Author response to referee comments and the resulting revisions of ms egusphere-2025-4756: "Methane releases across the Laptev Sea signaled by time-integrated biomarkers of aerobic methane oxidation"

Reference: https://doi.org/10.5194/egusphere-2025-4756

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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.

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*.

***Anonymous Referee #1*: https://doi.org/10.5194/egusphere-2025-4756-RC1**

This study utilizes lipid biomarker signatures from surface sediment samples to obtain a time-integrated signal of methane release events in the Laptev Sea. The results suggest that methane oxidation occurs across the Laptev Sea shelf, including the mid-shelf region that was previously known as a region of low methane emissions. This study is relevant for the community, and for better understanding methane cycling in the Arctic. Overall, it is very well-written and very thorough. I have several minor comments and suggestions regarding the manuscript.

> We are grateful for the supportive comments about the importance of this work for the community and how it improves the knowledge about Arctic methane cycling. Referee #1 provides below great suggestions about the possible hopanoid producers, including the possibility to expand that list to further improve the biomarker interpretation. We thank referee #1 for the suggested changes which positively influenced our revisions and helped improve the quality of the manuscript further.

Comments:

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 with this distinction. "Methane release" has been rephrased as "enhanced methane cycling" throughout the manuscript.

Lines 86-87: Based on previous studies, does methane-oxidation predominantly occur in the sediment or the water column? Or does this vary based on the site?

> To our current understanding, aerobic methane oxidation (AeOM) in this region has been observed in near bottom/sub-pycnocline waters of the outer Laptev Sea (Shakhova et al., 2015; Samylina et al., 2021) and in the inner Laptev Sea (Bussmann et al., 2021). Observations of AeOM and AOM has also been seen in Outer Laptev Sea sediments (Tikhonova et al., 2021; Savvichev et al., 2023). Based on these studies, with limited information on incubation-based rates of methane oxidation (both AeOM and AOM) and whether it mostly occurs in the water or sediments are variable between their studied stations. Similar studies remain to be made in other parts of the Laptev Sea to fully understand which process is of highest importance.
>
> Our study recognizes that both AeOM in the water column and the oxygenated surface sediments are possible, but cannot distinguish between the importance of the two with our biomarker approach. Both possibilities are already mentioned in the submitted ms on lines 86-87 and 402-403.

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 thank referee #1 for suggesting a clarification of the importance of the ebullitive versus diffusive fluxes of methane. Shakhova et al. (2010, *Science*) estimated that ~40 % of the methane fluxes to the atmosphere are from diffusive fluxes, and the rest from ebullitive fluxes. In our study, there are two main regions where ebullition is widespread, the OLS and the ILS. As evident from the very depleted δ^{13}C-hopanoids in the OLS (52.9±4.3 ‰, n =14) methane is incorporated into the hopanoid biomarkers across the samples of that region. Notably, the data also display strong evidence of AeOM where ebullitive fluxes dominate; while dissolved methane concentrations are variable, the median is still also in these bubble regimes very high at 350 nM in the ILS and 466 nM in the OLS (Fig.2), supporting that lots of dissolved methane is also here available for oxidation. Therefore, it likely does not affect the proxy-signature.
>
> Regarding the oxygenation of the water column/sediments, multi-year observations of oxygen (2015-2020) in the Laptev Sea water column displayed a usual oxygen saturation between 70-100 % (Xie et al., 2023, *Front. Mar. Sci.*). Sediments from the same stations that were sampled in our study displayed oxygen penetration depths between 0.2-1.8 cm (Maciute et al., 2025, *Env. DNA*). Taken together, this displays the possibility for AeOM throughout the oxygenated water column and in the oxygenated surface sediments, which has also been observed in previous studies (Shakhova et al., 2015, *Philos. Trans. R. Soc. A.*; Tikhonova et al., 2021, *Microbiology*; Bussmann et al., 2021, *Biogeosciences*). However, whether most of the oxidation occurs in the surface sediments or in the water column still remains unresolved, yet is of lesser relevance for the present study.

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).

We thank referee #1 for pointing out that not all *Methyloligella* and *Methyloceanibacter* are methanotrophs. We have consequently change lines 335-336 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*".

We also thank referee #1 for the very helpful suggestion to check whether *Methyloligella* and *Methyloceanibacter* produce hopanoids through accession no. WP_038942977.1. From this search three *Methyloceanibacter* species were found (*Methyloceanibacter methanicus, Methyloceanibacter ceanitepidi and Methyloceanibacter stevinii*). However, no *Methyloligella* species encoding *shc* (squalene-hopene cyclase) were found.

