

Laurent Biogeosciences Reviews & Response to reviewers:

We thank the three reviewers for their helpful comments and suggestions, which help to improve our manuscript. In response to the thoughtful and constructive comments from the reviewers, we made major revisions to this manuscript throughout all sections and most figures. A summary of these changes is given here, as well as the detailed response to reviewers below. The major changes include:

- 1) *Most of the reviewers pointed out that the results would be more relevant and interesting if the incubation time was longer in order to overcome the lag time before methane production that we observed. Unexpectedly, our results showed that after two months of incubation at 20degC, under anaerobic conditions, only one sample layer produced CH₄. We originally focused on a short-term incubation because we believe that it is essential to quantify C production under realistic timescale of a growing season (~60 days) in Kurungnakh Island because the aim of this study was to quantify the C production during the growing season under wet conditions communities and identify factors (microbial abundance, substrate availability) that would limit CH₄ production in this case study site. However, since only the active layer of the floodplain started producing CH₄, we decided to keep the incubation running to see whether the other cores would produce CH₄.*
- 2) *We've included this additional incubation data from days 68 to 363 of the extended experiment. We incorporated additional incubation data into the revised manuscript throughout and have produced a new figure with the cumulative production over a 363-day period. The revised manuscript now shows anaerobic CO₂ and CH₄ production over a 363-day period.
To summarize the results from this longer incubation period, the floodplain core produced CH₄ within the first 60 days due to the already established methanogen communities, as we showed in the initial manuscript. After 6 months of incubation, the permafrost layers from the Yedoma cores started producing CH₄. This important result was not included in the earlier manuscript version with the shorter incubation time, as noted by all the Reviewers. Old Figure 4 shows that the permafrost layer in P15 and P16 has a lower methanogen concentration than P17-A, so we attribute the difference in lag time primarily due to the time required for the Yedoma samples to activate the methanogen communities and to produce CH₄. We hypothesize that the lack of methanogens in the P16-A and P15-A could be due to the dry condition induced by the landscape position. This indicates that methanogenesis is unlikely established after permafrost thaw in these sediments unless colonized by methanogens and the lack of response of CH₄ to the glucose addition and continued anaerobic CO₂ production also reduces the likelihood that substrate availability limits CH₄ production despite the lower C abundance compared with the floodplain soil (Table 1).
Additionally, the results section has been clarified to distinguish missing versus zero data within the microbial dataset.*
- 3) *The introduction has been revised to address the concerns of the reviewers, to be more precise and specific about permafrost carbon, the permafrost carbon feedback, and earlier incubation studies. We both narrow the focus to findings from earlier incubation experiments and elaborate on the specifics of the findings regarding the landscape position. In detail, we include a definition, discuss what differs across landscape positions, and expand on the links between microbial abundance and CO₂ and CH₄ production.*
- 4) *We substantially revised the methods section to include more details addressing the criticisms of the reviewers and clarified terminology throughout the manuscript (e.g. "production").*
- 5) *We substantially revised the discussion in order to address the criticism from the reviewers about the overly broad implications of this study despite the limited number of permafrost cores. It is now substantially shorter. We remove Figure 5 (the conceptual diagram) in the revisions. We clarify throughout the manuscript that this is a case study based on permafrost cores from Kurungnakh Island, Siberia, Russia. We shortened and narrowed the discussion to a case study, which aims to understand and quantify the potential C production in this limited region by integrating information about the influence of landscape position, microbial data, and, soil parameters to understand the factors controlling C production in this site within the Yedoma dominated region. We would like to note, however, the importance of these particular permafrost cores collected at a remote field site in Arctic*

Siberia, and this data given that it is no longer possible to re-sample at these sites in the foreseeable future.

Reviewer 1:

The manuscript of Laurent and co-workers present data from an anaerobic short term incubation study of six samples from three different permafrost affected soils in a transect from ice-complex deposits into a floodplain in the Lena Delta, Russia. The authors incubated the samples at 4°C and 20°C, and measured for 60 days CH₄ and CO₂ concentrations. At the end of this incubation, they added glucose and measured for another week. Furthermore, they measured the abundance of mcrA genes (methanogenes) and pmoA genes (aerobic methane oxidizers).

We urgently need to better understand the consequences of thawing permafrost in the northern hemisphere on the global carbon cycle. In this respect, the study is concerned with an unquestionable important topic.

However, the main result of the study is that except for one sample, no consistent methane production was observed and that methanogens were still in the lag-phase during the short-term incubation experiment. This means that the experiment was too short to gain information about methanogenesis in most of the samples. Consequently, there is only limited information in the presented Q10 values for methanogenesis or the calculated CO₂:CH₄ ratios. The remaining results are mainly a confirmation of established knowledge. I suggest that the authors better elaborate, which new information or insights the reader gets from this study.

As explained in the main response, we aimed to simulate wet summer conditions during the growing season. Our results showed that after two months of incubation, only the active layer of the floodplain produced CH₄. The absence of CH₄ production for the other active layers at 20degC was unexpected. The lack of methanogens and CH₄ production after two months show that the methanogen communities were not established. Even though most of our samples did not produce CH₄ within the 2 months, we still believe it is important to capture the behaviour of these samples for C production during the growing season. We continued to measure this incubation experiment after the two months presented in this study, as mentioned in the overall summary above. These additional measurements from days 68 to 363 have been added to the study in response to this concern, raised both here and by the other reviewers.

