Author response to the associate editor to clarify implemented revisions after referee comments on ms egusphere-2025-4756: "Methane releases across the Laptev Sea signaled by time-integrated biomarkers of aerobic methane oxidation"

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We gratefully thank the referees for thoroughly reviewing and giving constructive feedback that helped to clarify the significance and importance of this manuscript during revision. Additionally, we thank the associate editor for additional suggestions that also helped to improve the manuscript clarity. We have thoroughly reviewed our author response and the referee comments once again to further improve the quality of our writing. The detailed implementations are described in this response letter. In addition to these changes, we have also corrected minor grammatical errors, added a recently published article to the reference list, and clarified a few sentences throughout the manuscript.

All reviewer comments are included below in *black italic font* each followed by our detailed author responses, formatted as indented blue text. Citations of our implemented changes in the manuscript are formatted as *indented italic blue text*. When citing whole paragraphs, newly added text that was not in the submitted ms text is highlighted with ***blue italic bold text*** (and is also included in the tracked changes document). Line numbers refer to line numbers in the clean revised manuscript version.

Implemented changes after suggestions from Referee #1:

Lines 23-24, 76, and 81: The authors interpret the d13C values of the hopanoids as a proxy for "methane release". In principle, yes, there does appear to be a correlation between methane concentrations and d13C values, however, this has not been thoroughly tested in diverse environmental settings and with different methanotroph communities. It would be more accurate to say that this proxy reflects aerobic methane oxidation, and therefore enhanced methane cycling. I would recommend rephrasing this in both the abstract and the manuscript by replacing "methane release" with "enhanced methane cycling" or "methane oxidation".

> We agree and have rephrased "methane release" and "emissions" as "*enhanced methane cycling*" throughout the manuscript when referring to the biomarker proxy, including in the manuscript title.

Lines 268-275: Based on these measurements the methane concentrations are highly variable between sites as you mentioned. Is most of the methane being emitted from the Laptev Sea from a diffusive or ebullitive flux? Previous studies have shown that aerobic methanotrophs are usually less efficient with oxidizing methane when the methane flux is mostly ebullitive. If the flux is mostly ebullitive rather than diffusive, do you expect this to influence the proxy signatures and your interpretations? Coming back to my previous comment, is the water fully oxygenated at all these sites? If so, do you expect most of the methane-oxidation to occur in the water column and sediment, and how would this influence the proxy signatures you obtain?

We have clarified the oxygenation of the water column and sediments related to the second part of the question. The newly added text is highlighted with ***italic bold text*** as follows (rev. ms line 91):

"*...**with the water column oxygen saturation ranging from 70-100% during expeditions between 2015 and 2020 (Xie et al., 2023). This combined with high CH_4 concentrations** indicat**es** the general possibility for AeOM."*

Lines 335-336: Not all members of the family Methyloligellaceae are considered methanotrophs. The majority of the classified strains appear to be methylotrophs, including Methyloligella and certain strains of Methyloceanibacter. You can still mention these as potential candidates for Type II methanotrophs, but you should add a sentence somewhere indicating that these are not all necessarily methanotrophs. Further, do you know whether Methyloligella and Methyloceanibacter have the capacity to produce hopanoids? It would be good to confirm this as this can influence some of your interpretations. You can check this by searching for the sqhc gene (accession no. WP_038942977.1) on the NCBI database, and then checking to see if either of these species contain the gene (see Richter et al. 2023 Biogeosciences for more details).

As a response to these helpful suggestions, we have corrected the sentence in lines 347-349 to:

*"**Candidates of Type II MOB were of the family Methyloligellaceae¸ with one identified genus known to produce hopanoids (Methyloceanibacter). However, it should be recognized that not all Methyloligellaceae are necessarily methanotrophs**."*

To ensure *Methyloceanibacter* was the only MOB-II hopanoid producer included in the results, we also revised the related results of isotopic mass balances, relative abundances of MOB-II (Fig.4; Fig.S1), and CH_4-related hopanoid endmembers (Fig.3).

