Authors' responses to reviews; replies and changes are provided in italics.

Age and Chemistry of Dissolved Organic Carbon Reveal Enhanced Leaching of Ancient Labile Carbon at the Permafrost Thaw Zone

Karis J. McFarlane[1], Heather M. Throckmorton[2,3], Jeffrey M. Heikoop[2], Brent D. Newman[2], Alexandra L. Hedgpeth[1,4], Marisa N. Repasch[1], Thomas P. Guilderson[1,5], Cathy J. Wilson[2]

[1]Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, Livermore, 94550, USA
[2]Earth and Environmental Sciences Division, Los Alamos National Laboratory, Los Alamos, 87545, USA
[3]Currently at Agilent Technologies, Lexington, 02421, USA
[4]Department of Geography, University of California, Los Angeles, 90095, USA
[5]Currently at University of California, Santa Cruz, 95064, USA

Correspondence to: Karis J. McFarlane (mcfarlane3@llnl.gov)

RC1

General comments:

This is a very interesting study and a nice dataset. While the ultimate conclusions of the study are not especially wide reaching, this is an interesting dataset and what appears to be a robust and interesting analysis. The manuscript is quite short, not necessarily a bad thing, but the introduction is quite long relative to the actual results/discussion. The discussion wouldn't be harmed by the addition of a bit more of a deeper discussion and exploration of wider implications.

It would have been nice to see the introduction focus more on studies that have considered headwater catchments specifically in the Arctic as there are a few in the literature now, although still lacking as noted here.

But overall, I don't really have any substantial criticisms of the manuscript other than the relatively minor points raised below. This is a sound study with some interesting and unique data adding to the relatively sparse number of studies looking into radiocarbon export in headwater streams in the Arctic.

-Thank you for your review and suggestions for improvements to the manuscript.
As pointed out in the reviewer comments, this is a small study and while we have added these additional studies (and others to our references, introduction, and discussion, we do not extensively expand on the discussion in our revision.

Specific comments:

L52, 56. Permafrost "thaws" rather than melting. *Done.*

L176. The Neff and Wild studies are of larger Arctic rivers, a better comparison would be other studies that look at DO14C in headwater catchments, e.g. https://doi.org/10.1088/1748-9326/aaa1fe , or see https://doi.org/10.1029/2020GB006672 for more 14C-centric studies. *Great suggestion, we do wish to point out that this trend is present in both headwater catchments and larger rivers, so this sentence now reads "…reported for Arctic headwater catchments (Dean et al., 2018) as well as Arctic rivers and their tributaries (Neff et al., 2006; Wild et al., 2019)." We have added also referred to the Dean et al 2018 paper to the Introduction: "For example, inland headwater systems showed an increase in the age of DOC seasonally as deepening of the active layer mobilized older soil C (Dean et al., 2018)." We also appreciate the suggestion to look at the Estop-Aragones paper and have added references to this paper in the introduction in two places where it was helpful.*

L225. It is very hard to see from Fig 4 how the correlation between DO14C and CH4 is positive, particularly for the surface samples. The surface samples look like a straight vertical line, were these log-transformed for the correlation? The positive relationship between DO14C and CH4 in the shallow samples look driven by a clustering of higher CH4 concentrations in July (circles), it would be useful to justify why you can group by timing in the correlations here but separate by timing in other places, e.g. Fig 2. *We see your point and agree that for the surface and shallow pore waters the correlation analysis isn't appropriate. The sentence now reads: "In surface and shallow pore waters, both DOC Δ14C values and CH4 concentrations decreased from July to September (Fig. 4a, p = 0.04) but DOC Δ14C values were not correlated with any of the other measured variables." The next paragraph discusses the deep porewater.*

R2

General comments:

The study presents porewater data for DOC/TN concentrations, SUVA values and 14C dates of DOC in July and September from drainages in Alaska. Authors relate their observations with leaching of DOC during the seasonal thaw that is labile. The introduction reviews general findings from literature and mentions concepts to characterize DOM composition (aromaticity, molecular weight, aliphatic compounds-microbial processing, vegetation-derived DOM). These concepts of DOM composition need to be more clearly and directly related with the proxies that authors report (C/N ratios and SUVA values) so that the reader can follow why authors conclude about the lability of their samples.

