Review on bg-2021-337
Anonymous Referee #2

Referee comment on "High peatland methane emissions following permafrost thaw: enhanced acetoclastic methanogenesis during early successional stages" by Liam Heffernan et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-337-RC2, 2022

The manuscript by Heffernan et al. looks at the effect of permafrost thaw on methane emission, pathways of methanogenesis and microbial community. They compare depth profiles of young and mature thermokarst bogs and the uncollapsed plateau. Based on isotope values of methane and methanogenic archaeal community composition it is concluded that acetoclastic methanogenesis is more important in the young bog with higher methane emission than in the mature bog.
The major strength of the manuscript of the manuscript is the multifaceted approach: CH4 and CO2 emissions during the whole growing season, isotope values of the gases, depth profiles of dissolved gases, depth profiles microbial communities in peat and porewater at two time points. These all help build a thorough picture of the large methane emission during thermokast formation where changes through the growing season and the peat profile are taken into account, together with the microbial successional dynamics. The manuscript is easy to read ang the figures are clear. I especially like Figure 1 on the experimental setup that shows well both the horizontal and vertical aspects of the sampling setup.

Thank you very much for your comments and feedback!

A potential weakness of the study is that the conclusions of the microbial community analysis focus on methanogens, but the analysis was carried out by primers that amplify both bacteria and archaea. This means that archaea and further methanogens form only a small fraction of the sequence reads. However, the read numbers and the proportion of archaea and methanogens in the dataset are reported well and suggest that there is on average around 900 methanogen reads per sample (I hope I got this right), which should be sufficient to cover methanogen diversity.

Yes, we used universal primers, targeting both archaea and bacteria. We made this choice in order to enable exploration of both the bacterial and archaeal populations before narrowing our focus on the methanogenic community for this manuscript. Because archaea are still adequately captured in our dataset, as the reviewer points out, on (average 1021 methanogen-related reads were captured per sample), we believe that our approach is sufficient for covering methanogen diversity. We will add these values to the methods section.

Major comments:

1. Based on Fig. S2, Methanosarcinales/Methanosarcinaceae/Methanosarcina were defined as acetoclastic methanogens Please clarify the basis of this definition. Methanosarcinales contains methanogens that can use acetate, H2+CO2 and methylated compounds. Even within genus Methanosarcina, not all species use acetate (Kendall & Boone 2006 https://doi.org/10.1007/0-387-30743-5_12). The family Methanotrichaceae consists of obligate acetoclastic methanogens, but based on Fig. S2 they were not detected?

Yes, as the reviewer points out, members of the Methanosarcinales do indeed perform multiple kinds of methanogenesis. We labelled them here as "acetoclastic" since they were only methanogenic members that we detected that were associated with acetoclastic methanogenesis. We did not detect Methanotrichaceae in our samples, using either SILVA or Greengenes (see response below re: SILVA). However, we agree with the reviewer that our labelling of Methanosarcinales as solely acetoclastic is mis-leading. We will re-label these as acetoclastic / hydrogenotrophic in Fig S2.

Reference:

Kendall M.M., Boone D.R. (2006) The Order Methanosarcinales. In: Dworkin M., Falkow S., Rosenberg E., Schleifer KH., Stackebrandt E. (eds) The Prokaryotes. Springer, New York, NY. https://doi.org/10.1007/0-387-30743-5_12

2. Do I understand correctly that the microbial analyses were based on one peat core per site per sampling month (so no replication within sampling month)? I understand that in such a multifaceted study it is not possible to cover everything perfectly, but how is it possible to test the effect of sampling month (L620-621) without replication?

Yes, correct. We only had one peat core per type of peat (YB, MB, peat plateau) per sampling month as well as per depth. We combined all samples between months together (i.e., all samples from YB, MB and peat plateau in June vs all samples from YB, MB and peat plateau in September) for statistical analyses because, utilizing a PERMANOVA, we found that these samples were not statistically significant between sampling months. However, using this approach, we were unable to more robustly confirm if sampling month had a significant impact on microbial community structure. We will add the caveat that we did not have replicate samples to test the robustness of this finding, and as such, additional study, with more samples, is necessary to verify this result.

Specific comments:

L84-85 Please clarify how the statement that two-thirds of CH4 comes from acetoclastic methanogenesis applies to peatlands. As far as I understand, Conrad 1999 is a general prediction, and Kotsyurbenko et al. 2007 cites several references to say most of methane in peatlands and even 100% comes from hydrogenotrophic methanogenesis?

Thank you for catching this! We will modify the text to: "according to a study conducted by Oremland in 1988, two thirds of methane produced in natural systems is attributed to acetoclastic methanogenesis. However, northern peatlands in particular have shown that the acetoclastic methanogenesis pathway is less relevant in producing CH_4 (Rooney-Varga et al., 2007), except for minerotrophic fens, dominated by vegetation such as Carex sp, where, especially in the upper layers of peat, acetoclastic methanogenesis dominates (Galand et al., 2005)".

