Review of "High peatland methane emissions following permafrost thaw: enhanced acetoclastic methanogenesis during early successional stages" by Liam Heffernan and others.

Summary:

The goal of this manuscript is to advance our understanding of the underlying controls of methane emissions from permafrost thaw in northern peatlands. Specifically, the authors assess how shifting ecological conditions (e.g., collapse of peat plateau and thermokarst bog formation) affect microbial communities, the amount of CH_4 released, and the d13C isotope composition of released CH_4. The authors also aim to determine how long elevated surface CH_4 emissions persist after thaw.

To answer these questions, the authors study peatland methanogenic community composition and methane emissions along a thaw gradient (intact peat plateau, thermokarst bog formed 30 years ago, and thermokarst bog formed 200 years ago) in discontinuous permafrost in western Canada. The authors analyzed methanogenic community composition down to 160 cm, measured dissolved CH_4 and CO_2 concentrations and d13C values down to 245 cm in the bogs sites, in addition to rates and d13C values of land-atmosphere CH_4 and CO_2 fluxes. Results from these analyses show that methanogenesis is primarily hydrogenotrophic, rather than acetoclastic, at both the young and mature sites. Young bog had isotopically heavier methane d13C values than the mature bog, suggesting that acetoclastic methanogenesis was more enhanced in the young bog. Young bog CH_4 emissions were 3x greater than the mature bog. These results imply that CH_4 emissions by acetoclastic methanogenesis will increase with continued thermokarst peat plateau collapse and thaw depth lowering in discontinuous permafrost over the next century. As thermokarst bogs mature and dry out, lower temperatures and lower substrate availability will lead to a dominance of hydrogenotrophic methanogenesis.

Recommendation:

This is an interesting study that aligns with the research focus of Biogeosciences, but further analysis, clarification, and a more robust discussion are needed before this manuscript can be accepted for publication. Below I describe my overall points of concern, and provide suggestions for the authors to improve the manuscript. I also found that the text needs editing and revision to be more easily understood by the reader, and thus I provide detailed line-byline comments that are more editorial in nature. Therefore, I recommend major revisions.

Thank you for your feedback! We below respond to your comments and suggestions to better revise our manuscript.

Major comments:

While the data and analyses reported in this manuscript appear robust and offer insight into the effect of thermokarst peat plateau collapse on greenhouse gas emissions, I feel that the authors have not presented any new ideas or conceptual models that help us relate Arctic landscape change to changes in carbon cycling. I find the discussion and conclusions to be very generalized, attributing the observed differences between young and mature bogs to "hydrological regimes, vegetation communities, and peat chemistry." Statements like this do not provide any insight to the specific mechanisms driving microbial community change.

With this manuscript, we present the first study to combine microbial and biogeochemical data to assess the influence of permafrost thaw on methanogenesis and CH_4 emissions along a space-for-time thermokarst bog transect. We will review and modify the discussion/conclusion to avoid very generalized statements and try to better link the biogeochemical and microbial community data.

Specifically, it would be useful to quantify the relationship between rate of water table lowering and CO2 and CH4 production rates/magnitudes. I suggest using the ages of the bogs to determine rate of change in environmental parameters, like thaw depth lowering, water table lowering, and temperature change.

This is an interesting thought; however, we do not think it reasonable to make any further extrapolation on how shifting ecological conditions will impact emissions using this specific dataset, which may be out of the scope of this study. A similar point was also made by RC3, wondering if we could determine how different conditions following thaw may influence total CH_4 emissions. To determine the relationship between magnitude of fluxes and site conditions we would need a larger, more comprehensive dataset that consists of either multiple years of data, or multiple sites, or both. This is the objective of a yet to be published study from this site, that includes 3 years of flux data and a secondary site. The objective of this study was to compare sites with different thaw histories, and thus differencing current ecological conditions. We will make the scope and objectives of the study clearer in the introduction to address both reviewers' concerns.

The data for this study were collected from three very localized sites, and it is not clear whether the processes driving CO2 and CH4 production are representative of the greater Arctic landscape. I think the authors need to use their dataset to dive a little deeper into the mechanisms of methanogenesis and transport to the surface (e.g., Throckmorton et al., 2015). I would also like to know exactly which archaeal communities are most important for greenhouse gas production and how they are changing along the thaw gradient (e.g., H j et al., 2008).

The study site is considered to be representative of boreal peatlands in the discontinuous permafrost zone in the Mackenzie River Basin of western Canada; see below for some references supporting this. In short, this area is comprised of intact peat plateaus interspersed with permafrost free bogs, fens, and ponds. Permafrost peatlands in this area are very similar to those found in the Hudson Bay Lowlands (Kuhry, 2008) and Alaska (Jones et al., 2017)

We do not think there is a single site or ecosystem that is representative of the greater Arctic landscape or northern circumpolar permafrost region. However, our study system does represent a globally significant organic carbon store that is vulnerable to permafrost thaw and potential mineralization into greenhouse gases. Peatlands in the Mackenzie River Basin are one of the three largest stores of organic carbon found in peatlands within the permafrost zone, the other two being the Hudson Bay Lowlands and the West Siberian Lowlands (Hugelius et al., 2020; Olefeldt et al., 2021). Within the sporadic and discontinuous permafrost zone of our study *region >15% of the total peat plateau area has thawed and formed thermokarst bogs in the last 30 years (Baltzer et al., 2014; Gibson et al., 2018). Projections for this area suggests total permafrost lost from plateaus by 2050 (Chasmer and Hopkins, 2017). Thus, we consider the results of this study results to be important.*