As a result of no known *Methyloligella* producing hopanoids, we have limited the MOB-II to *Methyloceanibacter* and revised the related results (isotopic mass balances, relative abundances of MOB-II, figure S1, figure 3 and figure 4).

Lines 344-354: The title of this subsection and the content seem unrelated. This section also sounds like it belongs more in the conclusions rather than at the start of the discussion.

The incorporation of this section and header is to highlight that enhanced methane cycling was displayed across the Laptev Sea in the lipid biomarkers even though there were regional differences. This is an important section to clarify that the proxy works in the Laptev Sea before discussing the details of each region. To clarify this the section heading 4.1 has been changed to:

"Indications of widespread enhanced methane cycling indicated by lipid biomarkers across the Laptev Sea"

Line 381-383: Are there hydrothermal vents in the ILS region?

Thermospores of hydrothermal origin have been observed in the outer Laptev Sea (Ståhl et al., 2024, *Geobiology*), but not in the Inner Laptev Sea.

To avoid confusion regarding hydrothermal vents, we have rephrased the sentence in line 381-383:

"*Methyloceanibacter constituted the only genera of MOB-II in the ILS (Fig. S1) and has to this date to our knowledge only been isolated from marine systems (Takeuchi et al., 2014, Int. Journ. System. Evol. Biol.; Takeuchi et al., 2019, PLoS ONE; Vekeman et al., 2016, Environmental Microbiology).* "

Lines 382-383: The reference that you site here (Takeuchi et al. 2014) says the strain of Methyloceanibacter is a methylotroph and not a methanotroph. Going back to my previous comment, maybe check to see if they produce hopanoids.

We thank referee #1 for the suggestion to search for the possibility of methane-related hopanoid production from *Methyloceanibacter*. As noted from the previous comment,

Methyloceanibacter methanicus, *Methyloceanibacter ceanitepidi and Methyloceanibacter stevinii* are capable of producing hopanoids, with *Methyloceanibacter methanicus* being a methanotroph (Vekeman et al., 2016, *Environmental Microbiology*). Therefore, *Methyloceanibacter* can be methanotrophs, but not necessarily. As referee #1 suggested earlier, we have now clarified this in lines 335-336 (see our previous comment).

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.

We thank referee #1 for the suggestion to clarify the source of the hopanoid signals of the ILS. Given the abundance also of "other hopanoid producers" in figure 4, we cannot fully exclude a terrestrial influence for the hopanoids. However, the MOB-II present in the ILS (*Methyloceanibacter;* Fig S1*)* have to our knowledge only been isolated from marine environments (Takeuchi et al., 2014, *Int. Journ. System. Evol. Biol.; Takeuchi et al.*, 2019, *PLoS ONE*; Vekeman et al., 2016, *Environmental Microbiology*). Additionally, the MOB-I in the ILS (*Marine methylotrophic group 2*, *Methyloprofundus*, *Milano-WF1B-03* and *pLW-20*) are associated with marine environments (e.g., Tavormina et al., 2015, *Int. Journ. System. Evol. Biol*; de Groot et al., 2025, *Biogeosciences*), although there is one report of *Methylosoma* having been observed in freshwater systems (Rahalkar et al., 2007, *Int J Syst Evol Microbiol*). Therefore, we conclude that the methane related signal in hopanoids of the ILS likely is predominantly from in situ production because of the presence of MOB associated with marine environments (Fig S1). However, "other hopanoid producers" diluting the methane related signal such as *Burkholderia*, *Jatrophihabitans*, *Bryobacter* and *Bradyrhizobium* are likely of terrestrial origin.

Regarding the methane in the ILS (likely stimulating the presence of methanotrophs), it is quite clear from the geochemistry that it is overwhelmingly stemming from coastal sediments as there are very high concentration gradients, intensive bubbling, and much lower concentration in the river waters (e.g., Shakhova et al., 2007, *J. Mar. Sys*; Shakhova et al., 2010, *Science; Shakhova et al.*, 2014, *Nat. Geo.*; Shakhova et al., 2017, *Nat. Comm*).

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.

