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 $\delta^{13}C$ 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 $\delta^{13}C$ values down to 245 cm in the bogs sites, in addition to rates and $\delta^{13}C$ 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 $\delta^{13}C$ 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-by-line comments that are more editorial in nature. Therefore, I recommend major revisions.

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. In= 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. 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.

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., Høj et al., 2008).

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). 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.

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).

Detailed comments:

Abstract

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

L 34-35: "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

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

Introduction

L51: "…are *thought to be* driven by…"

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)?

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.

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.

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

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

L219: remove "deep"

L221: "…devices were installed in each bog…" (since there are only two bogs, you don't need to keep repeating "young and mature bogs")

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

In general, the writing in section 2.2 needs to be improved to make the methods more clear for the reader.

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:

$$F = S*(PV/RTA) \quad (1)$$

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), . . .

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

L 302-303: It would sound better to say: "We measured the δ13C values of gas samples from both the flux chambers and atmospheric background."

Please use proper notation for stable carbon isotope values ($\delta^{13}C$), rather than saying "13C signatures"

L 307: "measured", not "quantified"

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

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

L321: Use "collected" rather than "taken"

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

L 330-331: Again, $\delta^{13}C$ values, rather than "13C signatures"

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

L335: "concentration range"

L337: "measurable range of the system"

L349: "Focusing"

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

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

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 alpha-c values.”

L412: Better subtitle would be “Statistical analyses”

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

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

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.

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

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

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.

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

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

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.

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.

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 methanogeneis pathways to each measurements. Using these estimates would allow for more quantitative comparison between methanogenesis pathways between the young and mature bogs.

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.”

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

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

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.

L718: up "to" the surficial…

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

L738: "also been observed"

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.

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.

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

Figures:

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

Figure 2: You need a legend showing which colors repreent 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.

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.

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

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.

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?

References cited above:

Høj, 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