each catchment. This is especially important for Mendenhall since it terminates in a proglacial lake. Understanding the location relative to local/micro-environments and non-glacial stream influence is relevant to water/DOC source, and residence/transit times. This would also be a good spot to describe the timing of your sample collection in relation to the seasonal evolution of the glacial hydrology and ultimately theoretical water/DOM sources. Do you expect the outflow at this time of year to represent an efficient drainage system with little influence from subglacial sources? If so, that means this paper is not really looking at the 'bulk glacier DOM pool' as the title suggests, and is instead looking at the lability of predominantly supraglacial DOM.

Thank you for the suggestion. As described above, we propose to add details of sampling location and glacier hydrology to text. We also suggest changing 'streamwater' to 'water' in the methods and throughout, in order to not mislead the reader as to the nature of the water samples at Mendenhall.

To clarify the meaning of 'bulk' here, this refers to the total DOC pool of samples, in order to distinguish it from the fractionated or respired component. As indicated by the Reviewer, we do intend to refer to the typical composition or all the possible compositions of supraglacial and outflow DOM. We suggest removing bulk from the title and from line 36 where the meaning may be confused. Nonetheless, given our sampling was focused on months of peak melt, we did capture DOM composition at the peak of the DOM flux from these glacier environments. In this way, sampling was designed to be representative of the period of time where the majority of DOM and bioavailable DOC export occurs. As such, we do maintain that these findings are broadly representative of these glacier outflows, although temporal variability could affect OC sources on a seasonal or annual basis (as acknowledged in text lines 233-240).

Table 1: report an indicator of uncertainty (including detection limits) for these analyses, here or elsewhere in the document. This is particularly important for the instances where you compare supraglacial DOM to outflow DOM.

Thank you for your comment. We propose to add the following details to the methods to clarify uncertainty:

"Concentrations of DOC were measured on a Shimadzu TOC-L_{CPH} analyzer following standard methods (Holt et al., 2023 and references therein). Before analysis, samples were sparged with air for five minutes at a flow rate of 0.08 L min^{-1} to remove DIC from solution. **Measured concentrations are based on 3 of 7 replicate injections with a coefficient of variance of <2%.** ^{13}C and ^{14}C were measured via isotope ratio mass spectrometry (IRMS) and accelerator mass spectrometry, respectively at Woods Hole Oceanographic Institution. For DOC isotopes, samples were UV-oxidized, and the resultant CO_2 cryogenically trapped for analysis. **$\delta^{13}C$ values measured by IRMS have a typical precision of <0.2‰ (Xu et al., 2021).** Estimates of the contributions from radiocarbon dead (-1000 ‰) versus modern (95 % of ^{14}C concentration in 1950 of NBS Oxalic Acid I normalized to $\delta^{13}C_{VPDB}$= -19 ‰) OC were calculated from fraction modern (F_m) values (Table 1), where the percentage of radiocarbon dead material was determined as 1-F_m (Stubbins et al., 2012). **Measurement error on F_m values ranged from 0.0017 to 0.0025 (Table 1), making little quantitative difference to calculated values (i.e., ‰ and yBP) and estimated source contributions."**

To clarify, the comparisons made between the surface and outflow composition are appropriate given these uncertainty levels. We maintain that molecular-level composition is relatively similar. For example, the relative abundance (RA) of aliphatic compounds between samples ranges from 21.0 – 34.2%. For comparison, past studies of global glacier outflows demonstrate this range is 8.6 – 71.5 %RA (Holt et al., 2024). Similarly, the isotopic composition of supraglacial respired OC was far younger (-30.8 compared to -146.2 – 232.9) and ^{13}C enriched (-15.5 compared to -18.3 - 19.5 ‰; Table 1), considering the uncertainty of measured values.

Figure 2: (a) and (b) are unnecessary since the same data are presented more efficiently in (c)

Thank you for the suggestion, we propose to remove panels a and b. We simply presented them in this way originally to aid accessibility and introduce stable and radiocarbon isotope data independently, but we are happy to remove them.

Section 3.2, line 157, line 210: given the increasing evidence for subglacial chemotrophic pathways, it's worth commenting on the likelihood of these sources of DOM to the outflows, especially whether the subglacial environment may be a source of old but labile DOM. Additional information on the subglacial hydrology/geology in section 2.1 will help frame whether these systems are conductive to chemotrophic DOM production. Are there previous studies at your sites that would justify ruling this source out? Perhaps this is what you're referring to in line 228, but I think it warrants a more thorough discussion than the statement in brackets on line 209-210.

Thank you for your comment. Previous studies at these sites have found no evidence for subglacial inputs at this time of year (peak melt), as indicated by similarity in isotopic signature and DOC concentrations and composition, and between the surface and outflow (Spencer et al., 2014; Stubbins et al., 2012). As explained in the text, we also note broad similarity in molecular and isotopic composition between outflows and the surface sample, indicating minimal inputs from aged subglacial sources and likely a predominately supraglacial origin of biolabile organics at the time of sampling. Although we cannot, and do not, rule out the possibility of some aged material originating from the subglacial environment, more research is required to validate this as a bioavailable carbon source at this location.