We note that after 6 months of incubation, methane was produced in more samples. Methane was produced consistently across the cores in the permafrost layers at 20degC after 6 months, in both the floodplain (P17) and the Yedoma cores (P15, P16). The active layer of the floodplain core produced methane at both 20degC and 4degC throughout the incubation. However, the active layer of the well-drained Yedoma core (P15) neither produced methane after one year, or at 20degC or with a substrate addition of glucose. The methane production rates of the samples at 4degC were overall very low. The samples that started producing CH₄ after one year of the incubation were those for which methanogenic communities were measured at the beginning of incubation. Therefore, although the methanogenic communities in the Yedoma permafrost layers needed more time to become active, these samples have a high potential for CH₄ production after a long thaw period.

This study is a case study in Kurungnakh Island. The geologic history of this site is well known, however few studies have worked on the potential CH₄ and CO₂ production after permafrost thaw at two temperatures. Here, we contribute to quantify and understand the potential C loss after permafrost thaw along a slope profile from the active and permafrost layers, and compare them with a floodplain in Kurungnakh Island. Our findings show that the landscape position plays a key role in the establishment of methanogen communities during the growing season. It is likely that under field condition, only the floodplain produce CH₄ during the growing season, this season might not be long enough for the upland and slope to establish methanogen communities. However, with longer thaw time they produce CH₄ in the permafrost layer. To our knowledge, these landscape position patterns have not been clearly shown for permafrost sites in Siberia.

Furthermore, the description of methods is in part insufficient to evaluate their suitability

We have substantially revised the methods based these comments to add further details.

and the references repeatedly do not support the statement in the text (see detailed comments).

We have substantially revised the methods and the references.

The discussion should substantially be shortened. In its current form its very lengthy, extensively repeats results and itself.

The revised discussion is both shorter and narrower and clarifies the importance of this study as a case study for the Lena Delta region.

The microbial data on methanogenesis are interesting but the importance of the microbial data about aerobic CH₄ oxidation remains obscure, since the experiments were done under anoxic conditions.

Thank you for this comment. We agree that the quantification of methanotrophs was not relevant for the study. We removed the data in the revised version.

Finally, I suggest clearly differentiating between production and emission. The data presented here are data on CH₄ and CO₂ production. There are no data on in situ CH₄ and CO₂ emissions. Particularly in the discussion, 'emission' is used for both the production in highly artificial laboratory incubations and in situ CH₄ and CO₂ fluxes. But incubations give only very limited information, if any, about in situ fluxes.

Thanks for this comment. We have changed "emission" by "production" throughout the manuscript unless otherwise necessary.

Specific comments:

L33: 822 Pg is the C in permafrost, not in permafrost soils. Please clearly differentiate between permafrost (permanently frozen) and permafrost soils (soils containing permafrost).

We revised this sentence and the introduction to specify differences in C stocks between permanently frozen and permafrost soils.

L34: Obu et al. 2019 reports that permafrost affected soils cover 14.6% of the northern hemisphere. 21.8% of the northern hemisphere is the permafrost region, i.e. the region where permafrost might be found (but not necessarily underlying 100% of the soils). Please clarify.

Revised this sentence during revisions and now focus on the extent of the permafrost C stock.

L38: Here is a misunderstanding of permafrost. The upper part of permafrost does not thaw in summer, in this case it would not be permafrost (see the definition given in line 34-35).

Revised as suggested: "the upper part of the permafrost affected soils thaws (active layer)".

L44: This sentence is unclear. Who is "providing decomposable C"?

We have significantly revised the introduction. This paragraph is deleted in the revised introduction.

L50: The review of Schuur et al., 2015 does not present data on aerobic CH₄ production. Better cite original data.

Removed during revisions (see major comment #3 above).

L79: The studies cited here report GHG production rates from incubation studies, which do not give much information about 'C emissions released from different landscape forms'. Please clarify.

We have significantly revised the introduction. This paragraph is deleted in the revised introduction (see major comment #3 above).

L81: The meaning of this sentence is unclear. Do you mean that microbes with a certain function may be active even if the redox conditions are not suitable for the respective process? Please clarify.

We have significantly revised the introduction. This paragraph is deleted in the revised introduction.

L85: This is a bit strange question in the context of this study. There are numerous studies on the importance of microbes and redox conditions on e.g. methane production and oxidation, but this study is not addressing redox conditions. Furthermore, in situ C emissions are strongly affected by vegetation, which is not mentioned at all. Please clarify.

We have significantly revised the introduction. This paragraph is deleted in the revised introduction.

L90: To prevent confusion, I recommend to replace 'emission' by 'production'. In that case, the reader does not expect data on in situ GHG fluxes.

We have changed emissions to production throughout the manuscript.

L133: Fuchs et al., 2018 determined the bulk density 'by dividing the dry weight of a sample by its original volume'. How may the bulk density be determined by the water content of the soil without knowing the volume of the sample? Particularly when the samples are not water saturated. Please explain.