Lines 383-387: Since it is unclear whether some of the MOB you have classified as Type II MOB are methanotrophs, I would say it is difficult to fully exclude terrestrial inputs in the ILS region. Do you have any other independent biomarkers that can give an indication of how much of your signal here is derived from terrestrial sources? In your next paragraph, you seem to indicate that terrestrial inputs are relatively high, so you might still have a terrestrial signal from your methanotrophs.

To clarify that the MOB-II are from marine sources and to more clearly discuss the terrestrial influence of MOB-II input we have revised the section "*Varying hopanoid sources in the Inner Laptev Sea hotspot region*" (lines 411-413) as follows:

"*Methyloceanibacter constituted the **only genus** of MOB-II in the ILS (Fig. S1) and **have to our knowledge only been isolated from marine systems** (Takeuchi et al., 2014, **2019; Vekeman et al., 2016**). "*

Line 392: Check your reference for the "10% bacteria". Belin et al (2018) was not the first paper to report this, this was already shown in previous studies.

The original sources, and the review article are now all included in this sentence as follows (lines 426-427):

"(Ourisson et al., 1979; Fischer et al., 2005; Racolta et al., 2012; Belin et al., 2018)."

Line 401: Could the high methane concentrations in the ILS region also be derived from the Lena River rather than in situ production in the ILS, or a combination of both? Based on your figure 3, it seems like the methane in this region should be very depleted. Could this tell us a bit more about the source of the methane in this region?

The clarification that the methane signal is from the marine system and not the Lena River is now included as follows (lines 435-437):

"It should also be noted that the high CH_4 concentrations in the ILS originate from an old biogenic source in the coastal region (Sapart et al., 2017) and not riverine input of CH_4 (Shakhova & Semiletov, 2007; Shakhova et al., 2010, 2014, 2017)."

Line 417: change to "here we show"

This sentence has been changed to *"here we show"* (line 397).

Line 429: Change "Our display" to "Our biomarkers"

This sentence has been changed to "*Our biomarkers*" (line 451).

Figure 1: The numbers for the stations are hard to read in the figure and against the subsea permafrost shading. Consider making the numbers black to make them easier to read. It would also be helpful if you could indicate the outer, mid-, and inner Laptev Sea regions in this figure.

Figure 1 has been revised according to the suggestions as follows:

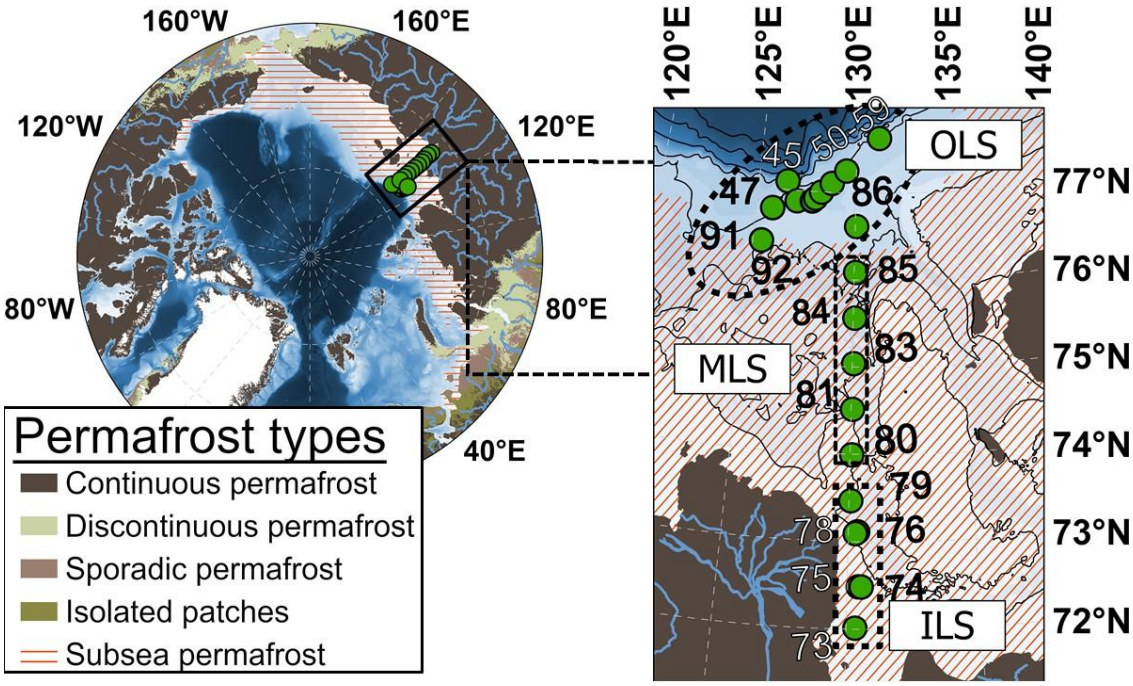
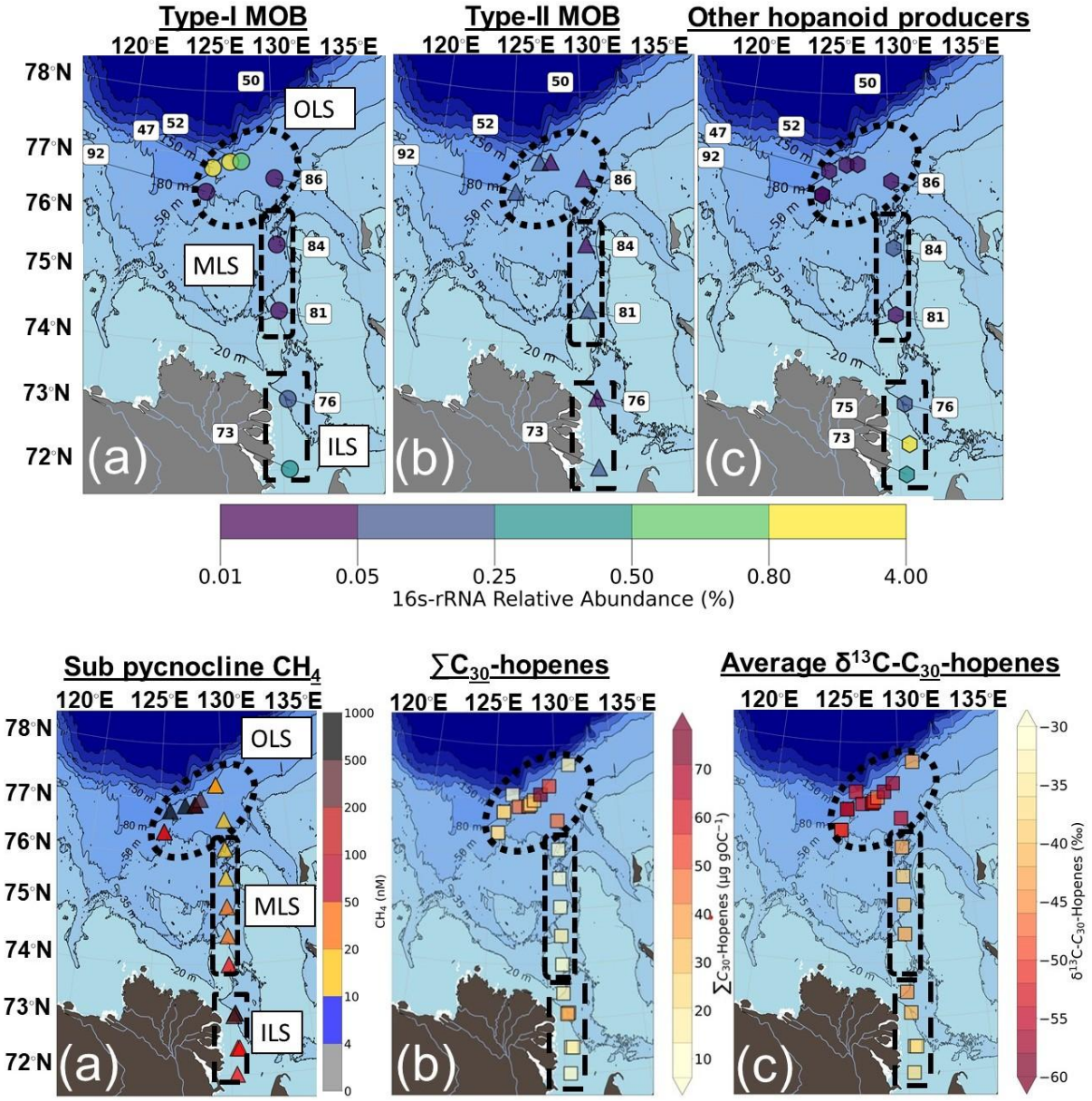