Regarding the mechanisms of methanogenesis and transport pathways to the surface, initially we considered the mass balance approach used by Thockmorton et al., (2015) as well by Corbett et al., (2013). We agree that this is a very interesting approach to answer questions regarding the pathways of anaerobic fermentation and decomposition, and vertical transport of the end-products of this decomposition. We use a similar approach in determining the pathways of methanogenesis responsible for dissolved concentrations of CH_4 at depth to that described in these papers. However, we do not follow a similar approach in assessing the transport of the resulting dissolved gases of anaerobic decomposition. We decided to not take such an approach as it was beyond the scope and objectives of our study. Our study focuses on assessing how shifting ecological conditions following permafrost thaw influence the structure and activity of the methanogen community, the pathways of methanogenesis, and surface CH_4 emissions. We do not focus on how dissolved CH_4 reaches the surface, or where in the peat profile the CH_4 emitted at the surface was produced. Rather, we focus on how methanogenesis and the microbial community responsible for methanogenesis is affected in the top 160 cm of a peatland following permafrost thaw, whether this results in greater surface CH_4 emissions, and for how long these surface emissions may last (decades to centuries). We believe that the combination of microbial data (16S) and biogeochemical data (dissolved concentrations, $\delta^{13}C$ signatures, surface emissions) from areas that have thawed 30 and 200 years ago in a thermokarst bog is novel, timely, and interesting.

Regarding which archaeal communities are most important for greenhouse gas production and how they are changing along the thaw gradient, we also agree that this is a very interesting and timely question. This question, however, is beyond the scope of our study. Here, we aim to address the influence that shifting ecological conditions, following permafrost thaw, has on methanogenesis specifically, not on anaerobic chemoheterotrophy in general. This indeed would be a fascinating topic for a future study that would include not just 16S data but also various other metaOmics. The dataset for this study is open and freely available, we would be more than happy to discuss the contribution of this data to any such studies in the future.

<u>*References – boreal western Canada*</u>

- *Bauer, I. E., Gignac, L. D., & Vitt, D. H. (2003). Development of a peatland complex in boreal western Canada: Lateral site expansion and local variability in vegetation succession and long-term peat accumulation. Canadian Journal of Botany, 81(8), 833–847. https://doi.org/10.1139/b03-076*
- *Beilman, D. W. (2001). Plant community and diversity change due to localized permafrost dynamics in bogs of western Canada. Canadian Journal of Botany, 79(8), 983–993. https://doi.org/10.1139/cjb-79-8-983*
- *Camill, P. (1999). Peat accumulation and succession following permafrost thaw in the Boreal peatlands of Manitoba, Canada. Ecoscience, 6(4), 592–602. https://doi.org/10.1080/11956860.1999.11682561*
- *Pelletier, N., Talbot, J., Olefeldt, D., Turetsky, M., Blodau, C., Sonnentag, O., & Quinton, W. L. (2017). Influence of Holocene permafrost aggradation and thaw on the paleoecology and carbon*

storage of a peatland complex in northwestern Canada. Holocene, 27(9), 1391–1405. https://doi.org/10.1177/0959683617693899

- *Vitt, D. H., Halsey, L. A., & Zoltai, S. C. (1994). The Bog Landforms of Continental Western Canada in Relation to Climate and Permafrost Patterns. Arctic and Alpine Research, 26(1), 1. https://doi.org/10.2307/1551870*
- *Vitt, D. H., Halsey, L. A., Bauer, I. E., & Campbell, C. (2000). Spatial and temporal trends in carbon storage of peatlands of continental western Canada through the Holocene. Canadian Journal of Earth Sciences, 37(5), 683–693. https://doi.org/10.1139/e99-097*
- *Zoltai, S. C. (1972). Palsas and Peat Plateaus in Central Manitoba and Saskatchewan. Canadian Journal of Forest Research, 2(3), 291–302. https://doi.org/10.1139/x72-046*
- *Zoltai, S. C. (1993). Cyclic Development of Permafrost in the Peatlands of Northwestern Alberta, Canada. Arctic and Alpine Research, 25(3), 240. https://doi.org/10.2307/1551820*

