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 and 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.

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.

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.

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?

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?

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).

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.

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

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

L718 Check missing letter in 'up t the'.