To reiterate, we note the dominant source of respired OC is young and ^{13}C enriched, with minimal inputs from aged OC regardless of origin (i.e., supra- or subglacial). We have noted the potential for subglacial OC contributing organics within the manuscript and this was meant to encompass subglacial microbial processes. Nonetheless, we propose to further clarify this as suggested below:

1. "Additionally, the relative similarity in molecular and isotopic composition (DOC and respiratory CO_2) between the supraglacial stream and the glacier outflow samples suggests that a portion of the bioavailable organic carbon exported from these glaciers originates from recent in situ production on the surface rather than subglacial sources (Table 1, Figures 2 & 3). However, subglacial sources such as paleosols and microbial chemotrophic processes could still contribute organic carbon to the outflow DOM pool."
2. "In outflows, ancient DOC compounds could also be derived from aged ice-locked, and subglacial sources (e.g., paleosols and microbial chemotrophic processes), although compositional and carbon isotopic evidence to support this conclusively is yet to be observed for Alaskan glaciers…."

Line 184-187: Without error bars or statistical analysis (Figure 4), I'm not sure you can claim these associations are significant or therefore make a justified link between the 13C and 14C signatures to the aliphatic content. Further, since the DOM characterization is not truly quantitative, RA was not measured before and after the incubation, and you do not present CO2 concentrations or quantify the DOC consumed during the incubation, I think it's a stretch to claim that "the 13C and 14C enriched source respired during the incubation may be relatively more abundant in the more aliphatic samples" and then extend that further in the abstract to say "Molecular-level analyses suggest respired OC was associated with the aliphatic-rich portion of the DOM pool".

Thank you for the comment. To clarify, we do not make the case that these trends are significant. Given the small number of samples, we agree statistical analysis is not appropriate and thus we cannot make the claim that the trends are significant. As explained in response to Reviewer 1, we have deliberately chosen not to quantify this shift, or statistically evaluate the trends, as to not mislead reader. This is why there are no regression lines or statistical analyses presented in Figure 4 or the text. We feel this is the appropriate caveat for the data presented in Figure 4. Although small in size, this data remains very interesting, and we hope that it acts as a first step in quantifying the drivers of bioavailability and organic carbon respiration in glacier ecosystems as we have noted in text (e.g., lines 238 onwards). This is one of the reasons we think this is a great dataset for a Brief Communications article to get this into the community, stimulating more research on this topic.

To make this clearer as suggested, we propose further refinements to the sentences discussing aliphatic content and $\delta^{13}C$-CO_2 in order to not mislead the reader:

1. Remove the sentence discussing molecular data from the abstract.
2. Edit the results and discussion as follows:
    a. Enriched $\delta^{13}C$-CO_2 values may be associated with an increased %RA of aliphatic compounds in the initial DOM pool, and in the case of the supraglacial stream appear to coincide with substantially younger CO_2 (Figure 4). Given the small dataset ($n = 4$), it is unclear whether this represents a compositional trend. However, the data suggest that the ^{13}C and ^{14}C enriched source respired during the incubations could contain a greater relative abundance of aliphatic compounds."
    b. "Furthermore, the supraglacial stream had the most aliphatic-rich DOM and the most ^{13}C and ^{14}C enriched CO_2 (Figure 2, 3 and 4). This is consistent with autochthonously sourced aliphatic organic matter (Musilova et al., 2017), and further suggests that respired organics were derived from in situ production."

Section 3.4: Do you know that there were no inorganic processes affecting CO2 concentrations/isotopic signatures, despite the 0.45 um filter? Glacial flour can have an ultra-fine component with high surface area, so it's theoretically possible. It appears you did not collect CO2 concentrations or DOC after incubation, which is a shame since that would allow for a simple mass balance calculation and help isolate/confirm the process(es) at play. An experimental control would have also addressed this issue. In the absence of these data and a robust experimental design with controls, I think you need to somehow make your case that inorganic processes and/or contamination are not contributing CO2 during incubation.

Thank you for your question. To clarify, samples for RCRS bioincubation were collected, filtered (0.45 µm), acidified (pH 2) and stored frozen. This is common methodology for DOC and DOM analyses of glacier waters, despite the high turbidity and fine glacier flour in glacier outflows. We did not observe any glacier flour in the samples after filtration, collection or when samples were thawed. As noted to Reviewer 1, we also did not observe any flocculation. Samples for RCRS incubation were acidified and sparged to remove inorganic carbon (IC) from solution before the bioincubation was started. Therefore, any IC (including those within any glacial flour <0.45 µm) would also be removed. As explained in response to Reviewer 1, an experimental control does not produce DIC as there is no active metabolism of organics. Therefore, we cannot send anything for isotopic analysis as there is no DIC produced and thus no CO_2 to capture. Furthermore, the observed isotopic shifts for glacier outflows and supraglacial samples were similar (Figure 3). Given surface samples have negligible glacier flour, this further indicates that any potential glacier flour and its influence on IC is not an issue. Additionally, all incubation containers were gas tight, using septa that are routinely used for the study of DIC and gases such as methane. Given their frequent use and testing, we have no reason to believe that atmospheric gases would be introduced into the gas-tight incubation bottles. Finally, as also noted to Reviewer 1, there was good recovery on a DIC standard (i.e. same concentration of DIC in solution and captured CO_2) and the vacuum lines are kept under vacuum and so any contamination associated with the lines during extraction would also be obvious. Taken together, this highlights the data presented is in no way an artifact of methodological design. We sincerely hope this alleviates the Reviewer's concern.

We thank you for your helpful comments and time spent on this manuscript, we hope, if the changes outlined here are adopted, you feel it is now suitable for publication.

Yours Sincerely,

Amy D. Holt

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