References - other

- *Kuhry, P. (2008), Palsa and peat plateau development in the Hudson Bay Lowlands, Canada: timing, pathways and causes. Boreas, 37: 316-327. https://doi.org/10.1111/j.1502-3885.2007.00022.x*
- *Jones, M. C., Harden, J., O'Donnell, J., Manies, K., Jorgenson, T., Treat, C., & Ewing, S. (2017). Rapid carbon loss and slow recovery following permafrost thaw in boreal peatlands. Global Change Biology, 23(3), 1109–1127. https://doi.org/10.1111/gcb.13403*
- *Hugelius, G., Loisel, J., Chadburn, S., Jackson, R. B., Jones, M., MacDonald, G., Marushchak, M., Olefeldt, D., Packalen, M., Siewert, M. B., Treat, C., Turetsky, M., Voigt, C., & Yu, Z. (2020). Large stocks of peatland carbon and nitrogen are vulnerable to permafrost thaw. Proceedings of the National Academy of Sciences of the United States of America, 117(34), 20438–20446. https://doi.org/10.1073/pnas.19163 87117*
- *Olefeldt, D., Heffernan, L., Jones, M. C., Sannel, A. B. K., Treat, C. C., & Turetsky, M. R. (2021). Permafrost thaw in northern peatlands: rapid changes in ecosystem and landscape functions. Ecosystem Collapse and Climate Change, 27-67.*
- *Baltzer JL, Veness T, Chasmer LE, et al (2014) Forests on thawing permafrost: fragmentation, edge effects, and net forest loss. Global Change Biology 20:824–834. doi: 10.1111/gcb.12349*
- *Gibson CM, Chasmer LE, Thompson DK, et al (2018) Wildfire as a major driver of recent permafrost thaw in boreal peatlands. Nature Communications 9:3041. doi:10.1038/s41467-018-1034 05457-1*
- *Chasmer L, Hopkinson C (2017) Threshold loss of discontinuous permafrost and landscape evolution. Global Change Biology 23:2672–2686. doi: 10.1111/gcb.13537*
- *Corbett, J. E., M. M. Tfaily, D. J. Burdige, W. T. Cooper, P. H. Glaser, and J. P. Chanton (2013), Partitioning pathways of CO2 production in peatlands with stable carbon isotopes, Biogeochemistry, 114(1–3), 327–340.*
- *Throckmorton, H.M., Heikoop, J.M., Newman, B.D., Altmann, G.L., Conrad, M.S., Muss, J.D., Perkins, G.B., Smith, L.J., Torn, M.S., Wullschleger, S.D. and Wilson, C.J., 2015. Pathways and transformations of dissolved methane and dissolved inorganic carbon in Arctic tundra watersheds: Evidence from analysis of stable isotopes. Global Biogeochemical Cycles, 29(11), pp.1893-1910.*

The 16S rRNA data appear to be underutilized, whereas the data could be used to test hypotheses presented by other studies (e.g., Hultman et al., 2015).

While it is true that 16S RrNA gene data may be underutilized in studies such as these, there are numerous constraints on what can be done with (and concluded in using) this kind of microbial taxonomic data in tandem with biogeochemical data. We therefore wanted to limit our interpretation and discussion to the methanogenic community so that we do not "overreach" with what our data could tell us about this system …

The hypotheses presented by the study exemplified (Hultman et al., 2015) is a more robust study in that it combines not just 16S microbial taxonomic data, but also metaOmics data such as proteomics, metatranscriptomics and metagenomics to specifically target the functional processes occurring in their system. With our dataset, we can explore the putative metabolisms involved, but with significant limitations, as 16S cannot be directly tied to microbial metabolic function. In an attempt to gain further insight into putative microbial function, we applied FAPROTAX, a bioinformatics tool that can predict ecologically relevant functions from 16S microbial taxonomic data (Louca et al., 2016, Sansupa et al., 2021), to our dataset. However, it was unable to resolve whether particular methanogenic pathways were taking place in different stages of thaw, and thus we chose not include this analysis. Instead the insight gleaned about the archaeal community composition from the 16S rRNA gene analysis, in conjunction with the isotopic signatures for CH_4 and CO_2, was ultimately more convincing in identifying the dominant methanogen pathways along our thaw gradient. There is previous precedent to combining 16S 16S rRNA gene and biogeochemical data in a similar fashion to this study to gain insight into changing microbial community structure (Ganzert et al., 2007; Saidi-Mehrabad et al., 2020; Cherbunina et al., 2021), as well as others that incorporate both 16SrRNA gene sequencing and targeted qPCR/metaOmics to more definitively determine more microbially-driven processes in permafrost (Wen et al., 2018, Unger et al., 2021).

References:

- *Lars Ganzert, German Jurgens, Uwe Münster, Dirk Wagner, Methanogenic communities in permafrost-affected soils of the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints, FEMS Microbiology Ecology, Volume 59, Issue 2, February 2007, Pages 476–488, https://doi.org/10.1111/j.1574-6941.2006.00205.x*

- *Saidi-Mehrabad, A., Neuberger, P., Hajihosseini, M., Froese, D., Lanoil, B.D. (2020). Permafrost microbial community structure changes across the Pleistocene-Holocene Boundary. Front. Environ. Sci. https://doi.org/10.3389/fenvs.2020.00133*

- *Cherbunina, M.Yu., Karaevskaya, E.S., Vasil'chuk, Yu.K., Tananaev, N.I., Smelev, D.G., Budantseva, N.A., Merkel, A.Y., Rakitin, A.L., Mardanov, A.V., Brouchkov, A.V., Bulat, S.A. (2021). Microbial and Geochemical evidence of permafrost formation at Mamontova Gora and Syrdakh, Central Yakutia. Front. Earth. Sci. https://doi.org/10.3389/feart.2021.739365*

- *Wen, X., Unger, V., Jurasinski, G., Koebsch, F., Horn, F., Rehder, G., Sachs, T., Zak, D., Lischeid, G., Knorr, K., Böttcher, M.E., Winkel, M., Bodelier, P.L., & Liebner, S. (2018).*

Predominance of methanogens over methanotrophs in rewetted fens characterized by high methane emissions. Biogeosciences.

- *Unger, V., Liebner,S., Koebsch, F., Yang,S., Horn,F., Sachs, T.,Kallmeyer, J., Klaus-Holger, K., Rehder,G., Gottschalk, P., Jurasinski,G. (2021). Congruent changes in microbial community dynamics and ecosystem methane fluxes following natural drought in two restored fens,*
  Soil Biology and Biochemistry 160: https://doi.org/10.1016/j.soilbio.2021.108348.