References:

Oremland R.S (1988) Biogeochemistry of methanogenic bacteria. Biology of Anaerobic Microorganisms (Zehnder AJB, ed), pp. 641–705. John Wiley, New York.

Galand, P. E., Fritze, H., Conrad, R., & Yrjälä, K. (2005). Pathways for methanogenesis and diversity of methanogenic archaea in three boreal peatland ecosystems. Applied and environmental microbiology, 71(4), 2195–2198. https://doi.org/10.1128/AEM.71.4.2195-2198.2005

Juliette N. Rooney-Varga, Michael W. Giewat, Khrystyne N. Duddleston, Jeffrey P. Chanton, Mark E. Hines, Links between archaeal community structure, vegetation type and methanogenic pathway in Alaskan peatlands, FEMS Microbiology Ecology*, Volume 60, Issue 2, May 2007, Pages 240–251, https://doi.org/10.1111/j.1574-6941.2007.00278.x*

L410-411 I think the Greengenes database hasn't been updated for a very long time? This might not be a big problem because methanogen nomenclature has not changed that much recently. However, I am still left wondering if using a newer reference database would have improved the taxonomic affiliations (for example by providing more detailed affiliations or affiliations to unidentified OTUs).

The reviewer is correct, the Greengenes database hasn't been updated since May 2013, however we also used the SILVA database to assign taxonomy to our ASVs and found that both SILVA and Greengenes captured a similar number of archaea (total of 51187 methanogenic read counts attributed to SILVA vs 51141 methanogenic read counts attributed to Greengenes). We will add these lines to the methods section as well. Also, the taxonomic resolution between both databases was also similar, identifying the same kinds of phyla, families and genus, and methanogens (i.e. methanoregula, methanosarcinales, etc,..) Given, these similarities, and the fact that methanogen nomenclature has not changed significantly as the reviewer points out, we ultimately chose to use Greengenes because it was able to resolve more methanogenic families belonging to methanocelalles and Methanomassiliicoccaceae compared to SILVA. We also note that the Greengenes database is still commonly used to explore methanogenic archaeal communities (Vanwonterghem et al., 2016, Lin et al., 2017, Carson et al., 2019).

References

- *Lin, Y., Liu, D., Yuan, J., Ye, G,m Ding, W. (2017). Methanogenic community was stable in two contrasting freshwater marshes exposed to elevated atmospheric CO2. Front Microbiol. https://doi.org/10.3389/fmicb.2017.00932*

- *Michael A Carson, Suzanna Bräuer, Nathan Basiliko, Enrichment of peat yields novel methanogens: approaches for obtaining uncultured organisms in the age of rapid sequencing, FEMS Microbiology Ecology, Volume 95, Issue 2, February 2019, fiz001, https://doi.org/10.1093/femsec/fiz001*

- *Vanwonterghem, I., Evans, P., Parks, D. et al. Methylotrophic methanogenesis discovered in the archaeal phylum Verstraetearchaeota. Nat Microbiol 1, 16170 (2016). https://doi.org/10.1038/nmicrobiol.2016.170*

L600, L606, L611: Are these PERMANOVA results or ANOSIM results? In the methods only ANOSIM is mentioned (L444), and L617 and L621 mentions ANOSIM instead of PERMANOVA. Were both ANOSIM and PERMANOVA used and why? PERMANOVA should be the more robust alternative (see vegan documentation). Please also give the R or R2 values for PERMANOVA/ANOSIM results in addition to p values to give the reader an idea on the magnitude of the difference.

We used both ANOSIM and PERMANOVA as a method to test significance, since they are similar analyses (although one is more robust, as the reviewer points out). The fact that PERMANOVA was left out of the methods was an oversight. All statistical tests using ANOSIM will be converted to PERMANOVA, with the corresponding R2 reported. Furthermore, the results from the PERMANOVA matched those from the ANOSIM test, and so our conclusions do remain unchanged.

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L611 Figure S2b is cited here but Fig. S2 has no a or b panels?

Thank you for catching this typo! Figure S2 is a standalone figure with no a) or b) panels. The text will be modified to reflect this change (referring to Figure S2 rather than S2b).

L620-621, L693 Microbial community diversity -> microbial community composition or microbial community structure (because 'diversity' often refers to alpha diversity).

We will change the corresponding lines to "microbial community structure / microbial community composition" as suggested, to avoid referral towards alpha diversity.

L718 Check missing letter in 'up t the'.

Thank you for catching this typo! This text will be edited to "…despite similar peat stratigraphy amongst the surficial vegetation…"