-Thank you for your review and suggestions for improvements to the manuscript. We agree with your comments and have made the changes suggested here, including the additional references you've provided. You also make several important points that we address in the revision as we agree they improve the manuscript:

1) *Clarification of how we relate our measurements to lability of DOC. Thank you for identifying this issue. We have included in the revision a more developed explanation of how characteristics (SUVA and DOC:DON ratio) of the dissolved organic matter chemistry can relate to decomposability and the extent of microbial processing of organic matter. This addresses several comments (in the abstract, introduction, and results/discussion) related to how we interpret our data to suggest a highly labile DOC source and differences with age across the drained thaw lake basins. For the sake of clarity in this response, we interpret higher DOC:DON and SUVAs to reflect fresh vegetation inputs, which should have: 1) higher C contents relative to N (these ratios converge with microbial processing); 2) higher SUVA 440, indicating more high molecular weight compounds (which break down into smaller molecular weight compounds with microbial processing); and 3) higher SUVA 254 and 350, indicating greater aromaticity and lignin content – compounds that are present in plant C inputs and break down with microbial processing. We also connect these characteristics to biolability using references from the literature.*
2) *Additional context is provided for expected differences in DOC quantity and quality in different drainage types, including DTLB's. This is accomplished through revisions to the Introduction and the Results and Discussion sections, including the addition of new resources and additional focus in the Introduction on smaller catchments as had been requested by the other reviewer. We have added contextual information regarding how the DTLBs change with development into the interpretation of our results.*

Specific comments:

Abstract

Line 23 – Specify which "biogeochemical indicators" you mean to make the statement less vague. *We have added:* "… *(including dissolved methane concentration, δ13C, and apparent fractionation factor)….*"

Line 24-25 – Based on the data and its interpretation, it is unclear how authors conclude that the old DOC is highly labile. *We largely address this comment by revisions to the manuscript Introduction and Results and Conclusions sections to improve on the interpretation of the data. We did revise the following sentence in the abstract to help clarify, but as the reviewer noted, we did not directly measure biolability." C quality indicators reflected a DOC source rich in high-molecular weight and aromatic compounds, characteristics consistent with vegetation-derived organic matter that had undergone little microbial processing, throughout the summer and a weak relationship with DOC age."*

Introduction

Line 27 – Consider adding a more recent reference: https://bg.copernicus.org/articles/11/6573/2014/ *Done.*

Line 41 – Consider replacing "exceeds" for "may exceed" to not overgeneralize as not all data in the cited references support DOC export being greater than NEE. For example, the study of Billet 2004 shows DOC export is greater but the data in the study of Christensen 2007 is less clear and for the study of Roulet 2007, DOC export exceeds some years but NEE is on average greater. *We agree, very good point. Done.*

Lines 44-51 – The main message in this paragraph is that old DOC is labile thus relating permafrost thaw with potentially large C loss downstream. It may be also worth adding other studies that indicate that little old C via DOC seems to be mobilized or mineralized in thawing ecosystems - see references below:

https://onlinelibrary.wiley.com/doi/epdf/10.1111/gcb.15756

https://www.nature.com/articles/s41467-020-15511-6

This is a good point. The references provided for lability in this paragraph were all laboratory incubations, which we have now specified through minor edits to this paragraph. We've revised the final sentence in this paragraph as follows: "In fact, increasing terrestrial DOC loads have been linked to increased CO2 emissions from aquatic systems (Lapierre et al., 2013), though field studies suggest biological activity in Arctic aquatic and anoxic systems may be fueled largely by modern C (Dean et al., 2020; Estop-Aragonés et al., 2020; Tanentzap et al., 2021)." We've added the synthesis paper by Estop-Aragones et al., 2021, which provides nice context for this statement. Estop-Aragones et al., 2021 also report that in oxic systems it does seem that thaw results in microbial utilization of older C and so we have added this reference to the statement about drier systems in the paragraph following the one this comment referred to (we had originally only referred to Pastor et al., 2003 here).