The study relies heavily on statistical analysis, but it is not clear that the authors are testing specific hypotheses with their analyses. Further hypothesis testing will help to elucidate some of the other processes driving carbon cycling along the thaw gradient.

The hypotheses that we aim to answer, are specified in the introduction (Lines 130-136). There, we state that we hypothesize "(1) shifting ecological conditions along the permafrost thaw gradient results in a successional microbial community and a restructuring of the methanogenic community, and (2) the warmer conditions in the young bog, along with the exposure of previously frozen peat, will result in a greater relative abundance of acetoclastic methanogens throughout the depth profile, and subsequently greater overall CH4 emissions."

To test our first hypothesis, our 16S microbial data was used to test whether there is evidence of distinct groupings of methanogen communities using NMDS and ANOSIM (L434-444) down to 160 cm depth in areas that have thawed 30 and 200 years ago. We then used RDA and variance partitioning (L445-471) to test how biogeochemical and site data from these two different thawed areas influence the 16S data and methanogen community structure. Using our dataset, we unfortunately cannot get more specific than this without further metaOmic data or qPCR data.

To test our second hypothesis, we used ANOVAs and Bonferroni post-hoc tests on linear mixed effects models (L422-433) to test for differences in the concentrations and $\delta^{13}C$ signatures of surface gas fluxes and dissolved gas depth profiles down to 245 cm between the two thawed areas.

Could the reviewer be more specific regarding what they mean by testing and how they deem these tests to not be sufficient in addressing our hypotheses? We welcome any suggestions for further hypotheses they would consider testing with the dataset available to us.

I also find that the authors make comparative statements that are not supported by statistically significant differences (e.g., in dissolved chemistry parameters). This section of the results is misleading, and also leads to some misleading interpretations of the data (e.g., L853-854).

We will better highlight the pore water chemistry parameters that are statistically different between the sites (pH and DOC). We will further discuss those parameters that are not statistically different, and which have large standard deviations associated with them (SUVA and TDN). We will also better highlight that the differences in lability associated with the young and mature bog is also inferred from previous work at this study site (Heffernan et al. 2021), but also from work at other closely related sites with similar vegetation communities (Burd et al., 2020).

References

- *Heffernan, L., Jassey, V. E. J., Frederickson, M., MacKenzie, M. D., & Olefeldt, D. (2021). Constraints on potential enzyme activities in thermokarst bogs: Implications for the carbon balance of peatlands following thaw. Global Change Biology, 27, 4711–4726. https://doi.org/10.1111/gcb.15758*
- *Burd, K., Estop-Aragonés, C., Tank, S. E., & Olefeldt, D. (2020). Lability of dissolved organic carbon from boreal peatlands: Interactions between permafrost thaw, wildfire, and season. Canadian Journal of Soil Science, 13(February), 503–515. https://doi.org/10.1139/cjss-2019-0154*

Detailed comments:

Abstract

L 32: "(~30 and 200 years since that, respectively)"

We will change L32 to read:"~30 and 200 years since thaw, respectively

L 34-35: "high throughput 16S rRNA gene sequencing"

We will change L34-35 to read:"~…high throughput 16S rRNA gene sequencing"…

L 39-40: It would be helpful to give values or the difference between the mean values of the young vs. mature sites

We will add these values to the abstract

L 42: It would also be useful to give the measured CH4 fluxes in the abstract

We will add the rates of CH4 fluxes to the abstract

L43-45: Be more specific on what the interactions are. I assume that different interactions between ecological conditions and methanogen communities can also reduce CH4 emissions. What exactly are favorable conditions for methanogens and what is the implication for future CH4 emissions as these thermokarst bogs continue to age and turn more hydrogenotrophic?

We will be more specific about what interactions we are talking about here. Namely, we will say that warmer temperatures, higher water table, and hydrophilic vegetation in the young bog are favourable for enhanced CH_4 emissions. It will now read as
"Our study suggests that interactions between the methanogenic community and hydrophilic vegetation, warmer temperatures, and saturated surface conditions enhance CH4 emissions in young thermokarst bogs, but these favorable conditions only persist for the initial decades after permafrost thaw."

<u>Introduction</u>

L51: "…are thought to be driven by…"

We will change L51 to read: "~…are thought to be driven by", as suggested.

L 64: Can thermokarst formation also expose frozen C to aerobic microbial decomposition(e.g., to CO2)? Do we know whether aerobic or anaerobic decomposition result in greater greenhouse emissions? What about the role of methane oxidation by aerobic bacteria or anaerobic archaea (e.g., In't Zandt et al., 2020)

Yes, thermokarst formation can expose previously frozen organic matter to aerobic respiration, resulting in increased CO_2 emissions (Schädel et al., 2016). However, thermokarst formation in peatlands is characterized by ground subsidence, resulting in saturated surface conditions (Camill, 1999). These saturated surface conditions result in previously frozen peat being exposed to anoxic conditions. Peatlands are a wetland ecosystem where anoxia is the dominant redox condition and anaerobic decomposition is the main form of decomposition.

In general, aerobic respiration occurs at a faster rate than anaerobic respiration as has been shown for other, non-peatland, thermokarst ecosystems (Schädel et al., 2016). We will add some text to reflect this in the introduction. This new text will read
"Redox conditions following thermokarst formation are an important control of decomposition, with 3-4 times as C mineralization occurring as aerobic respiration compared to anaerobic respiration (Schädel et al., 2016)"

We address CH_4 oxidation in the manuscript (L798-807) but will elaborate further. Our study objectives were not to explore the relationship between ecological conditions following thaw and aerobic respiration at the surface in peatlands, but rather the anaerobic processes beneath the water table. The In't Zandt et al., 2020 study focuses on thermokarst lakes in ice-rich Yedoma deposits. While the role of CH_4 oxidation within anaerobic lake sediments in these systems is an interesting one, we do not think it entirely relevant to our study. In thermokarst affected permafrost peatlands, CH_4 oxidation has been shown to be closely linked with redox potential associated with the water table position (Perryman et al., 2020). This is included in our discussion of oxidation in the manuscript.