Thank you for pointing this out. First, we apologize for the wrong reference, which may have caused this confusion. We corrected the reference by "Fuchs 2019". In this thesis, Fuchs plotted the bulk density in relation to the absolute water content of one thousand samples and was able to calculate a transfer function from absolute water content to dry bulk density. To calculate the bulk density, he divided the dry weight of a sample by its initial volume. With the data from the bulk density and the water content, they established a transfer function to determine the bulk density of a sample when the volume is unknown. As we explained in the manuscript, we did not calculate the bulk density, but estimated it according to this transfer function. This transfer function is in the supplementary material XY. In addition, the samples used for our study come from the same area as most of the samples used to establish this correlation. We changed the manuscript and explained the bulk density estimation in more details.

L162: Please explain in more detail how the CO₂ and CH₄ production rates were determined. Did you consider DIC in the soil water? At pH > 7 this might be more than in the headspace. How did you calculate rates from single concentration measurements? I could not find a method in the cited reference (Robertson et al., 1999) that enables the determination of production rates from single gas concentration measurements.

For samples with pH>7, water contents are very low (Table 1), so we assumed that a negligible amount of CO₂ was stored as DIC in the sample water. However, we agreed that this might underestimate C mineralization and now mention it in the text.

We did not calculate the production from single gas measurements, but used the change in concentration of CO₂ and CH₄ over the incubation time. We first converted the concentration from ppm to µg/L using the ideal gas law and then used a linear regression between each measurement point to calculate the change in concentration over time, then calculated the mineralisation rate with the headspace and the volume of the dry content. (after Robertson et al., 1999, Exchangeable Ions, pH and Cation Exchange Capacity, p. 266-267)

L164: As the equation is written G_f gives the factor by which glucose addition increases gas production, the unit is not %.

The equation has been changed to have %.

L205: P16-F has a EC of 479 µS cm⁻¹.

Revised as suggested.

L215: <0.3% L219: ... P17-A P17-F

Revised as suggested.

L301: Could you give the detection limit of your mcrA quantification? Can you measure 76 gene copies per gram?

The detection limit of the mcrA quantification is $4,3 \times 10^3$ copies per gram. We edited Figure 4 by adding the expression “non detected” and “below detection limit”.

L329: Is there a concentration of carbon below which it may not be decomposed? Please explain.

C mineralization depends on the quantity and the availability of the OC. This means that samples with very low C content can have high turnover if the C is easily decomposable. Therefore, it is complicated to say that there is a threshold below which C mineralization does not occur. Here, we clarified that our C and N contents from our samples are in the same range as those in the study of Strauss et al (2013), and thus, the bioavailability of OC should not be a limited factor for C mineralization.

L332ff: This is correct as long as sufficient sulphate or nitrate is available, which is generally not the case in terrestrial soils. The reason for low methanogen abundance is probably rather the high redox potential in these soils.

We deleted this part in the revised version.

L385: Please explain what you mean by ‘favourable to C mineralization’.

We compared our soil characteristic results with other studies to elucidate whether TOC and N content were limited factors for C mineralization. Other studies showed that with similar, or even lower, TOC and N content C mineralization was possible, and therefore we concluded that C mineralization was not limited by the quality and/or quantity of the OM. Hence, by “favourable to C mineralisation” we mean “not limiting for C mineralisation”.

We added more recent studies to the references to support this statement.

L417: Which discrepancies do you mean? ‘Cumulative emissions’ (production) are the consequence of the observed production rates. Please explain.

We revised this sentence to explain that there is high variability in CH₄ production rates within floodplain environments. We compare our results of CH₄ production (cumulative production and production rates) with the ones from Herbst (2022). However, the sentence construction is not clear and we have changed it to make it easier to understand.

L419: What do you mean by ‘methane conditions’. Please explain.

We did not mean “methane conditions”, but “methane production”. Thanks for noting this mistake.

L448f: In a completely anaerobic incubation experiment, landscape position might not be relevant since CO₂ production depends on C and N availability. However, at in situ conditions the redox potential differs and hence likely also CO₂ production. Please clarify.

Thank you for this remark. Here, we were referring in anaerobic incubation experiment. We clarified this sentence.

Fig 5: This figure gives no new information or concept. It is quite similar to several figures that have been published previously, even from the same region. Furthermore, the current manuscript gives no information about in situ fluxes. I suggest removing it.

We agreed that this figure was not relevant. We removed it.

Reviewer 2:

This paper aims to address some major knowledge gaps on a consequential subject--namely, the controls on potential carbon feedbacks from warming in permafrost regions. These controls are poorly understood due to the many interacting factors (e.g. temperatures, redox conditions, organic matter quality, composition of legacy

microbial communities, etc.) that affect CH₄ and CO₂ release, and this paper does contribute somewhat to the knowledge base. However, I found this paper to be lacking in terms of the strength of the findings.

First of all, only three cores were analyzed, despite the heterogeneity of the landscape. This is partially compensated by comparisons with other studies, including an extensive discussion of the results in comparison to other cores from the same region analyzed by Herbst (2022).

