

Response to Reviewer 1:

The authors investigated the effect of the thaw gradient of permafrost in a peatland in Alaska on the CO₂ and CH₄ fluxes, combining environmental variables like water table depth, seasonal thaw depth, soil temperature and soil moisture with changes in microbial community composition based on the 16S rRNA gene sequencing and analysis of methanogenic communities based on the *mcrA* gene and measures $\delta^{13}\text{C}$ -CH₄ signatures released from thaw and from deeper porewater. This study addresses relevant scientific questions within the scope of BG, integrating “biological, chemical, and physical processes in terrestrial life with the geosphere, hydrosphere, and atmosphere”. The experiment is well-designed. The authors concluded that combining $\delta^{13}\text{C}$ -CH₄ signatures with characterisation of the changes in methanogenic communities in response to thawing allowed a more reliable interpretation of methane cycling processes.

A taxonomic shift in methanogenic communities indicated an increasing contribution of hydrogenotrophic methanogen taxa, which may reveal a change towards hydrogenotrophic methanogens observed at sites in the advanced stages of thawing. In contrast, based solely on the isotope results, the false conclusion might be drawn that acetoclastic methanogenesis is dominant. Their study indicates that, based on the model used, approximately 40% of the methane produced is oxidised before leaving the soil profile, altering the interpretation of isotopic results and possibly explaining the discrepancy between the results obtained.

Overall, the results described are interesting; however, there is a lack of a more critical discussion of the methods used.

We thank reviewer 1 for their thorough review of our manuscript and their insightful comments. We have addressed all of their concerns below and feel that their comments have greatly improved the quality of our manuscript.

(1) The estimated percentage of oxidised CH₄ is valid only for the variables assumed in the model.

We thank reviewer 1 for this comment. In the Results section, we clarified that our 40% value is only relevant under the assumptions of our Rayleigh fractionation model by changing the wording to:

“Under the assumptions of the Rayleigh fractionation model and an enrichment factor of -15‰, apparent CH₄ oxidation in the active thaw margin was 40% (95% CI: 35.3-47.2%).” Lines 395-397

In the discussion, we changed the wording to be more explicit about the limitations of this calculation:

“Our results support the potential for this mechanism in the active thaw margin, with the Rayleigh fractionation model estimating that approximately 40% of CH₄ present at 100 cm was oxidized before reaching the atmosphere. This estimate is

dependent on the assumptions and variables used in the model and should be interpreted as apparent rather than absolute oxidation. The enrichment of $\delta^{13}\text{C}$ at shallower depths (Figure 4) is consistent with CH_4 consumption during upward transport in the soil and indicates that oxidation may represent a substantial CH_4 sink and meaningfully alter the isotopic composition of emitted CH_4 .” Lines 580-585

We also softened the wording in the conclusion:

“However, isotopic enrichment of emitted CH_4 and Rayleigh fractionation modeling indicate that CH_4 oxidation likely occurred before CH_4 reached the atmosphere, obscuring the underlying production pathways.” Lines 606-608

(2) The *mcrA* analysis was carried out using forward reads, and

We thank the reviewer for this comment. We stated the reason for only using forward reads in the Methods section but have added a clause to highlight this limitation:

“We used forward reads only because there was not sufficient overlap with the reverse reads after quality trimming, so taxonomic patterns should be interpreted with consideration of the reduced sequence coverage and potential limitations in taxonomic resolution.” Lines 273-275

(3) the presence of a given physiological group, as determined by DNA, should be interpreted with greater caution as evidence of actual functional activity.

We thank the reviewer for pointing out the limitations of our study in this regard and have highlighted these limitations in a few areas of our discussion.

Section 4.2 lines 543-546:

“The strong association between *Methanoregula* relative abundance and CH_4 emissions suggests that this taxon may contribute to CH_4 production under thawing conditions, although community presence alone does not demonstrate metabolic activity or quantify its contribution to CH_4 production. Isotopic variation further indicates additional controls beyond production pathway alone.”

Section 4.2 lines 561-554:

“*Methanothrix* (an obligate acetoclastic methanogen in the Methanotrichaceae family) and *Methanosarcina* (a mixotroph in the Methanosarcinaceae family) did not vary across thaw stages. This could indicate that acetate availability did not increase with thaw progression and/or that acidic peat bog conditions constrained acetoclastic methanogenesis, however, this is speculative since our methods only measured relative abundance, not metabolic activity.”

Section 4.2 lines 568-569:

“Thaw appears to favor methanogenic taxa adapted to H_2/CO_2 substrates, suggesting a potential shift towards hydrogenotrophic methanogenesis under thawing conditions.”

(4) It is also necessary to describe in detail the potential impact of the spatial separation of the Permafrost Plateau, with stable and early thaw stages, from the Active Thaw Margin

(intermediate, advanced) on the results obtained. It is understandable that in the field it is not possible to plan everything as one would in a laboratory, and that data collection is fraught with methodological problems, but these concerns should be discussed.

We thank the reviewer for this comment. We added a short paragraph at the end of section 4.1 to describe the spatial separation of the thaw stages:

“As with all space-for-time substitution designs, differences across thaw stages may also reflect variation in hydrology, vegetation, microtopography, and soil properties between these landscape positions, many of which are associated with increasing time-since-thaw. However, our study design does not allow these effects to be fully separated from thaw itself. Results across the thaw gradient should be interpreted as reflecting both direct and indirect outcomes of permafrost thaw.” Lines 529-532

(5) Raw NGS data should be deposited in an appropriate scientific repository, and the data access number should be included with the article before final acceptance of the manuscript. The journal Biogeosciences favours reliable, public repositories that comply with the FAIR principles and use persistent identifiers

We thank the reviewer for this comment and have deposited all raw NGS data in study in the NCBI Sequence Read Archive (SRA) and have included the accession number in the Code and Data Availability section at the end of the manuscript (BioProject PJRNA1540908).