<u>References</u>

- *Schädel, C., Bader, M. K. F., Schuur, E. A. G., Biasi, C., Bracho, R., Capek, P., et al. (2016). Potential carbon emissions dominated by carbon dioxide from thawed permafrost soils. Nature Climate Change, 6(10), 950–953. https://doi.org/10.1038/nclimate3054*
- *Perryman, C. R., McCalley, C. K., Malhotra, A., Fahnestock, M. F., Kashi, N. N., Bryce, J. G., ... Varner, R. K. (2020). Thaw Transitions and Redox Conditions Drive Methane Oxidation in a Permafrost Peatland. Journal of Geophysical Research: Biogeosciences, 125(3). https://doi.org/10.1029/2019JG005526*

L133: Does colonization cause fresh, labile inputs of carbon here? I'm not sure what specific process is increasing the amount and temperature sensitivity of CH2 emissions.

Yes, colonization of hydrophilic vegetation following thaw is associated with an increase in labile inputs. This increase in labile inputs can increase methanogenesis, and thus, the sensitivity of methanogenesis to temperature. We cite references in the text (L113) that address this and shaped this hypothesis.

L130: Can you be more specific about the "shifting ecological conditions"? Only be more specific if your results allow you to link methane emissions to specific conditions.

The shifting ecological conditions associated with permafrost thaw in peatlands is outlined in the text above (L62; L87-91; L102-104) and at our study site in the methods section below (Section 2.1 L147). We will add a new, thorough, and clear definition of what we mean by ecological conditions in relation to the conditions found in the young and mature bog at our study site

L197: "…is drier than the young bog, with …"

We will change L197 accordingly

L217: "…young and mature bog stages, ~1 m from the nearest collar."

We are unsure about what change is suggested, the suggestion is exactly similar to the current text.

L219: remove "deep"

We will remove "deep" from L219.

L221: "…devices were installed in each bog…" (since there are only two bogs, you don't need to
keep repeating "young and mature bogs")
We will remove this repetition as suggested.

L221-222: "…where three dissolved gas samples were collected, two from 5-95 cm depth and a third from 115-245 cm depth."

We will add this change to the text as suggested.

In general, the writing in section 2.2 needs to be improved to make the methods more clear for the reader.
This section will be re-written to improve clarity .

L272-280: It is better practice to first present he equation, the define and give values for all variables. Here, there is a mix of information given before and after the equation. I recommend changing to: “The rates of CH4 and CO2 land-atmosphere fluxes (F) were calculated following:
F = S*(PV/RTA) (1)
Where S is the slope of the linear regression fitted to the gas concentration measurements over time inside the flux chamber (units). P is the atmospheric pressure (0.96 atm), . . .

We will change this text accordingly.

L 285-286: You should write this out using the equation tool.

We will change this text accordingly.

L 302-303: It would sound better to say: “We measured the d13C values of gas samples from both the flux chambers and atmospheric background.”

We will change this text accordingly.

Please use proper notation for stable carbon isotope values ($d_{13}C$), rather than saying “13C signatures”

We will change “^{13}C isotopic signatures” to “$\delta^{13}C$” throughout the text.

L 307: “measured”, not “quantified”

We will change L307 as specified.

L302-311: This paragraph could be written more clearly and concisely. Use of passive voice here makes it difficult to read.

We will edit this text to improve clarity.

L315: should this be “(1/[CH_4])” to denote that it is the concentration of CH4?

We will change this text accordingly, here and throughout the manuscript.

L321: Use “collected” rather than “taken”

We will change L321 to read as “Dissolved gas samples were collected…”

L 329: "concentration measurements" rather than "concentrations"

We will change this as suggested.

L 330-331: Again, d13C values, rather than "13C signatures"

Addressed above.

L321-340: Again, needs to be written more clearly.

This section will be edited to improve clarity.

L335: "concentration range"

We will change L335 as specified.

L337: "measurable range of the system"

We will change L337 as specified.

L349: "Focusing"

We will change L349 as specified.

L372: please write out what PVDF stands for(Polyvinylidene difluoride)

We will change L372 to "...pore size Polyvinylidene difluoride (PVDF) membrane…"

L424: "We performed these tests to assess whether thaw stage…"

We will change L424 accordingly.

L430: "Similarly, we tested for significant differences between the depth profiles in the young versus old bogs with respect to dissolved CH4 and CO2 concentrations, d13C values, and alphac values."

We will change L430 to: "Similarly, we tested for significant differences between the depth profiles in the young versus old bogs with respect to dissolved CH4 and CO2 concentrations, d13C values, and alpha c values…"

L412: Better subtitle would be "Statistical analyses"

We will change the subtitle to "Statistical Analyses", as suggested.

L434: do you need to mention the instrument again? The illumine miseq is already mentioned in the methods section

Good point- we will remove the additional mention of the Illumina Miseq

L448: There are other key studies that should have been used in the comparison

We provide additional references, Kendall & Boone (2006) and Zhang et al., (2020). In case further studies exist we are not yet aware of, we would be glad to include them and kindly ask the reviewer to provide more suggestions here.