Line 52 – Replace "melting" for "thawing", also in line 56 *Done.*

Line 59 – Do you mean "surface soils" here? *No, we mean lateral export to surface waters, but "surface waters" can be cut here for clarity and to improve sentence flow. We have removed "surface waters" from this line.*

Line 62 – It seems the statement of DOC export increasing with streamflow is based on the spring thaw. The relation of DOC export being water rather than carbon limited is thus based on the fact that large DOC pools accumulate throughout several months in winter and then are flushed during snowmelt. As currently phrased it seems that this relation would also occur during other hydrological events such as after precipitation. Consider rephrasing as "This seems to be the current case for the Arctic as DOC export by streams and rivers largely occurs during the increase in streamflow during snowmelt, implying that DOC transport and production is water, not carbon, limited (REFS)" or alike. *Yes, good point. We have revised the sentence as follows: "This seems to be the current case for the Arctic as DOC export by streams and rivers largely occurs during the increase in streamflow associated with snowmelt, implying that DOC transport and production is water, not carbon, limited [Refs]."*

Line 69 – Add "age" after "provide insights into the" *Done.*

Line 74-79 – Consider adding before this last sentence that age typically increases with depth and that the flowpath associated to seasonal thaw may thus be reflected in the 14C-DOC downstream. *We have added a statement to this effect and an additional one to illustrate the concept with an example from the literature: "Soil C age typically increases with depth and so deepening flow paths associated with seasonal thaw may be reflected in the 14C-age of DOC downstream. For example, inland headwater systems showed an increase in the age of DOC seasonally as deepening of the active layer mobilized older soil C (Dean et al., 2018)."*

Line 89 – Rephrase sentence for clarity. As stated, it reads as the biodegradability decline from January to December rather than seasonally. *We have removed "January to December", which we agree is confusing. Note we have substantially revised this entire paragraph to address the issues raised regarding the need to link the indicators more explicitly we use to our expected results.*

Line 100 – Rephrase. The part of "do not provide information about the locations within their watersheds..." is unclear *Our thought here is that because the streams integrate processes across the watershed that drains to the point of sampling, we miss the ability to identify specific source areas, the potential biogeochemical hotspots that contribute disproportionally to the C fluxes detected in the streams. We have added "specific" to the sentence such that it now reads: "The information gained from stream water samples is valuable, but these streams integrate processes across the landscape and do not provide information about the specific locations within their watersheds where older or more labile C is being mobilized, limiting our ability to attribute these observations to processes."*

Line 99-115: Authors state their expected findings in this paragraph. The introduction has reviewed broad aspects of DOC cycling but it is hard to relate with these expected findings at the end of the paragraph. Specifically, 1) why do authors expect DOC to become more enriched in aromatics over the course of the summer? If this is related with the degree of microbial processing, it would be helpful to spell out more clearly this relation in the introduction. 2) Also, the first time that the concept of thaw lake appears is here and it seems reasonable to introduce it before to better understand the stated expectation.

To address point 1, we expected DOC to become enriched in aromatics because more DOC would be coming from unprocessed soil organic matter as the active layer deepened. We have tried to improve the clarity of how we use our indicators of DOC composition by being more specific throughout the manuscript but also be revising the Introduction paragraph on seasonal changes in DOC composition and lability to more specifically address the indicators we and others use, justify our expected results regarding seasonal changes, and to provide better context for our results and discussion.

To address point 2, we have modified the Introduction paragraph before the one where we describe our study and expected results, the paragraph on the effects of permafrost thaw on DOC chemistry and biolability, to describe differences more broadly in DOC content and chemistry with differences in watershed characteristics and the extent of thaw – we have included here an introduction to the the concept of DTLBs.

Methods

Line 119 - Please describe what you mean by “drainages” or where were samples collected from. In soils/sediments from channels and streams or in soils adjacent to water channels? Just *This comment seems incomplete. We have changed this to “watershed drainages” rather than simply “drainages” to attempt to be clearer. The rest of the paragraph explains from where and how the samples were collected.*

Line 121 – This is unclear - what are internal and external drainages? *Agreed, we were referencing to the Barrow Environmental Observatory but this is confusing and not necessary and we have changed “internal and external drainages” to “drainages”.*

Line 145 – The statement of calculation of SUVA follows after a statement about absorption coefficients. For clarity, please state how you calculated SUVA and whether you used "absorption coefficients" (from your Equation 1) or "spectral absorbance". *We now specify that the absorption coefficients from Equation 1 were used to calculate SUVA.*

Line 157 – Add "Dissolved oxygen" for DO *Done.*

Line 163 – Add "significance level" *Done.*

Results and discussion

Figure 2 caption – Specify units of C/N ratio: either mass (g/g) or mols (mol/mol) *Done, it is by mass.*