Figure 3: In the caption you say the shaded "gray zone" but in the figure it looks "green" to me. Consider changing this. All of these figures are showing the same things but the varying scales are a bit confusing. Consider making this into one large figure that contains all of the same information to make it easier to read.

The text in the Fig 3 caption "*shaded gray zone*" has been changed to the correct color "*shaded green zone*".

Figures 4 & 5: It would be helpful if you could indicate the ILS, MLS, and OLS regions on these figures. It would make the figures easier to interpret and to know which station numbers and data points belong to which region.

Each subregion of the Laptev Sea is now indicated in figures 4 and 5 as follows:



Implemented changes after suggestions from Referee #2:

- *Hopanoids are not unique to methanotrophic bacteria. Although this study includes measurements of bulk δ13C-OC and presents a stable carbon isotope mixing model with defined end members, the interpretation would be strengthened by more explicitly contextualising the δ13C- C30 values within the existing AeOM literature. In particular, it would be helpful to include reported δ13C- C30 ranges from other methane seep studies where hopanoids have been used as proxies for methane release. This information could be included in both the Introduction and then in the discussion, comparing results with existing literature values. In addition, providing δ13C- C30 ranges from related environments, such as peatlands and lacustrine systems which are mentioned in the Introduction, would offer useful broader context. By providing this broader context it will make it easier to assess the AeOM results and improve the clarity and interpretability of the results throughout the manuscript.*
- *In relation, the introduction would benefit from more clearly outlining previous studies that have applied the δ13C- C30 proxy to infer methane release, including a brief summary of their main findings. At present, it is not entirely clear whether this proxy is well established or in early stages, only one study is mentioned (van Winden et al., 2020. This ambiguity comes from the third paragraph of the introduction, which is a key section for framing the proxy and one of the most important paragraphs in the manuscript. It would benefit from being rewritten.*

> The point is well taken; to address the comments, we rewrote and clarified this important part of the Introduction (lines 77-86). The newly added text is highlighted with ***italic bold text*** as follows:
>
> *"Generally, **enhanced CH_4 cycling** has been linked to decreasing $\delta^{13}C$ values of hopanoids (e.g., **Inglis et al., 2019**; van Winden et al., 2020; **Yan et al., 2025**). Therefore, $\delta^{13}C$-hopanoids may be used as a time-integrated **signal of enhanced CH_4 cycling**. In particular, hopanoid carbon chain lengths $\leq C_{30}$ are often more depleted in ^{13}C compared to C_{31}-hopanoids and therefore closely associated with MOB production of hopanoids (Inglis et al., 2019). **While the proxy has been widely used to study methane dynamics in past climates**, contemporary system calibrations of $\leq C_{30}$ hopanoids only exist in peatlands (**$\leq C_{30}$ hopanoids between -21 and -45‰;** Inglis et al., 2019) and lacustrine systems (**diploptene ranging from -38.8 to -68.8‰;** Davies et al., 2016). The lack of a large-scale comparison of CH_4 and hopanoids in marine systems leaves uncertainties when interpreting $\delta^{13}C$-hopanoids as tracers of CH_4 cycling in geological records. In summary, $\delta^{13}C$-hopanoids in oxygenated surface sediments can be an informative tool to constrain a time-integrated **signal of enhanced** CH_4 **cycling on a years-decade scale**, complementing observations of CH_4 concentrations in **seawater which is highly variable over much shorter time-scales**."*