References:

- *Kendall MM, Boone DR. Cultivation of methanogens from shallow marine sediments at Hydrate Ridge, Oregon. Archaea. 2006 Aug;2(1):31-8. doi: 10.1155/2006/710190. PMID: 16877319; PMCID: PMC2685590.*
- *Zhang, CJ., Pan, J., Liu, Y. et al. Genomic and transcriptomic insights into methanogenesis potential of novel methanogens from mangrove sediments. Microbiome 8, 94 (2020). https://doi.org/10.1186/s40168-020-00876-z*

L492-497: These are not statistically significant differences, so you cannot say that measurements were higher in the mature bog than the young bog. These parameters are statistically identical between the two bogs.

Statistically, pH and DOC are different (ANOVA; $p < 0.05$). We will clearly outline that these are statistically different and provide the test results. For the rest of the results that are not significantly different, we will clearly state this and also provide the test results. However, we will still state which averages are higher, particularly for SUVA and TDN, which have large standard deviations associated with them. While statistically not different, the differences in SUVA and TDN between stages of thaw may be sufficient to impact the microbial community structure (Bradley et al 2017). Nevertheless, we agree that we must point out more clearly where we found significant differences and where there were only insignificant trends or tendencies.

References

- *Bradley JA, Anesio AM and Arndt S (2017) Microbial and Biogeochemical Dynamics in Glacier Forefields Are Sensitive to Century-Scale Climate and Anthropogenic Change. Front. Earth Sci. 5:26. doi: 10.3389/feart.2017.00026*

L503: “below the water table”, rather than “under”

We will change L503 accordingly.

L504, 505: “Dissolved CH4 concentrations”, rather than “concentrations of CH4”

We will change L504 & L505 accordingly.

L507: What was the peak concentration in the mature bog? It’s difficult to compare the concentrations between the two bogs because you report different types of measurements.

Peak concentration in the mature bog was 6,800 µmol L^{-1}. This will be added to the text

L509-510: It's not clear that the mature bog had higher CO2 concentrations, so this is confusing.

Figure 2 shows that the mature bog has higher CO_2 concentrations, and we state in Lines 510-511 that the peak values were higher in the mature bog and provide these concentrations. " Again, the mature bog had overall higher concentrations, peaking at 1,500 µmol L-1 at 85 cm while the young bog peaked at 1,200 µmol L-1 at 95 cm (Figure 2b)."

L517: Again, use the delta notation rather than writing "13C isotopic signatures."

Addressed above.

L527: "Distinct" is a strong word to use here, given that there are many d13C measurements with overlapping uncertainty in both CO2 and CH4 d13C profiles.

The depth profiles of both $\delta^{13}C$-CO_2 and $\delta^{13}C$-CH_4 are statistically different from one another both above (L533; ANOVA ($F (1, 92) = 17.25$, $P < 0.001$) and below the water table (L536); ANOVA $F (1, 99) = 5.33$, $P < 0.05$) thus we consider distinct to be an appropriate word.

L533: It is not immediately apparent that "F" is the ANOVA F-test statistic. In the methods section, please introduce that you will use the F-test static to compare the two profiles statistically. It's also not clear what the (1, 99) and (1,92) subscripts indicate.

We will clearly state in the methods section that we are using ANOVA and will report the F statistic throughout when reporting the results from our ANOVA. We will also add "ANOVA" to the results section when reporting the F statistic.

L553-554: Each d13C measurements represents a mixture of two sources (acetoclastic and hydrogenotrophic). It would be much more informative to make a two end-member mixing model and estimate the relative contributions of the two methanogenesis pathways to each measurements. Using these estimates would allow for more quantitative comparison between methanogenesis pathways between the young and mature bogs.

We agree that an end member mixing model would be an interesting way to answer the questions we are addressing in the manuscript. Unfortunately, we do not have the correct dataset to do so. To perform an end-member mixing model we would need the specific fractionation factors associated with acetoclastic and hydrogenotrophic methanogenesis at our site. These fractionation factors vary considerably across sites (Conrad, 2005), thus we cannot use values from the literature. To determine these fractionation factors we would need to either perform an in situ labelling experiment or have a high resolution of $\delta^{13}C$ data of organic matter at each depth from where we have dissolved CO_2 and CH_4 concentrations. As we do not have either of these an end member mixing model approach is unfortunately not suitable to our study.

References:

- *Conrad, Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and a proposal, Organic Geochemistry, Volume 36, Issue 5, 2005, Pages 739-752, ISSN 0146-6380, https://doi.org/10.1016/j.orggeochem.2004.09.006.*

L567-568: Rather than saying "maximum ecosystem respiration in the mature bog was found…", better to say "Ecosystem respiration rates were elevated from June to August, and decreased in September."

We will change this line to "Ecosystem respiration rates were elevated from June to August, and decreased in September", as suggested.

L570-577: There are some grammatical mistakes and misuse of punctuation that make this difficult to read.

L570-L577 will be corrected to read as: "...emissions (sum of CH_4 and CO_2 emissions) released as CH_4 were an order or magnitude greater in the young bog than in the mature bog stage, at 18 and 2% respectively. This resulted from both the young bog's higher CH_4 emissions and lower ecosystem respiration (Figure S3). The $\delta^{13}C$-CH_4 signature of CH_4 emissions (intercept values from Keeling plots) in the young bog were significantly greater than those observed in the mature bog (Figure 3c; $F(1, 4) = 20.67$, $P < 0.05$), suggesting a greater influence of acetoclastic CH_4 production. The average isotopic signature in young bog CH_4 emissions ($n = 4$) was -66.5 ± 1.4‰ (Figure 3c), whereas the average from mature bog emissions ($n = 4$) was -78.5 ± 5.6‰ (95% CI)."