We thank referee #1 for the presence of earlier literature on this, and to not only cite the review article. The sentence has been changed to "A mixture of hopanoid sources is to be expected, as ~10 % percent of bacteria can synthesize hopanoids (Ourisson et al., 1979, *Pure Appl. Chem*; Fischer et al., 2005, *Geobiology*; Racolta et al., 2012, *Proteins*; Belin et al., 2018, *Nat. Rev. Micro.*)."

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?

> We thank referee #1 for suggesting a clarification of what the methane source is in the ILS. As discussed above, Shakhova et al. (2010, *Science*) and others showed decreasing dissolved methane concentrations through the main outflow from the Lena River, and that the methane concentrations increased in coastal waters of the ILS. In fact, the concentrations are strongly elevated in the coastal waters relative to in the river water, hence why the source must be in the coastal marine system where there is widespread ebullition observed. Additionally, isotopic evidence points towards an old biogenic source in this region, very likely from subsea permafrost (Sapart et al., 2017, *Biogeosciences*).

Line 417: change to "here we show"

> This sentence has been changed to "*here we show*" (line 417).

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

> This sentence has been changed to "*Our biomarkers*" (line 429).

Comments on figures:

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.

> Thank you for these helpful suggestions. The outer, mid- and inner Laptev Sea regions have been added to display the different study regions according to the referee suggestion. The station numbers were initially changed to filled black numbers according to the referee suggestion. However, this made station "45" and "75" harder to read. Therefore, the station number colors were changed to yellow with a black outline to enable a clearer distinction to the subsea permafrost lines in the background. Please see draft of revised Fig below.