As explained in the general answer, this study is a case study in Kurungnakh Island. The geologic history of this site is well known, however few studies have worked on the potential CH₄ and CO₂ production after permafrost thaw at two temperatures. Here, we contribute to quantify and understand the potential C loss after permafrost thaw along a slope profile from the active and permafrost layers, and compare them with a floodplain in Kurungnakh Island. Our findings show the landscape position plays a key role in the establishment of methanogen communities during the growing season. It is likely that under field condition, only the floodplain produce CH₄ during the growing season, this season might not be long enough for the upland and slope to establish methanogen communities. However, with longer thaw time they produce CH₄ in the permafrost layer.

As mentioned above, our study is a case study of Kurungnakh Island. We worked with three cores, but we analysed those cores at two depths and incubated them at two different temperatures. We would like to point out that there is still a lack of data regarding incubation studies in Siberia, and only few studies have been working with the active layer and permafrost layer, and two incubation temperatures. Finally, to establish pan-arctic dataset and compare data throughout the Arctic, smaller studies are essential.

However, the bibliographic entry for Herbst (2022) did not include a link to that manuscript, and I was unable to find it through a web search. Is that manuscript planned to be published in the near future?

The Master's thesis from Herbst (2022) is now available via a permalink on the AWI preprint server EPIC, we added the link in the bibliography section.

Second, the conclusions about microbial abundances do not seem supported by the sparse amount of data shown in Fig. 4. This issue might be partially addressed by better delineating zero abundances vs. truly missing values in the figure, but that depends on how much of the data is actually missing. (see specific comments below)

Thank you for this comment. Based this comment and the one below, we edited Figure 4 and clarified which data was missing and which is zero. We revised the text to clarify that no methanogen was detected for the samples without values and that these should be interpreted as zero values. Methanogen were found only in permafrost layers of P15 and P16, and the active layers of P15-P17. After one year of incubation, the results show CH₄ production only for the samples where methanogens were detected (except P15-A).

Finally, the Discussion and Conclusions include numerous statements about how the results can improve predictions of greenhouse gas release under permafrost thaw, but the most significant result (higher methanogenesis in the floodplain active layer) doesn't seem to directly address the effects of permafrost thaw, as the P17-A sample is from the active layer of a floodplain--unless that floodplain location is part of a thermokarst feature, or that sample was formerly part of the permafrost before active layer deepening; but this is unclear from the site description.

Given this comment and the feedback from the other reviewers about the discussion and the shortcomings of the incubation length (major revisions summary #2, #5), we have changed the scope of the discussion to focus more on the relevant results. Additionally, we are now able to quantify the potential effects of permafrost thaw by demonstrating that the lag time before methane production in these thawed upland soils exceeds the length of the current growing season, so CH₄ production will likely not be an immediate effect of this yedoma thaw under an active layer deepening scenario unless there is some abrupt thaw and landscape change.

Specific comments:

li 83-85: Remove extraneous reference text outside the parentheses (3 occurrences).

Thanks for the comment, we have corrected the text.

li 132-133: After looking at Fuchs et al. (2018), I could not find any information about the "relationship between absolute water content and bulk density."

Thank you for pointing this out. First, we apologize for the wrong reference, which may have caused this confusion. We corrected the reference by "Fuchs 2019". In his thesis, Fuchs determined the bulk density and the water content of one thousand samples from the Lena Delta. To calculate the bulk density, he divided the dry weight of a sample by its initial volume. With the data from the bulk density and the water content, they established a transfer function to determine the bulk density of a sample when the volume is unknown. As we explained in the manuscript, we did not calculate the bulk density, but estimated it according to this transfer function. In addition, the samples used for this study come from the same area as the samples used to establish this correlation. We changed the manuscript and explained the bulk density estimation in more details.

li 142-143: How much sterilized tap water was added to the low-moisture samples?

We calculated the amount of water to add to reach 30% of water according to the water content, and the weight (dry and wet) of the samples. Therefore, the amount of added water differs for each sample.

li 164-165: Does the "cumulative emissions" used for calculating the Glucose Factor also include the time before the day 60 glucose additions? If so, this factor might be overly sensitive to random variations in production rates before the additions. (Also, the wording of this sentence is unclear. Suggested rephrasing: "The impact of glucose on CH₄ and CO₂ production was quantified as a glucose factor, calculated using the cumulative C emissions at 67 days:")

This is a very good point. In the revisions, we will calculate the glucose factor only after glucose addition and see if we have different values. If so, we will modify the results and the discussion based on the results.

li 167-168: Related to the above, the phrase "total CH₄ production rate at i days" implies an instantaneous rate measured at several (i) timepoints, as opposed to cumulative only at 67 days (from line 164). Which method was used? (If only cumulative, then the phrase "at i days" seems extraneous.)

Thank you for this comment. We used only the cumulative C emissions. Therefore, we deleted the phrase "at i days" and replace the words "production rate" by "production".

li 173: I assume "after glucose addition" means at Day 67? Please clarify.

We clarified the text by adding "67 days".

li 202: Typo ("Kuskal-Wallis" should be "Kruskal-Wallis").

Thanks for the comment, we corrected the text.

li 216: "the lowest [C:N] were in P17": Did you mean P16 (Table 1)?