L674-678: If these variables only explain 18.4 and 4.3% of methanogenic community structure variation, then what other variables are important here? It seems like the analysis needs to go farther/data are inconclusive.

It is important to note that these variables are <u>significant</u> in influencing methanogenic community structure as determined by our backward stepping model, As such these two variables (distance to water table and thaw stage) were only mentioned because these were most important and relevant in determining methanogenic community structure, utilizing our backward stepping model. The analysis would not be as statistically robust if we were to include other, non-significant variables that were used as part of this model (i.e., DOC, temperature, enzymatic activity estimate depth, and etc, as described in L453 of the methods). We will add a line to mention that the remaining variation may be constrained by these non-significant parameters, and likely others not measured here. We have added the caveat that microbial community structure cannot always be fully explained by the discrete set of environmental parameters measured in any one study, and unconstrained variation may be further explained by plant-microbe and individual microbe-microbe interactions that we did not quantify (Boon et al., 2014).

<u>Reference:</u>

- *Boon, E., Meehan, C. J., Whidden, C., Wong, D. H., Langille, M. G., & Beiko, R. G. (2014). Interactions in the microbiome: communities of organisms and communities of*

genes. FEMS microbiology reviews, 38(1), 90–118. https://doi.org/10.1111/1574-6976.12035

L709-710: Would be worth mentioning that 14C measurements of CO2 and CH4 would help answer the question of whether the emissions are derived from decomposition of fresh, labile DOM or old, previously frozen peat.

We will add in text citing previous 14C-CO2 work that was performed at the study site (Estop-Aragonés et al 2018), which showed little to no evidence of aged carbon contributing to surface CO2 emissions. Other studies at similar thermokarst bog sites in western Canada have also found little to no evidence of aged carbon contributing to CH4 emissions at the surface (Cooper et al 2017) which we will also include in the text.

References

- *Cooper, M. D. A., Estop-Aragonés, C., Fisher, J. P., Thierry, A., Garnett, M. H., Charman, D. J., et al. (2017). Limited contribution of permafrost carbon to methane release from thawing peatlands. Nature Climate Change, 7(7), 507–511. https://doi.org/10.1038/nclimate3328*
- *Estop-Aragonés, C., Czimczik, C. I., Heffernan, L., Gibson, C., Walker, J. C., Xu, X., & Olefeldt, D. (2018). Respiration of aged soil carbon during fall in permafrost peatlands enhanced by active layer deepening following wildfire but limited following thermokarst. Environmental Research Letters, 13(8). https://doi.org/10.1088/1748-9326/aad5f0*

L718: up "to" the surficial…

We will change L718 accordingly.

L720: "drier", rather than "relatively drier." Using the word "drier" already implies a Comparison

We will change L720 accordingly, to avoid repetition.

L738: "also been observed"

We will change L738 accordingly.

Conclusions:

L853-854: The authors cite a "greater availability of plant leachates" but the DOC and DN data suggest no statistically significant differences in plant leachates between the young and mature bog. This is not a good explanation for the observed shifts in methanogenic communities.

We will better differentiate how we use our data as well as that from the literature to discuss our results. In this study, we only measured a small suite of DOM parameters and thus, rely upon previous literature at this site and similar locations to provide further support for our interpretations. We have two distinct vegetation communities and surface inundation conditions. Previously, both of these factors have been shown to influence the quality and quantity of plant derived DOM, which in turn has been shown to significantly influence microbial community composition (Laiho, 2003; 2006; Robroek., et al 2016; Bragazza et al., 2015; Ernakovich et al., 2017; Burd et al., 2020). Thus, we believe this to be a logical and appropriate explanation for the differences we observe within the microbial community. We will make these connections clearer in the text.

References

- *Laiho, R. (2006). Decomposition in peatlands: Reconciling seemingly contrasting results on the impacts of lowered water levels. Soil Biology and Biochemistry, 38(8), 2011–2024. https://doi.org/10.1016/j.soilbio.2006.02.017*
- *Laiho, R., Vasander, H., Penttilä, T., & Laine, J. (2003). Dynamics of plant-mediated organic matter and nutrient cycling following water-level drawdown in boreal peatlands. Global Biogeochemical Cycles, 17(2). https://doi.org/10.1029/2002g b002015*
- *Robroek, B. J. M., Albrecht, R. J. H., Hamard, S., Pulgarin, A., Bragazza, L., Buttler, A., & Jassey, V. E. (2016). Peatland vascular plant functional types affect dissolved organic matter chemistry. Plant and Soil, 407(1-2), 135–143. https://doi.org/10.1007/s1110 4-015-2710-3*
- *Bragazza, L., Bardgett, R. D., Mitchell, E. A. D., & Buttler, A. (2015). Linking soil microbial communities to vascular plant abundance along a climate gradient. New Phytologist, 205(3), 1175–1182. https://doi.org/10.1111/nph.13116*
- *Ernakovich, J. G., Lynch, L. M., Brewer, P. E., Calderon, F. J., & Wallenstein, M. D. (2017). Redox and temperature-sensitive changes in microbial communities and soil chemistry dictate greenhouse gas loss from thawed permafrost. Biogeochemistry, 134(1–2), 183–200. https://doi.org/10.1007/s1053 3-017-0354-5*
- *Burd, K., Estop-Aragonés, C., Tank, S. E., & Olefeldt, D. (2020). Lability of dissolved organic carbon from boreal peatlands: Interactions between permafrost thaw, wildfire, and season. Canadian Journal of Soil Science, 13(February), 503–515. https://doi.org/10.1139/cjss-2019-0154*

L861: lower temperatures in the mature bog? I assume that the water in the young bog helps to absorb more heat, but this should be made clear in the manuscript.