- *In line of the points raised above, the sections discussing the inner shelf region (ILS) would benefit from reconsideration and restructuring. Based on the δ13C- C30 values*

reported for ILS, the AeOM signal appears relatively weak. Values that are only moderately depleted (e.g., not more negative than ~−40‰) quite likely reflect mixing between multiple bacterial carbon sources rather than a distinct methanotrophic signature, making it difficult to draw firm conclusions. Strengthening this discussion would likely require clearer consideration of (i) the relative roles of aerobic versus anaerobic methane oxidation, (ii) potential mixing with terrestrially derived organic matter delivered by the Lena River, and (iii) the limitations of the δ13C- C30 proxy in this specific setting where you have a large river. In addition, the interpretation in terms of MOB I versus MOB II would be more convincing if it was explicitly contextualised using the δ13C- C30 literature ranges as well as typical values for non-methanotrophic bacteria. Providing these comparative ranges would make it much easier for readers to assess whether the ILS signal is consistent with methanotrophy or more likely reflects mixed sources.

> As clarified in the author response, most of these distinctions were already included in the submitted ms. Nevertheless, in order to clarify the in situ signal of MOB and the moderate depletion of δ^{13}C-hopanoids we have revised this section and added a new comparison to Freeman et al. (2014) in the new version. The newly added text is highlighted with ***italic bold text*** as follows (lines 411-421):
>
> *"Methyloceanibacter constituted the **only genus** of MOB-II in the ILS (Fig. S1) and **have to our knowledge only been isolated from marine systems** (Takeuchi et al., 2014, **2019; Vekeman et al., 2016**). Thus, the MOB-II **present** in our study is likely an in situ signal of AeOM rather than of coastal influence as was found in the Kara Sea/Yenisei River (de Jonge et al., 2016), despite MOB-II generally dominating terrestrial ecosystems (Hanson & Hanson, 1996; Inglis et al., 2019**). Noteworthy, $\delta^{13}C$-C_{30} hopenes values ~-40 ‰ has in contemporary peatland settings been interpreted as a strong indication of enhanced CH_4 cycling related to MOB-II (Inglis et al., 2019). In fact, even $\delta^{13}C$-diploptene as high as -35 ‰ has been interpreted as an indication of AeOM in the Cariaco Trench (Freeman et al., 1994), due to co-occurring autotrophic biomarkers with a $\delta^{13}C$ composition of >4 ‰ higher than diploptene (Freeman et al., 1994). Therefore**, the less depleted $\delta^{13}C$-C_{30} hopenes, yet in similar concentrations to the OLS, can be an indication of MOB-II and **shows that** a proxy-derived CH_4 signal in coastal regions needs thorough system knowledge to depict the source. "*

The results appear to show a consistent geographic trend from the inner shelf region (ILS) to the outer shelf region (OLS), with organic carbon concentrations decreasing, δ13C- OC values becoming less negative (from approximately −26‰ to −23‰), and δ13C- C30 values becoming more depleted (from ~−39‰ to ~−52‰). The manuscript would benefit from a more integrated discussion of these spatial trends and the processes that may control their origin, as this could help unify the Results and Discussion sections and strengthen the overall interpretation.

> The geographic trend of organic carbon and δ^{13}C-organic carbon have been implemented in the revised manuscript. The newly added text is highlighted as ***italic bold*** as follows (lines 428-435):
>
> *"This study thereby indicates that a dilution from non-methanotrophic terrestrial sources may contribute to the higher $\delta^{13}C$-C_{30} hopenes, due to the vicinity of the Lena*

River delta and higher relative abundance of non-methanotrophic hopanoid producers compared to MOB. ***This is further strengthened by δ^{13}C-OC around ~26‰, indicating a larger terrestrial loading to the sediments of the ILS.*** *Hopanoid source apportionment displayed that 78±7 % of hopanoids in the ILS are related to non-methanotrophic hopanoid synthesis* ***and 16S-rRNA data indicate that the "other hopanoid producers" are partly of terrestrial origin (Fig.4; Fig.S2)****. Consequently, the presence of hopenes more enriched in ^{13}C is also an indication of an additional contribution from non-methanotrophic hopanoid sources, rather than reflecting lower CH_4 cycling in this region."*

2. Line by line comments

Introduction

Line 71- 73: This sentence is quite long and could be rephrased for clarity, as it is currently difficult to follow the main point. More generally, this paragraph would benefit from including a reported range of δ13C- C30 values for AeOM from the literature, which would help clarify how AeOM is being diagnosed in this study.