Line 186-187 – What does it mean that DOC increased from July to September "in samples from the thaw table depth"? Do you mean the "deeper samples"? Rephrase for clarity. *We changed this phrasing to “in deep porewater”.*

Line 191 – Based on the data presented so far (14C-DOC, DOC concentration, DOC:DON, DON concentration), it is unclear why "undecomposed" fits in the statement. Also, it is unclear why "vegetation-derived C" fits in the statement. It is not simply "organic carbon"? Please rephrase or provide information that allows understanding how you link the measurements with those adjectives/sources. *We have rephrased to “unprocessed organic matter” and have used the term “unprocessed” in the Introduction to describe organic matter attributed to vegetation/litter and unprocessed soil organic matter. We have introduced DON to the Introduction in the context of organic matter source and quality. We have added a sentence to explain our logic and clarify this point so that the last few sentences of this paragraph read: “An increase in microbially-derived or highly processed organic matter should have included an increase in DON and decrease in DOC:DON ratio as microbially-derived organic matter has lower C:N ratios than plant litter and soil organic matter (Cleveland and Liptzin, 2007) and C:N ratios decline with* *increased microbial processing of litter and soil organic matter (Lavallee et al., 2020; Moore et al., 2011). Thus, these patterns are consistent with increased mobilization of unprocessed organic matter from old permafrost as the thaw table deepened from an average of 34 cm in July to 43 cm in September."*

Figure S1 and S2 – Please correct the units on the x axis of Figures S1 and S2 *Thank you for catching this – we have changed the units to mg L-1.*

Line 215-217 – How do authors conclude the last part of the sentence "that has not previously undergone microbial processing and may be biolabile." The paper does not provide any experimental evidence of DOC lability. If authors want to relate their SUVA values with the degree of lability, they should describe and put in context the relation of SUVA vs lability observed in other studies. Clarifying how authors relate SUVA values with lability would help to clarify this. *We have addressed microbial processing elsewhere and here, we have clarified that others have found a correlation between SUVA and lability: "These trends likely reflect in situ production of DOC at depth from thawing permafrost that has not previously undergone microbial processing and may be biolabile because DOC biolability (the amount of DOC lost during incubation) has been shown to be positive correlated to SUVA254 (Mann et al., 2015)."*

Line 223 - Replace "were" for "was" *Thank you for pointing out this error in grammar. We have changed "relationship…were" to "relationships…were".*

Line 226 – Explaining the potential reasons of these results and their relations is missing. In its current state, the results and correlations are presented without any further explanation. Why do CH4 concentrations are greater in July than in Sept? Why do 14C-DOC is higher ("younger DOC") in July than in Sept? Suggestion: Is it possible that both CH4 and 14C-DOC are related to vegetation seasonal patterns with more active vegetation in July releasing younger substrates (higher 14C-DOC) that are preferentially used resulting in CH4 production and higher soil concentrations? This influence of vegetation could be stronger in shallow depths which could explain why these relations are only observed in samples of the top 10 cm and not deeper down (30-40cm). *R1 also pointed out an issue with this sentence (L225-226). We have removed the correlation results – this is not a meaningful correlation between 14C and CH4 concentration, but a shift in surface and porewater chemistry from June to September. The sentence now reads: "In surface and shallow pore waters, both DOC Δ14C values and CH4 concentrations decreased from July to September (Fig. 4a, p = 0.04) but DOC Δ14C values were not correlated with any of the other measured variables."As noted in the paper, CH4 concentrations were presented and discussed in Throckmorton et al., 2015. Our point here was intended to be a relatively minor one to place the new data in the context of the other geochemical data available for these samples and to lead into the following paragraph focused on the deep porewater. We think this change to this sentence helps clarify our point concisely.*

You pose interesting questions, here! As discussed by Throckmorton et al., there does seem to be a shift not only in the methanogenesis pathway (more acetoclastic in July and more hydrogenotrophic in September) but also in the depth where most of the CH4 is being produced (in shallow porewater in July to deep porewater in September). Throckmorton et al. also suggested this might be driven by a shift in C source related to vegetation inputs. We have looked *to see if we observed correlations between CH4 concentrations and the DOM chemistry variables we measured. If the increase in CH4 was driven by plant inputs, we would expect to see a correlation with the indicators of fresh plant organic matter sources (higher CH4 with higher DOC:DON or SUVAs) – but we did not observe any correlation between CH4 concentration and OM chemistry. An alternative to the plant C inputs hypothesis, is that the CH4 values may be higher in July than in September because of a buildup of acetate over the winter when cold temperatures should inhibit acetoclastic methanogenesis, but we did not measure acetate at our sites and this a speculation. We do not think our data are strong enough to support one of these explanations over the other and so have chosen not to add this to the discussion as we have not added anything new to what was discussed previously in the Throckmorton et al paper.*