We have soil temperature measurements from the site (Figure S1) to show this and provide results from L483-491 on soil temperatures. We also highlight how the thermal properties of the drier surface-peat reduce the temperature in the mature bog (L723).

In the conclusions, it would be useful to mention the relative contributions of acetoclastic and hydrogenotrophic methanogenesis to methane emissions in the two bogs.

This has been addressed above (comment for lines 553-554).

Figures:

Figure 1: would be helpful to label photos d and e with "mature" and "young"

We will add these labels to enable easier interpretation of the plots.

Figure 2: You need a legend showing which colors represent young vs. mature bog. It is better practice to not rely on explanation in the figure caption, but to give the reader essential information in the figure itself. I would suggest using a different shape for the data points for one of the bogs too.

We will add a legend to this figure in panel Figure 2b.

Figure 2(a): It would be helpful to write "CH4 concentrations" and "CO2 concentrations" or use concentration notation ([CO2], [CH4]). I also don't understand the arrows. Why are they pointing to specific points? Don't these represent the top of the permafrost/bottom of active layer? It would make more sense to have additional horizontal lines rather than arrows, unless that looks too busy with the water table levels.

We will change to Dissolved CH_4/CO_2. The unit (μmol L^{-1}) demonstrates that these are concentrations.

The arrows are explained in the figure caption on L523-525; they indicate the thaw transition depth at both sites. We tried using horizontal lines to indicate this, however it made the figure too crowded and messy. We do not focus too much on this transition within our results and discussion, but rather, the water table position as it is more important to our study. Thus, the water table position is prominent in the figure but the thaw transition is not.

Figure 2(f): To help guide the reader, I suggest using background shading and labels to identify the regions of acetoclastic and hydrogenotrophic methanogesis

We appreciate this suggestion, and had previously tried this, but ultimately, the shading made the figure too busy and unclear. Because the range of α_C values for acetoclastic and hydrogenotrophic methanogenesis overlap, the shading becomes difficult. We instead provide a label for each line within the figure and the range associated with each pathway in the text. This is in the same format as Hornibrook et al (1997, 2000).

References

- *Hornibrook, E. R. C., Longstaffe, F. J., & Fyfe, W. S. (1997). Spatial distribution of microbial methane production pathways in temperate zone wetland soils: Stable carbon and hydrogen isotope evidence. Geochimica et Cosmochimica Acta, 61(4), 745–753. https://doi.org/https://doi.org/10.1016/S0016-7037(96)00368-7*
- *Hornibrook, E. R. C., Longstaffe, F. J., & Fyfe, W. S. (2000). Evolution of stable carbon isotope compositions for methane and carbon dioxide in freshwater wetlands and other anaerobic environments. Geochimica et Cosmochimica Acta, 64(6). https://doi.org/10.1016/S0016-7037(99)00321-X*

Figure 4: I like this figure, but it could be arranged differently to take up less space on the page – the circles and triangle legend could go below the color bars, or the entire legend could go on top of the plot and the text can wrap around the right side of the figure.

We will edit this figure, as suggested.

Figure 6: In the text, you say that you assess only thaw stage and distance to water table, but in the caption you say that you explore both biotic and abiotic factors. What else was included in this analysis that is not shown in the plot that seemingly explains more of the methanogenic community variation?

We describe the other factors that went into this analysis in the methods section at L453 of the methods. We will reference this section in the figure caption.

References cited above:

H j, L., Olsen, R. & Torsvik, V. Effects of temperature on the diversity and community structure of known methanogenic groups and other archaea in high Arctic peat. ISME J 2, 37–48 (2008). https://doi.org/10.1038/ismej.2007.84
Throckmorton, H.M., Heikoop, J.M., Newman, B.D., Altmann, G.L., Conrad, M.S., Muss, J.D., Perkins, G.B., Smith, L.J., Torn, M.S., Wullschleger, S.D. and Wilson, C.J., 2015. Pathways and transformations of dissolved methane and dissolved inorganic carbon in Arctic tundra watersheds: Evidence from analysis of stable isotopes. Global Biogeochemical Cycles, 29(11), pp.1893-1910.
Data show a temporal shift in methanogenesis pathways, from acetoclastic in July to hydrogenotrophic in September.
Hultman, J., Waldrop, M., Mackelprang, R. et al. Multi-omics of permafrost, active layer and thermokarst bog soil microbiomes. Nature 521, 208–212 (2015). https://doi.org/10.1038/nature14238
Active layer communities expressed genes and proteins involved in obtaining energy and nutrients from a diversity of aerobic and anaerobic processes and were equipped with functions for survival under freeze–thaw conditions. The bog represented a different scenario with a very high measured rate of methanogenesis and correspondingly high relative abundances of genes, transcripts and proteins involved in methanogenesis, thus demonstrating the potential linkage between molecular data and ecosystem level process rates