Yes, that is correct, we meant P16, thanks for your comment.

Table 2: Typo in second-to-last row of first column (first occurrence of "P17-F" seems like it should be "P17-A").

Thanks for the comment, we corrected the text.

li 247: It would be clearer to cite Table 3 (from which the 42.53 ± 15.79 value is directly derived) in addition to Figure 3.

Revised as suggested.

li 251-252, "P15 and P16 behaved similarly, with higher CH₄ production for the active layer at 4 °C than at 20 °C": This doesn't appear to be true for P16, based on its active layer Q10 being >1 (see Table 2).

Thank you for pointing out this mistake. P16-A has higher CH₄ production at 20°C. We corrected the manuscript.

li 253, "...and no difference for the permafrost layer": This also seems surprising, given that for P16 (Fig. 2b), the blue line (permafrost at 4 degC) is noticeably higher than the red (permafrost at 20 degC).

Even though the blue line is higher than the red one, the values of the production rates from the samples at 4°C are still very low and cannot be considered as a real methane production from the samples (samples are still in lag time). In addition, the error bars for the permafrost layer at 4°C and 20°C overlap, meaning that the values cannot be statistically considered as significantly different (supported also with the statistic test). We discussed this in the section 4.1.

Figure 2: Several comments:

- Dashes are missing from the lines in Fig. 2b.

Thank you for this remark. We modified the figure.

- In Fig. 2c, due to the very high Active Layer 20 degC values, it's impossible to see what's happening with the other samples. Would it be possible to create another version of this panel (perhaps for the Supplement) with the very high CH₄ values removed, so that the differences in the other lines can be seen?

Thank you for this good remark. We added a zoom in version in the supplementary figures to see the behaviour of the other samples.

- Some of the plots, particularly Fig. 2f, show negative CO₂ production rates. How would you explain these?

These negative production rates appear mainly the days where we flushed the samples, therefore it is likely due to the flushing. In the revisions, we replace this figure with one showing the cumulative CH₄ and CO₂ production where the trends are clearer.

Figure 4, li 320-321, "Absence of values for some samples is due to either low DNA concentration or failure in qPCR run.": Can you indicate on the figure (maybe using a symbol) which empty values were due to which cause (low concentration vs. failed qPCR run)? This delineation of zero vs. missing values would help a lot with the interpretation of this figure, as a zero (or below detection limit) concentration still represents the information that concentrations were low, as opposed to not measured at all.

Thank you for this helpful comment. We edited Figure 4 based on your comment, by adding the expression "below detection limit", or "not detected".

li 330: Wrong table references for CO₂ production (and move "Table 1" reference to line 329 or 331 about C and N contents)?

Revised as suggested, and Figure 2 added to support "low CO₂ production throughout the incubation".

li 390-391, "methanogen concentration before incubation showed the highest numbers in the floodplain (Figure 4c)": I can't tell whether this statement is supported by Figure 4, as the zero values aren't distinguished from an absence of measurement (see Figure 4 comment above). If all the empty values are actually missing (i.e. due to

failed qPCR), then no direct comparisons of the pre-incubation samples would be possible between P17 and the other sites.

Half of the values from the “pre-incubation” were not measured. In the revised version, we carefully compared the values and specified that the microbial results were in line with the CH₄ production, but missing values (likely due to too low concentration) make it difficult to establish a comparison with certainty.

li 396, "little change in methanogen quantity after 60 days of incubation": This doesn't appear true for the P16 permafrost layer incubated at 20 degC, which had much higher mcrA (Fig. 4b).

Yes, that is correct. We changed the text according to the comment and specify that this does not apply for P16 at 20degC.

li 404, "after permafrost thaw": Are the portions of the floodplains sampled by Herbst (2022) part of thermokarst features?

The samples incubated by Herbst (2022) were not part of thermokarst features. They incubated samples from the floodplains, and samples from the permafrost layer belonging to the same cores.

li 451: Typo; "three time" should be "three times".

Revised as suggested.

li 461-463: Invalid sentence structure; did you mean for the end to read "CH₄ production will likely increase" ?

Yes, you are right, thanks for pointing out this mistake.

li 472, "methanotrophic": Did you mean "methanogenic" ? or both?

We meant “methanogenic”. We changed the manuscript.

Supplementary Figure 1: Which incubation temperature is shown here (or is it an average of both)?

The incubation temperature shown in this figure is 20°C. We added the temperature to the figure title and the figure.

Reviewer 3:

This manuscript presents data from a short-term anaerobic incubation study. The authors present results from six individual samples from three different locations. The goal of the study is to understand the potential effects of temperature, “landscape position”, and the addition of glucose to CH₄ and CO₂ production from the soil samples. The premise of the study is interesting and timely.

However, the incubation time of the experiment appears to have been too short, as the methanogens were still in lag-phase. Based on the information presented in the study, I found the links between their results and conclusions unconvincing.