Figure 4 – Correct units of x axis in panel 4a. It probably should be mM or mmol/L? *Done (it is mM).*

Line 247-254 – It is unclear how this paragraph adds to the story. I guess the point of this paragraph is to discuss about the dominance of hydrogenotrophic vs acetoclastic at the site but it seems all the information is based on previous studies and poorly related with the findings presented in the paper. Consider reworking this paragraph and merge it with the previous one where the alpha fractionation data is presented. *We see your point and have merged this paragraph with the previous one, deleting the middle sentence about sample limitations as we agree it does not add to this paper.*

Line 271 – Decreasing DOC "concentrations" or "14C-DOC"? Please clarify *We added “concentration”.*

Line 273-275 – Apart from the age, can authors explain how DTBLs differ in other ecological characteristics among young, medium, old and ancient? Is there a vegetation succession during the maturing of these ecosystems? Is it commonly expected to have shallower thaw depths in younger DTBL? Such additions may help to better interpret the ecological relevance of the differences in DOC concentrations and SUVA presented in Figure 6. *This is a good point. We have revised this paragraph to better place our results into the context of how DTLBs differ with age. We have added early in the paragraph, after reporting our observed increase in the active layer depth with DLTLB age: “Others have reported an increase in the organic layer thickness with increasing DTLB age (Bockheim et al., 2004; Hinkel et al., 2003), although no difference was reported for active layer thickness with basin age (Bockheim and Hinkel, 2005).” We have revised the later portion of the paragraph as follows: “However, our observed shifts in DOC chemistry are consistent with patterns of ecological succession and landform development for DTLBs. In our study region, young and medium aged basins (< 300 years) contain relatively productive plant communities dominated by grass and sedge species, while overall productivity declines coincide with ponding and polygonal ground development in old and ancient basins (Hinkel et al., 2003). Such declines in vegetation productivity should result in decreased carbon input to soils and in decreases in the indictors for vegetation-derived, unprocessed organic matter, including the SUVA values observed in this study. In fact, the degree of soil organic matter decomposition increased with increasing DTLB age based on decreasing fiber content (Bockheim et al., 2004; Hinkel et al., 2003) and extractable organic matter (Bockheim et al.,* *2004). We suggest that our observed trends reflect a decline in readily decomposable carbon (e.g., vegetation-derived, unprocessed organic matter) and increase in the degree of organic matter decomposition as DTLB's age." We have also revised the second-to-last paragraph of the introduction to introduce DTLB's age as something of interest when considering differences in DOC production and chemistry across the landscape and moved the statement about DOC concentration decreasing with lake development (the Prokrovsky et al., 2011 reference) to this introduction paragraph. This provides a little more context to the idea that watershed characteristics and the extent of permafrost thaw might change DOC flux and chemistry and allows us to focus this paragraph in the results and discussion on the DTLB's.*

Line 275 – Does Figure 6a present only "deep pore water samples"? If so, please include in the caption of Figure 6 as it currently only mentions "DOC concentration across depths", which is unclear. *We agree, this is unclear. In fact, the figure is for all samples, but the significant trend is for deep pore waters only. We have changed figure 6a so that it shows only the deep pore water and have clarified that all panels of figure 6 show deep porewater only in the figure caption.*

Line 280 – Authors refer to "the degree of organic matter decomposition". This goes back to a previous comment - authors should more clearly state whether high or low SUVA values are associated with higher or lower lability based on previous findings. **This would avoid the confusion of thinking that high SUVA results in lower lability** but rather here it seems that authors relate the SUVA values with the quantity of vegetation derived C, which seems to decline with DTBL aging. Please clarify how you relate SUVA values with lability and DTBL aging in your study. *In this paragraph, we have expanded the discussion of how DTLBs change as they age and have added clarifications. We have added additional clarifications in the introduction and results, and we hope that all of these changes contribute to addressing this issue.*

Line 296 – Replace "older DOC and younger DOC" for "old and young DOC"? *Sure, done.*