R1

This study presents a comprehensive dataset to characterize the rates and control of methane production across thermokarst lakes of different ages in interior Alaska. The authors conducted geochemical analyses and incubation experiments with sediment cores collected from a young (BTL) and an older (GSL) thermokarst lake. They observed elevated methane production rates at BTL, which was correlated with higher carbon lability for thermal induced reactions measured by Rock Eval analyses. They discussed how methane production varies with lake evolution and sediment depth, and also the influence of permafrost thawing on microbial activity. By comparing the depth-integrated methane production rates, they propose mechanism of how lake age and thawed talik thickness affect methane production rates and fluxes. The experiments were well-designed and the methods were generally sound. However, I have a few comments that need to be addressed before acceptance of the manuscript.

We thank the reviewer for the constructive comments. We addressed all the concerned as outlined below.

Major Comments:

(1) The stable isotopes of dissolved inorganic carbon in BTL were much more enriched in 13C than GSL, and the authors interpreted this as a result of methanogenesis. However, both methane concentrations and production rates were quite similar at two sites. So I wonder if methanogenesis could lead to such a large difference in 13C-DIC between two sites. Or if this could be related to the source of DIC. I also notice that both data of 13C-DIC and 13C-CO2 were present in Table S1, but I am not sure how were 13C-CO2 measured.

Indeed, one of the interesting features of using the 13C-DIC in thermokarst lakes is that it can really help us point out the differences between those two lakes. We could argue for a different source for the DIC if we were in a different setting, but here the soil does not contain significant carbonates that could “seep” into the DIC pool. Therefore, an in-situ difference seems more logical. Methane production rates at BTL are indeed at the same order of GSL, but they are much greater in large part of the sediments. These increased rates are imprinted in the $\delta^{13}C_{DIC}$, as the isotopic composition is much more sensitive than the concentrations difference and the uncertainty of their measurements. The isotopic composition of GSL suggests insignificant imprint of methanogenesis. This leads its $\delta^{13}C_{DIC}$ values to be influenced from oxidation of organic carbon, which might include methane oxidation, as indicated by the negative values. These points were emphasized and discussed in detail in the revised Discussion chapter.

Regarding the carbon isotopic measurements: $\delta^{13}C_{CH4}$ and $\delta^{13}C_{CO2}$ were both measured from the headspace of the sediment subsamples using the PreCon and Gas Bench II interface of DeltaV GS-IRMS of Thermo. The $\delta^{13}C_{DIC}$ was measured from the porewater sample after adding acid and producing headspace using the Gas Bench. This was clarified in the revised version.

(2) Source of methane. The observed δ13CCH4 values from the incubation experiment were mostly >-60 ‰ particularly in BTL, with many of them >-50 ‰. This seems contrary to the biological production of methane with such positive δ13CCH4 values. Any explanation for this? Do you have a parallel killed control sample for incubations and how do they like?

Methane with stable isotopic compositions that are more positive than -60‰ and even more positive than -50‰ is typical of produced biological methane in high energy environments. i.e.

environments where organic carbon substrates are still quite labile as terrestrial environments. While this is typical of thermokarst lakes where labile Org C is available, it doesn't often happen in marine sediments where the bulk of the literature on methane has been established. Microbial methane in marine sediments is usually more negative because sulfate reduction typically consumes a large portion of the labile organic carbon before methanogenesis can get to it because of the large pool of sulfate from seawater in the pore spaces. So, with labile organic carbon available in thermokarst lakes, more energetically favorable pathways are used i.e. acetoclastic methanogenesis (as compared to hydrogenotrophic methanogenesis that dominates marine sediments). This produces methane less isotopically depleted relative to the source material, but still in the typical range for microbial methanogenesis (Whiticar et al., 1986; Liu et al., 2025). Also, even slight methane oxidation, as suggested above by the 13C-DIC, can contribute to these more positive values, as shown also in other lake sediments (e.g. Sivan et al., 2011). Finally, killed control samples from BTL and GSL and other lakes do not produce any methane at all. We have shown this in previous papers e.g. Lotem et al., 2023 L&O; Pellerin et al., 2022 GCB). This explanation was also added to the revised Discussion.

(3) Following the above comment, it would be nice if the authors could include more discussion about the importance of different methane production pathways.

We agree that the production pathways are an important part of the story. However, this was discussed in our previous studies on these lakes (e.g. Lotem et al., 2023 L&O; Pellerin et al., 2022 GCB). We have therefore clarified shortly the above points in the revised version and also referred the readers to the discussions on pathways elaborated in previous papers.

(4) Similar observations about the control of organic matter on methane production have been reported previously, which can be cited in this work.

Zhuang et al. 2018. Relative importance of methylotrophic methanogenesis in sediments of the Western Mediterranean Sea. Geochim. Cosmochim. Acta 224: 171-186.

Maltby et al. 2016. Microbial methanogenesis in the sulfate-reducing zone of surface sediments traversing the Peruvian margin. Biogeosciences 13: 283-299.

Berberich et al. 2020. Spatial variability of sediment methane production and methanogen communities within a eutrophic reservoir: Importance of organic matter source and quantity. Limnol. Oceanogr. 65: 1336-1358.

We thank the reviewer for suggesting adding these references, and we have updated the text to integrate these works.

(5) It is kind of confusing for the use of methane fluxes in Fig. 7. From my understanding, the production rates did not necessarily mean the emission flux from sediments to the water columns. I did not say the comparison was invalid, but please better justify it.

We totally agree and we updated the figure caption.

(6) Some figures such as Fig. S1 to Fig. S4 that contain important information should move to the main text rather than buried in the supplementary.

We agree that the figures contain important information, and should, at least partly, move to the main text, as also suggested by the second reviewer. Accordingly, we moved Fig. S1 and Fig. S2 to the main text in the revised version.

Minor Comments:

Line 37: Remove the comma.

Done

Lines 54, 317, 412: Revise and format the brackets.

Done

Line 154: What was the purpose of the additional 3 mL sample? Please clarify.

Done

Line 225: Should be "200 ℃".

Done

Figure 2: Please indicate what A, B, C, and D represent in the legend.

Done

Lines 345 and 351: The term in situ should be used consistently and italicized throughout the text.

The term *in situ* was checked throughout manuscript and updated accordingly.

Figure 7: The figure is blurred, and the resolution needs to be improved.

Done

Lines 403–407: This sentence is vague and confusing. When you talk about significant difference, you need statistical analysis to support it.

This sentence refers to methane production rates. We have rephrased this sentence to try to capture the fact that methane production rates that we measured in this study (based on concentration increase) were robust but not enough to differentiate between the rates at GSL and BTL in the deep talik. However, we believe that the two lakes biogeochemical cycling function very differently based on the other lines of evidence mentioned above. This was clarified in the revised text.

Figure 8: Please adjust the figure layout, as the overlapping text affects readability. Please provide statistics and coefficients in the figure or text.

We adjusted the figure layout. We also added the statistics and coefficients to the figure.