As explained in the main response, we aimed to simulate wet summer conditions during the growing season. Our results showed that after two months of incubation, only the active layer of the floodplain produced CH₄. The absence of CH₄ production for the other active layers at 20degC was unexpected. The lack of methanogens and CH₄ production after two months show that the methanogen communities were not established. Even though most of our samples did not produce CH₄ within the 2 months, we still believe it is necessary to capture the behaviour of these samples for C production during the growing season. We continued to measure this incubation experiment after the two months presented in this study, as mentioned in the overall summary above. These

additional measurements from days 68 to 363 have been added to the study in response to this concern, raised both here and by the other reviewers.

I think this could be improved by adding more details and specificity to the methods section, in particular.

We have substantially revised the methods based on these comments to add further details.

More details about the “landscape position” of the sample site would be helpful (i.e. slope, aspect, vegetation cover, etc). I think it would be helpful to consider the scope of the experiment when formulating conclusions.

There is a table with descriptions of the three different sites (see supplementary table 1) in the supplemental materials. In the revisions, we have added pictures of the sites and more details regarding the distance to the rivers surrounding the sample sites. In addition, during the revision, we clarified throughout the manuscript that this is a case study in the Lena River Delta, proposed a hypothesis that these trends may occur at other sites within the permafrost region, and generally narrowed the scope of the discussion and conclusion.

Six samples (from three locations) were incubated for ~70 days. While interesting, there are not enough data points presented in this study to draw meaningful conclusions for permafrost landscapes as a whole.

As mentioned above, our study is a case study of Kurungnakh Island. We worked with three cores, but we analyzed these cores at two depths with laboratory replicates and incubated them at two different temperatures. We would like to point out that there is still a lack of data regarding incubation studies in Siberia, and only a few studies have worked with the active layer and permafrost layer, and two incubation temperatures. Finally, to establish a pan-Arctic dataset and compare data across the Arctic, smaller studies are essential. Nevertheless, we agreed that it is not possible to draw conclusions for permafrost landscapes and use this case study to generate a hypothesis that the landscape position is an important control on the potential for methane production with permafrost thaw in the broader permafrost region.

I strongly suggest that the authors simplify the sentence structure throughout the article. I think that the article can be substantially shortened by removing redundancy and superfluous information/sentences. Most instances of conjunctive adverbs (however, finally, on the other hand, likewise, etc.) should be removed.

Thanks for this feedback. Overall, we found the specific comments from reviewer 3 to be really constructive and helpful particularly in the discussion section. We have carefully revised the manuscript to simplify the sentence structure throughout and to focus it on the question of potential CO₂ and CH₄ production across different landscape positions in this permafrost landscape.

Also consider the difference between GHG “emissions” and “production” and change your wording accordingly.

We changed “emission” by “production” when necessary and reworked the sentence structure.

Line 10: “release more greenhouse gases”. More compared to what? I suggest you remove “more” or be more specific.

Removed during revisions in order to simplify and be more precise.

Line 11: “to address the large heterogeneities of GHG releases”. Spatial heterogeneities? Temporal heterogeneities? I suggest you be more specific here.

Removed during revisions as above.

Line 11: I suggest you reformulate this sentence. Your study is not really addressing “large heterogeneities of GHG releases”. You are trying to understand what the relationship might be between GHG emissions and soil parameters and what factors might be causing large ‘spatial’ heterogeneities in GHG emissions from permafrost landscapes.

Removed during revisions in order to simplify and be more precise.

Line 13: Two depths? Do you mean sediment from two depths from three Lena Delta cores? I suggest you be more specific/clarify.

From each core, samples were collected at two depths. We specified by replacing “two depths” by “Active layer and permafrost layer samples from three cores...”

Line 15: “Samples from located in upland or slope positions”. Typos here.

Revised as suggested.

Line 16: Same typo as above “from located in”

Revised as suggested.

Line 18-19: I suggest you rewrite/simplify this sentence and make it easier to read.

Revised as suggested.

Line 20: In addition, our study identified different CO₂ production...

Revised as suggested.

Line 23: Suggestion: Climate change is causing increasing temperatures and permafrost thaw, which might lead to increases in the release of greenhouse gases CO₂ and CH₄.

Revised as suggested.

Line 36: “Due to the low temperatures, the organic matter...”

Revised as suggested.

Line 36: The statement that all permafrost soils act as a C sink is misleading. Check out:

Elder et al. 2021 <https://doi.org/10.1029/2020GB006922>; Anthony et al., 2021 <https://iopscience.iop.org/article/10.1088/1748-9326/abc848/meta>; etc.

Removed during revisions.

Line 40: Consider using an oxford comma throughout the article. It is the standard and will really improve the clarity of your sentences.

Thank for your comment.

Line 47: The paragraph beginning on line 47 is two sentences long. Consider merging it with the preceding paragraph.

Removed during revisions as above.

Line 53: Consider rewriting this sentence to reduce the number of commas and clauses. Currently, it is difficult to read.

Removed during revisions as above.

Line 57: Consider eliminating both instances of “able to produce”. It is not necessary (i.e., not all soils produced the same quantity of CH₄...)

Removed during revisions as above.

Line 59: Suggestion: “Even though several factors controlling C decomposition have been ...”. I would consider rewriting this sentence to make it more neutral.

Removed during revisions as above.