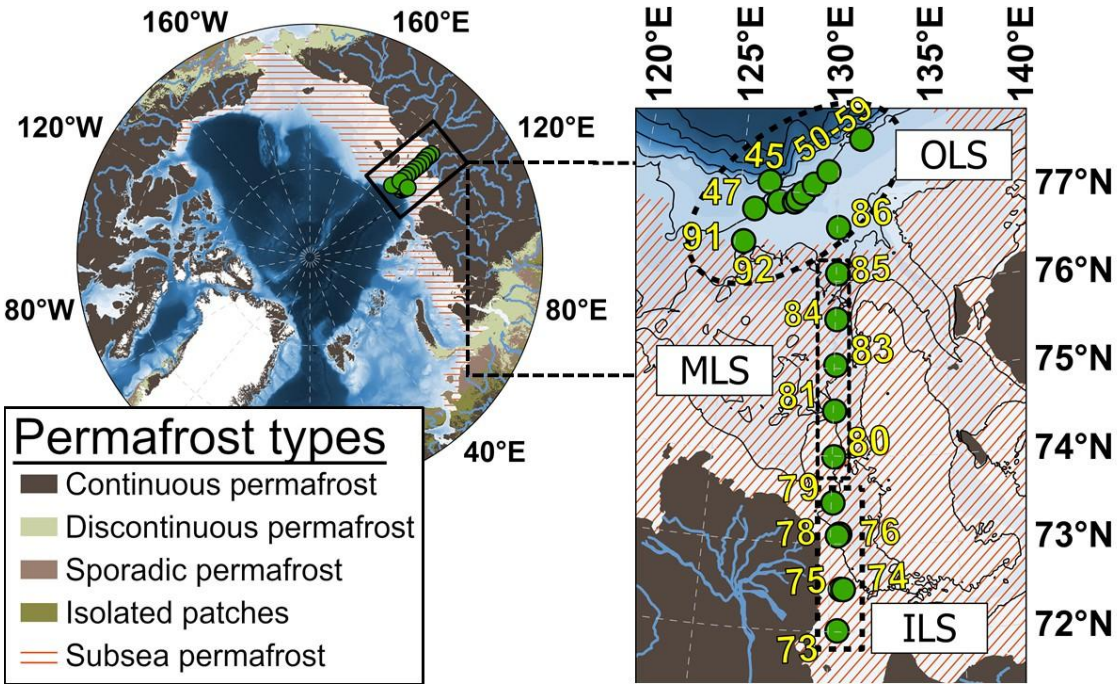
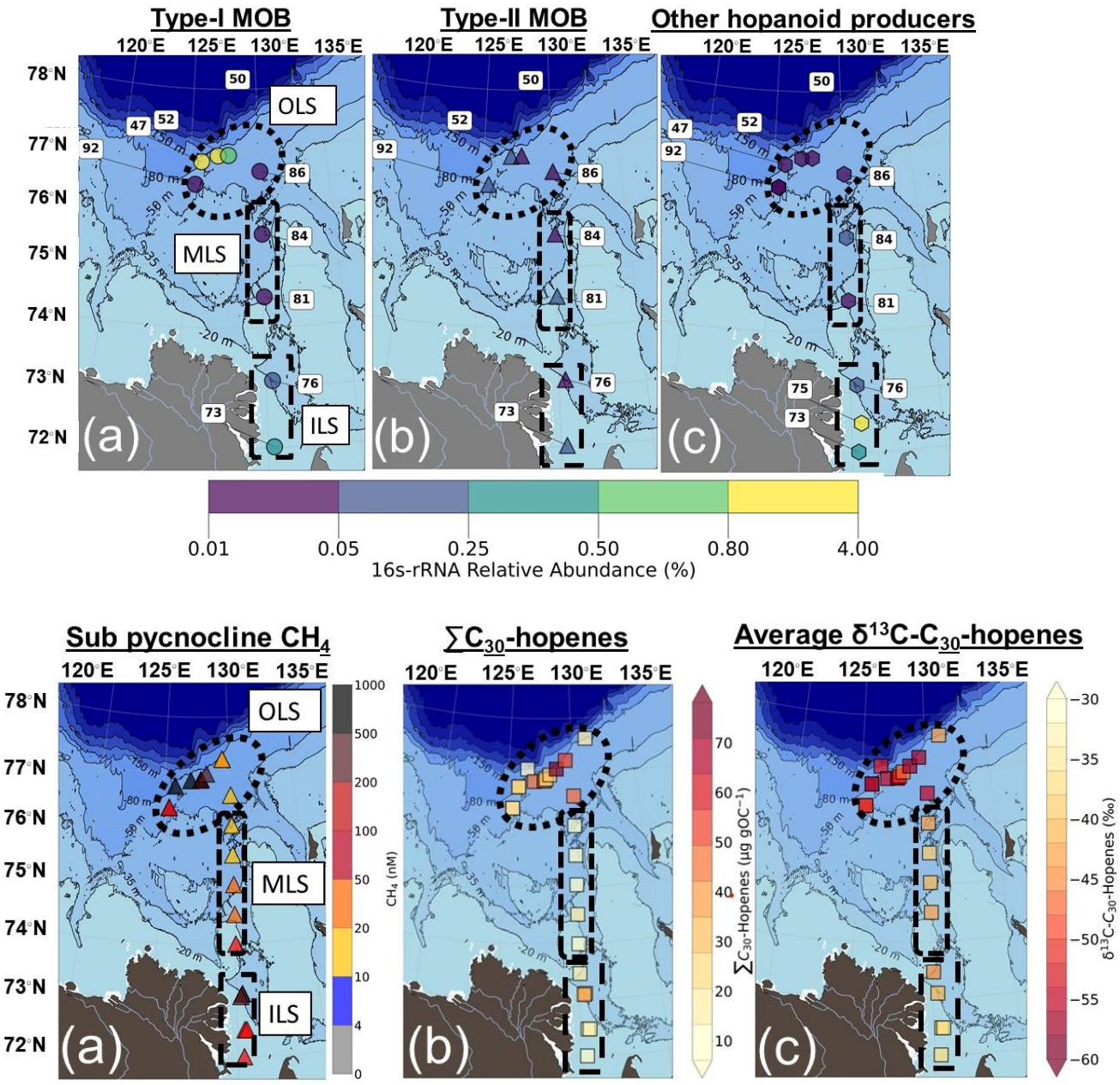


Figure 3: In the caption you say the shaded "gray zone" but in the figure it looks "green" to me. Consider changing this. All of these figures are showing the same things but the varying scales are a bit confusing. Consider making this into one large figure that contains all of the same information to 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*". We are unsure what the referee is suggesting with "one large figure". We attempted to make the figure with one subplot, but the result contained to many boxes, arrows etc., making the result hard to interpret. Therefore, we have kept the figure as three subplots with one displaying the overview (a), the second the different isotopic endmembers and included isotope fractionation (b), and the third our data plotted on top of the endmembers (c). The reason behind the varying x-axis scales is to make it easier to visualize details of the results in the overview (a) panel.

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.

> Figure 4 and 5 have been changed according to referee #1 suggestions. For clarifications, see the figures below.



Thank you for detailed and thoughtful review comments that certainly was a good support for us to improve the ms.