Line 75: This sentence illustrates the point above. When stating that "a larger presence of methane has been linked to decreasing $\delta^{13}C$ values of hopanoids," you need to specify the $\delta^{13}C–C_{30}$ range associated with high methane presence, based on published studies.

Line 76: The text mentions that this approach is "generally used," but only one relatively recent reference (Van Winden et al., 2020) is cited. Including additional references would help support the idea that this is a well-established and widely applied method.

Line 76: The phrase "intensity of time-integrated" could benefit from a brief clarification. A short explanation or example would help readers better understand what is meant here.

Line 79: Introduce with few words or a short sentence the concept of contemporary system calibrations for hopanoids and why are they important for interpreting geological records.

Line 96: Since time-integrated proxies appear to be a central concept in this study (and are also linked to the point raised in line 76), it would be very helpful to introduce and explain this concept more explicitly earlier in the manuscript.

We rephrased the paragraph related to the comments above. The newly added text is highlighted with ***italic bold text*** as follows (lines 71-87):

"Thus, hopanoid analyses are frequently combined with compound-specific isotope analysis of stable carbon isotopes ($\delta^{13}C$) to relate these biomarkers to CH_4 cycling. ***The isotopically depleted stable carbon isotopes of CH_4,*** *$\delta^{13}C$-CH_4 (as low as -90 ‰; Milkov & Etiope, 2018)* ***is used*** *to differentiate CH_4-derived hopanoids from other sources.* ***Thereby, C_{30}-hopanoids (hopanoids with 30 carbon atoms) have been used as a proxy to infer enhanced CH_4 cycling in past climates*** *(e.g., Hinrichs, 2001; Hinrichs et al., 2003; Birgel and Peckmann, 2008; Sun et al., 2022; Blumenberg et al., 2024; Yan et al., 2025)* ***and contemporary systems*** *(Davies et al., 2016; Inglis et al., 2019). Generally,* ***enhanced CH_4 cycling*** *has been linked to decreasing $\delta^{13}C$ values of hopanoids (e.g.****, Inglis et al., 2019****; van Winden et al., 2020;* ***Yan et al., 2025****). Therefore, $\delta^{13}C$-hopanoids may be used as a time-****integrated signal of***

***enhanced CH_4 cycling**. In particular, hopanoid **carbon** chain lengths ≤C_{30} are **often** more depleted in ^{13}C compared to C_{31}-hopanoids and therefore closely associated with MOB production of hopanoids (Inglis et al., 2019). **While the proxy has been widely used in past climates**, contemporary system calibrations of ≤C_{30} hopanoids only exist in peatlands **(≤C_{30} hopanoids between -21 and -45 ‰;** Inglis et al., 2019) and lacustrine systems (**diploptene ranging from -38.8 to -68.8‰;** Davies et al., 2016). The lack of a large-scale comparison of CH_4 and hopanoids in marine systems leaves uncertainties when interpreting $\delta^{13}C$-hopanoids as tracers of CH_4 cycling in geological records. In summary, $\delta^{13}C$-hopanoids in oxygenated surface sediments can be an informative tool to constrain a time-integrated **signal of enhanced** CH_4 **cycling on a years-decade scale**, complementing observations of CH_4 **concentrations in seawater which is highly variable over much shorter time-scales**. "*

Line 99: When referring to "high concentrations," specify what is being measured (e.g. methane concentrations) for clarity.

Line 101: The second hypothesis is somewhat unclear. When referring to "lower concentrations of higher hopanoids," it is not clear whether this refers to lower $\delta^{13}C–C_{31}$ values. Since the manuscript focuses primarily on $\delta^{13}C–C_{30}$, this section may benefit from clarification and consistency in terminology.