Line 61: What do you mean by “a single temperature”. As opposed to temperature profile with depth? Can you be more specific?

Removed during revisions as above.

Line 62-63: Suggestion: “Therefore, the relationships between different temperatures, landscape positions, and C production under anoxic conditions are not well understood.”

Removed during revisions as above.

Line 62: Consider defining “landscape position”. It is not clear to me what you mean by this term. Can you be more specific? Same for “different temperatures”. Do you mean the natural spatial heterogeneity of ground temperature in permafrost landscapes (i.e., cooler temps under forest cover, temperature profiles with depth, etc.)?

In the revision, we defined landscape position as a specific geomorphology component of the landscape (e.g. upland, mid-slope, floodplain, as shown in Fig 1b), which affects factors like soil moisture and site drainage. As well, we specified that “temperature” stands for the temperature of permafrost thaw.

Line 64: consider replacing “form” with “type” and “amount” with “quantity”

Removed during revisions as above.

Line 64: released

Removed during revisions as above.

Line 72: Consider adding Hughes-Allen et al., 2021 to the list of references as it discusses specifically differences in GHG emissions from different types of thermokarst lakes. <https://doi.org/10.1002/lno.11665>

Thank you for the reference but we now have removed this paragraph to focus on incubations.

Line 77: I think you are overstating the lack of studies/info here. A quick google search turned up many studies from the last three years describing both experimental studies and in-situ analyses.

Removed during revisions as above.

Line 84: Typo. “King”

Removed during revisions as above

Line 84: Consider finding a different term for “C control”. It’s not clear what you mean here.

Removed during revisions as above.

Line 85: Typo. Citation doubled “Koch, Knoblauch, et Wagner 2009”.

Revised as suggested.

Line 86: You start discussing methods here without yet discussing the objectives of your study. Consider reordering these sentences/paragraphs.

We revised the introduction to address the objectives of the study first.

Line 88: You mention landscape positions often, but again, you never define this variable. Please consider defining/being more specific.

Thanks for this critique. We now clearly define this and focus on this aspect in the revised introduction.

Line 89: Define “short term”. Days, weeks, months?

Removed during revisions as above.

Line 93: microbesif microbial community composition? Quantity of microbes? Please be more specific.

Removed during revisions as above.

Line 107: I suggest you make “The soil sampling was carried out...” the beginning of a new paragraph.

Revised as suggested.

Line 107: I strongly suggest that you break up this sentence into two shorter sentences. End the first sentence where the colon is.

Revised as suggested.

Line 109: You can remove “after excavating the active layer”

Revised as suggested.

Line 112: Does “with a well-drained upland soil profile” apply to the topography of all three sites? The sentence should be restructured so that it ends with “respectively”.

“well-drained upland soil profile”, applies for the upland and the slope.

Line 110-113: You mention twice that the cores were chosen based on their location within the local topography. I think you can reorder/rework these sentences to make it flow better.

As suggested, we reworked the sentence construction.

Line 115: replace “another” with “one”

Revised as suggested.

Figure 1: I suggest that you add Figure sublabels (i.e., a, b) so that you can reference them in the figure caption.

Thanks for the suggestion. Revised as suggested.

Line 127: “Electrical conductivity and pH were measured from pore water for better comparison between samples.” Better comparison compared to what? A different type of method? This sentence isn’t super clear to me.

We revised this sentence in the new version.

Line 128: I think this equation would be more readable if it was presented in normal equation form (i.e., inline equation)

Revised as suggested.

Line 131: how many samples is one series?

The instruments can both analyse 90 samples for one series. For each analyse (TC and TN), the samples were measured together. We revised as this clearly caused confusion.

Line 132: Can you describe the relationship?

Thank you for pointing this out. First, we apologize for the wrong reference, which may have caused this confusion. We corrected the reference by “Fuchs 2019”. In his thesis, Fuchs determined the bulk density and the water content of one thousand samples from the Lena Delta. To calculate the bulk density, he divided the dry weight of a sample by its initial volume. With the data from the bulk density and the water content, they established a transfer function to determine the bulk density of a sample when the volume is unknown. As we explained in the manuscript, we did not calculate the bulk density, but estimated it according to this transfer function. In addition, the samples used for this study come from the same area as the samples used to establish this correlation. We changed the manuscript and explained the bulk density estimation in more details.

Line 135: I suggest that you use “organic material” rather than “organics”.

Revised as suggested.

Line 136: I suggest that you remove “In the end”.

Revised as suggested.

Line 142: I suggest that you keep the passive voice here that you are using throughout the methods. For example, “Sterilized tap water was added to samples with a moisture content of less than 30% to limit the effect of gas dissolution (Henry’s Law).

Revised as suggested.

Line 152: I suggest you say, “The effects of glucose are usually observed within less than 48h”.

Revised as suggested.

Line 153: How are you measuring the gas? And do you mean one week as in 7 days or one working week as in days.

Described in the following section.

Line 156: Ok now I see the gas section. Maybe just add that it is describe in the following section.

Revised as suggested.

Line 162: I suggest that you eliminate “Finally”

Revised as suggested.

Line 172: I suggest that you keep the tone neutral here. Eliminate “We decided”. Just explain what you did.