Other comments:

Gene names, such as *mcrA*, should be written in italics throughout the text, whilst protein names should remain in normal typeface.

We thank the reviewer for this helpful comment and have italicized all gene names in the text.

L.225: Check the sequence for 926R primer. It looks like you pasted the sequence for a different primer (806R). Additionally, in the supplementary materials, you used another primer 896R. Is it correct? The accuracy of the data in the supplementary materials should be checked.

We thank the reviewer for catching these errors. We updated the sequence for the 926R primer: CCGYCAATTYMTTTRAGTTT and added the relevant citation (Quince et al., 2011). We also corrected the Supplemental to read 926R instead of 896R.

L. 293 vs L. 375: what was the final number of 16S rRNA samples: 71 or 62? Methodological data referring to the initial number of samples and the numbers remaining after each stage of data preparation should be presented in a single place

We thank the reviewer for noting this discrepancy in the final number of 16S rRNA samples which was 62. We removed the total number of samples from the Methods section and now only state the final (corrected) sample numbers in the results section.

The interpretation of Figures 6 and 8 could be enhanced by a brief description of the direction of the environmental-factor gradient at each melting stage. In particular, it is worth noting that the distinction between stable/early and intermediate/advanced communities essentially corresponds to changes in thaw depth, groundwater level, temperature, and CH₄ flux. Moreover, the figure caption should define all the environmental vectors shown in the graph.

We thank the reviewer for this comment. We added the following description to the captions of Figures 6 & 8:

“The environmental vectors indicate the direction of increasing values of each variable. The distinction of stable/early and intermediate/advanced communities corresponds to an environmental gradient characterized by increasing active layer depth, water table depth, soil temperature and CH₄ flux toward intermediate and advanced thaw stages.”

In response to the second part of this comment, the captions for both Figures 6 & 8 already define the environmental vectors shown in each graph (i.e., Soil T = soil temperature).

L. 397-400: The authors list five types whose relative abundances change significantly. However, in the discussion, they refer to only one of them (Bacteroidota). Besides, in microbiology, classification at the phylum level is useful for the general characterisation of communities and for comparing communities of microorganisms in different environments. Therefore, it is good that fig. 7 is presented. However, such classifications are usually too general to allow for detailed functional interpretation, as presented in Discussion (L. 467-469), because individual phyla include taxa with highly diverse metabolic characteristics. To identify the groups of microorganisms most likely responsible for the observed changes in community composition, the authors should analyse changes at lower taxonomic levels, such as the family or genus level. It is also possible that a significant trend at the phylum level is largely due to a strong response from one or more dominant taxa at lower taxonomic levels.

We thank the reviewer for this comment. We updated section 4.1 to address the five phyla mentioned in the results, but we only discuss Bacteroidota in further detail to keep our manuscript concise:

“The concurrent declines in Acidobacteriota, Actinobacteriota, and Pseudomonadota and increases in Chloroflexi and Bacteroidota indicate broad restructuring of the bacterial community along the thaw gradient. Among these changes, Bacteroidota increased more than tenfold from stable to advanced thaw stages (Figure 7)...” Lines 507-509

At the request of the reviewer, we investigated which lower-level taxa were responsible for the increase in Bacteroidota and found that this increase was primarily driven by SR-FBR-L83 which belongs to Ignavibacteriales. We added this to our results section:

“Bacteroidota exhibited more than a tenfold increase in relative abundance from stable to advanced thaw stages which was primarily associated with the family SF-FBR-L83 within the Ignavibacteriales.” Lines 430-431

We also added this detail to the discussion and emphasized that community composition cannot identify functional activity:

“Among these changes, Bacteroidota increased more than tenfold from stable to advanced thaw stages (Figure 7), with the increase primarily associated with Ignavibacteriales. Members of this order have been associated with the degradation of complex polymers, including cellulose, hemicellulose, and chitin, suggesting a potential role in the initial breakdown of complex plant-derived organic matter under thawed conditions (Bei et al., 2021; Pan et al., 2023; Lapébie et al., 2019). However, community composition alone does not demonstrate functional activity. Increased substrate degradation could have increased H₂ availability from substrate fermentation, giving hydrogenotrophic taxa a competitive advantage (Philben et al., 2020; Wilmoth et al., 2021).” Lines 509-515

L. 418-419 The non-linear trend in methanogen alpha diversity is potentially interesting, but has not been discussed. Both Shannon diversity and ASV richness decline from stable to intermediate thaw, then increase markedly during the advanced thaw phase. The authors should discuss potential ecological explanations for this phenomenon.

We thank the reviewer for this comment and have added a short paragraph in section 4.2 to discuss the non-linear trend in methanogen alpha-diversity:

“There was a non-linear response of methanogen alpha-diversity to thaw which decreased from stable to intermediate thaw before increasing in advanced thaw. The initial decline from stable to intermediate thaw may reflect more selective environmental conditions as the permafrost thawed, favoring a smaller subset of methanogenic taxa adapted to changing water tables, temperatures, and substrate conditions. However, the increase in diversity with advanced thaw could suggest a transition to a different ecosystem state where greater nutrient availability from groundwater inputs and vegetation, along with increased environmental heterogeneity, support more abundant methanogen communities. However, because our data represent community composition rather than metabolic activity, these mechanisms remain theoretical.” Lines 548-555

Additional changes were made to the manuscript at the request of Reviewer 2. Please see the track changes document.