The paragraph related to the two comments above was revised.newly added text is highlighted with ***italic bold text*** as follows:

*"We hypothesize that known CH_4 ebullition hotspots in the Outer Laptev Sea and Inner Laptev Sea (OLS and ILS), where >1000 nM dissolved CH_4 concentrations have been observed (Shakhova et al., 2010, 2014; Steinbach et al., 2021), have high concentrations **of AeOM-tracing C_{30} hopanoids (diploptene, hop-17(21)-ene, neohop-13(18)-ene and diplopterol) with** low $\delta^{13}C$ values. In contrast, we hypothesize that the mid-shelf region without any discovered CH_4 hotspots, yet with dissolved **CH_4 concentrations** in the range 10-60 nM (Fig.2) display lower concentrations of **C_{30}** hopanoids **more enriched in ^{13}C**. "*

Line 158: While the sampled interval (1–2 cm slice) is provided, it would be helpful to also report the mass of material used for δ13C- OC analyses.

The mass of sediment used for $\delta^{13}C$-OC analyses has been added (line 167).

Line 160: Please clarify what is meant by "Ag capsules." If "Ag" is an abbreviation, it should be defined at first use.

Ag capsules has been changed to *"silver capsules"* (line 168).

Line 218: Indicate how much sample material was used for 16s rRNA analyses

The typical mass used for 16S-rRNA analyses has been added (line 227).

Line 283: The results suggest that OC concentrations decrease from ILS to MLS to OLS. If this reflects the geographic order, presenting the results consistently in that sequence will improve clarity of the manuscript.

The order of presenting the results has been changed throughout to 1) OLS, 2) MLS and 3) ILS to stay consistent with the rest of the results. Therefore, the order of discussion in 4.2 has been changed as well to match the results.

Line 290 to 294: There is a relatively large uncertainty associated with OLS values, can you provide an explanation? Perhaps in the methodology. Additionally, the statement that OLS and ILS have similar concentrations, while MLS and ILS show no significant difference, is somewhat confusing and could be rephrased for clarity.

The results have been revised. The newly added text is highlighted with ***italic bold text*** as follows (lines 299-304):

"The highest concentrations were found in the OLS (35±20 µg gOC^{-1}; n=15; Fig.5). ***Relatively high*** *$\sum C_{30}$-hopenes concentrations were also present in the ILS, with no significant difference compared to the OLS (18±11 µg gOC^{-1}; n=6; Fig.5; Supplementary Table 4). In contrast, the concentrations of $\sum C_{30}$-hopenes were significantly lower in the MLS (10±4 µg gOC^{-1}; n=4; Fig.5)* ***compared to*** *the OLS, but* ***did not show*** *significant****ly*** *different* ***concentrations*** *compared to the ILS (Supplementary Table 4).* ***The large standard deviation of hopanoid concentrations in the OLS and ILS is likely related to the presence of main "hotspot" stations, causing a log-normal distribution of the data****. "*

Line 303: If −57‰ represents the most depleted δ13C value observed at OLS stations, state more explicitly. You might also consider only reporting mean values with standard deviations, or instead providing ranges for OLS, MLS, and ILS, rather than listing individual station values. Whichever approach is chosen, stay consistent across the results section.

The presentation of the results has been revised according to the referee suggestion and now only includes averages and standard deviations. The newly added text is highlighted with ***italic bold text*** as follows (lines 312-313):

"The $\delta^{13}C$-C_{30}-hopenes were lowest in the OLS with a mean±standard deviation of -52.9±4.3 ‰ ***across the region*** *(Fig. 5)."*

The conclusions might benefit from more cautious wording, emphasising that the δ13C- C30 –methane release proxy appears robust for MLS and OLS, but that in areas influenced by large terrestrial inputs (such as the Lena River), this proxy likely needs to be complemented by additional lines of evidence.

The related section of the conclusions has been revised. The newly added text is highlighted with ***italic bold text*** as follows (lines 489-493):

"This is attributed to a higher relative abundance of MOB-II that utilize both CH_4 and CO_2 for hopanoid production, isotopic mixing with non-methanotrophic bacteria, ***partly of terrestrial origin****, producing hopanoids, but also less active AeOM in the ILS hotspot.* ***This indicates that complementary evidence to $\delta^{13}C$-C_{30} hopanoids and thorough system knowledge is necessary in coastal settings in the vicinity of large rivers.*** *"*

Several sentences throughout the manuscript are quite long (three lines or more). Breaking these into shorter sentences would improve readability.