Revised as suggested.

Line 170-175: I think this paragraph can be cleaned up to be more specific and easier to understand.

Based on your comment, we simplified and made this paragraph more understandable.

Line 202: check spelling Kuskal-Wallis

Revised as suggested.

Line 207: I suggest “All soil samples, except P15-F, had a pH between 6.5-7.5.”

Revised as suggested.

Line 211: Do you mean TOC weight percent?

Yes, we specified this in the method section.

Line 242: ...CH₄ production at either 4°C or 20 °C

Revised as suggested.

Line 242: CH production rates were consistently below...

Revised as suggested.

Line 249: I don’t think you mean emissions here, rather production

Revised as suggested.

Line 255: Very long sentence. I strongly suggest that you rewrite it to focus on succinctness and clarity.

We reworked the sentence construction to have something more succinct and easier to read.

Results section: Limit the results section to the actual results. Currently, you are mixing in some discussion elements. These should really be saved for the discussion section.

Revised as suggested.

Line 296: Error in figure cross reference

Thank you for the remark. We corrected the cross reference.

Line 325: I suggest you write 1-2 overview discussion sentences rather than restating the results section.

Thanks for this feedback, we incorporate this in the newly revised discussion section.

Line 325-330: This section is really heavy on words like “nevertheless, likewise, however, etc.” These should be used more sparingly for easier reading. I also believe that you can reduce this paragraph to two sentences.

Thanks for your comment. We revised the manuscript according to your comment.

Line 330-339: Very nice paragraph and interesting. Can you expand more here, especially the relationship between C and N and anaerobic CO₂ production?

C mineralization is mainly controlled by the bioavailability of the OM and microbial communities. Under anaerobic conditions, diverse microbial communities are able to decompose the OM to CO₂. Therefore, the CO₂ production is mainly controlled by the quality (N) and the quantity (TOC) of the OM. Here, our results followed the trend of the TOC contents.

Line 340: Can you clarify the sentence?

We expected a large increase of CO₂ production rate after the glucose addition. However, only a slight increase of CO₂ production was observed at 20degC for P15 and P16. Therefore, we tried to understand why the CO₂ production did not increase after the glucose addition.

Line 351: what is lysis?

In this case, lysis means decomposition. We replaced this word in the manuscript as it caused confusion.

Line 352: A concluding sentence would be nice/helpful to wrap up the ideas you present in the preceding section.

Thanks, we revised the discussion to pay careful attention to the sentence and paragraph construction.

Line 363: I think it would be helpful to define lag time much earlier in the paper.

We defined “lag time” after the first use, e.g. section 3.2.1. but because it is important, we discuss this further in the revised introduction.

Line 373: I don't think it's appropriate to make this leap from your study to this general statement that glucose availability is not a driving factor for CH₄ production in mineral soils.

That is true. We removed this sentence in the revision.

Line 380-382: Interesting ideas. Can you expand more here, especially on topographic position? I am not seeing the link between topographic position and the results/factors influencing CH₄ CO₂ production that you discuss in this section.

In this section we discuss the behaviour of CH₄ production under anaerobic conditions. Here, we explain that the lack of CH₄ production for P15 and P16 was mainly due to no established methanogen communities. If the methanogen community was small, but established, we would expect to have community growth after the glucose addition. However, since nothing happened, we concluded that this result was an additional support to our hypothesis, e.g., the absence of CH₄ production for those samples was because the methanogen was not active (or not active enough). We explain this lack of activity with the actual environmental conditions of sample sites due to the landscape position (cf section 4.2). Based on this feedback, we discuss this point more thoroughly in the revised discussion

Line 385: There are many newer available articles which discuss this subject. Check out

Roy Chowdhury, Taniya & Berns, Erin & Moon, Ji-Won & Gu, Baohua & Liang, Liyuan & Wullschleger, Stan & Graham, David. (2021). Temporal, Spatial, and Temperature Controls on Organic Carbon Mineralization and Methanogenesis in Arctic High-Centered Polygon Soils. *Frontiers in Microbiology*. 11. 10.3389/fmicb.2020.616518.

Thank you for the reference, we added more recent references.

Line 400-405: Rather than summarizing the Herbst study so specifically, can you give a more general summary and explain how their result relate to yours and why they might differ?

Yes, that is a good idea. Overall, Herbst et al. (2022) uses samples collected in the same area as ours but exclusively from floodplain soils, and did very similar incubation experiments using anoxic conditions at 20 C. Therefore, we consider this study as a comparison to complete our dataset. The results of Herbst (2022) showed rapid establishment of methanogen communities in floodplains. The lower production rate might be due to lower TOC and TN contents.

Line 406: I suggest “confirm” rather than “are in line with”

Revised as suggested.

Line 410-415: I think this paragraph can be streamlined and made more concise. Please be specific about how the results/conclusions of the studies you discuss are related to your results.

Thanks for this suggestion. We revised this paragraph to focus on the results from this study, including references to the figures showing these results, the conditions observed in the field. Then we use the observations from other field-based measurements as a comparison. The in-situ measurements show similar trends to what we observed in our incubations. We also compare their explanations for these high CH₄ fluxes to our findings.