We have shortened several sentences throughout the manuscript, see details in the tracked changes document.

Line 301: "Strikingly" may sound somewhat strong; "remarkably" could be a suitable alternative.

Strikingly has been changed to "*remarkably*" (line 311).

Figure 2: A scale bar should be included, reduce panel labels (a, b, c, d) size. The methane concentrations colour scale makes it difficult to distinguish values between ~50 and 300 nM; adjusting the colour scheme may help. Indicating OLS, MLS, and ILS on the map and summarising key spatial trends in the caption would improve interpretability.

The panel sizes have been included and the color gradients have been changed to ensure better visibility. A new scale bar for concentrations has been added.

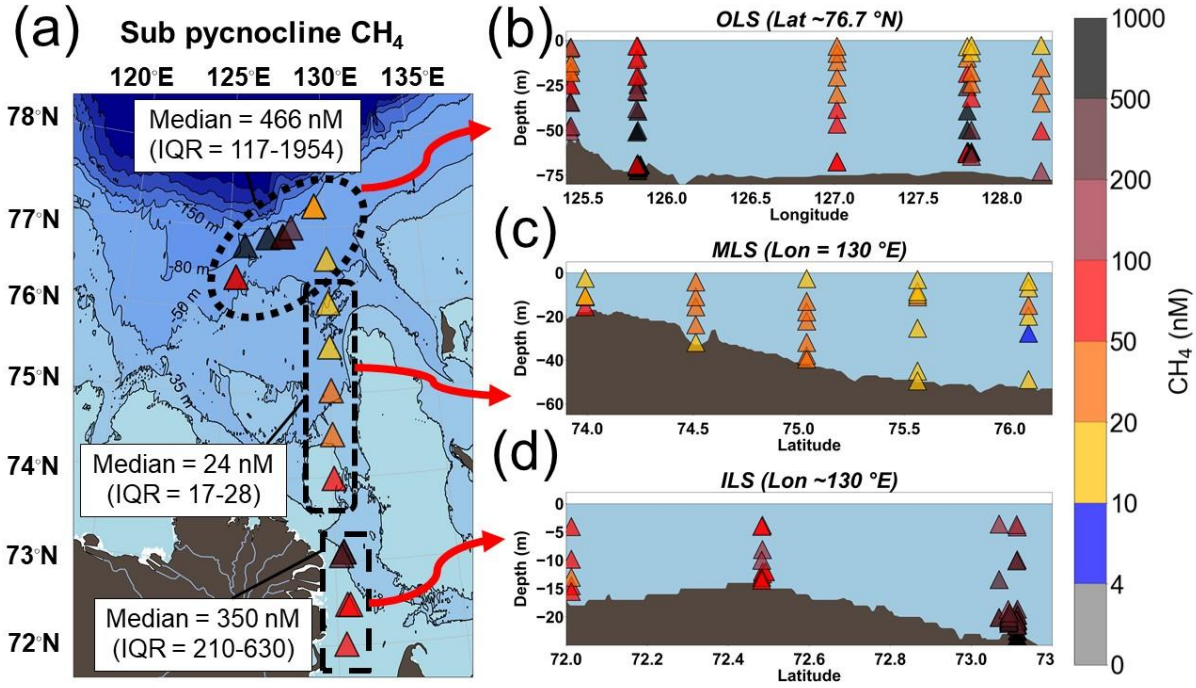


Figure 3: Panel labels (a, b, c) should be included directly in the figure, not only in the caption. The shaded area referred to as "grey" in the caption appears green in the figure and should be made consistent. Please also indicate the literature sources of the hopanoid end-member values in the caption and clarify that the shaded areas are based on semi-quantitative estimations.

Panel labels have now been included in the figure. All the literature sources of the endmembers are now included both in supplementary tables S6 and S7 and in the figure 3 caption.