

Interactive comment on “Biogeochemical and microbiological evidence for methane-related archaeal communities at active submarine mud volcanoes on the Canadian Beaufort Sea slope” by Dong-Hun Lee et al.

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Received and published: 7 June 2018

Reviewer #1: Manuscript ID: bg-2018-91 Biogeochemical and microbiological evidence for methane-related archaeal communities at active submarine mud volcanoes on the Canadian Beaufort Sea slope

Lee and coworkers investigated the biogeochemistry Pingo-like structures (associated with gas/ mud emissions) in the Beaufort Sea, in particular the imprints of the anaerobic oxidation of methane. In figure 1 these structures are shown, figure 2 shows

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representative GC-runs, fig. 3 the complete specific results for archaeal lipids. Figure 4 shows results from GDGT analysis, in particular the ring distribution of numbers and a phylogenetic tree of important community members. The concentrations and isotopic compositions of specific compounds as well as (relative) number of 16S sequences are presented in the supplements, although they are by far more interesting than exemplary GC runs. Hence these data should be displayed in the main text. Most results from the supplementary table and Supplementary Figure 1 are essential to the study – they show that sulfate methane transition zones are in the upper 20 to 50 cm. Somehow this is not further discussed in the manuscript – although this is core to the biogeochemistry.

Reply: We appreciate for the generally positive opinion of the reviewer about our manuscript. As the reviewer mentioned, we provided all the figures and tables necessary for the manuscript. As suggested by the reviewer, we will move Fig. S1 and Table S1 into the main text in the revised version, but Fig. 2 into the Supplementary information as Fig. S1. Although we already mentioned the steep sulfate depletion related to AOM processes in the shallow depths (line 339-342) in the submitted version, we will extend the discussion on the sulfate methane transition zone in the revised version.

Although certainly quiet active - AOM has relatively little impact on total organic carbon contents of the sediment, yet lipid and microbial composition data shows presence and activity of key organisms. The data is there - but the discussion of it needs to be strongly revised. The biogeochemistry of AOM as suggested in the title / abstract are -so far - not really covered.

Reply: The reviewer is right that AOM had a comparably little impact on the TOC content but this is not unusual for AOM settings. However, we do not agree with the reviewer for the point mentioned above. In our opinion, we discussed the AOM presence based on the bulk/biomarker and microbial composition in the submitted version. However, we will strengthen this part of discussion extending the biogeochem-

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istry of AOM in the revised version. For example, we will incorporate a new aspect connecting relatively depleted $\delta^{13}\text{C}$ values of archaeol and biphytanes with the activity of chemoorganoautotrophs.

Below I discussed some findings in more detail “Evidence for AOM:” The best evidence for AOM here is the depletion of sulfate, the presence of highly depleted lipids and larger sequence numbers in respective horizons. The rest is not very meaningful “However, organic carbon contents and $\delta^{13}\text{C}_{\text{TOC}}$ values of the three sediment cores investigated spanned a narrow ranges of 1.2 ± 0.1 wt.% and $-26.4 \pm 0.6\%$ ‰ respectively (see Table S1 and Fig. S1), without the negative isotopic excursion that has often been associated with methane-derived biomass from AOM in MVs (e.g. Haese et al., 2003; Werne et al., 2004).” AC: It is quite normal that the total organic carbon content and the total carbon isotopic composition are only slightly influences. I wonder why the TOC data are shown in the main text but those of specific lipids Abundance and isotopic compositions of GDGTs are always very abundant in sediments but they derive with very distinct isotopic compositions around $\delta^{13}\text{C}$ -25 permil from the water column. The Dataset by Lee et al., clearly shows that AOM shifts the GDGT isotopic composition from -25 to around -45 permil. Although this is for sure less than the -60 permil of methane, it is a clear imprint on the isotopic composition. Those data should be transferred into the main text. Moreover assuming an origin of the ANME derived GDGTs from head to head condensation of archaeol lipids (i.e. c.f. Kellermann et al., 2016; Org Geochem) one could determine the contribution of archaea to the GDGT pool assuming similar concentrations of both compounds.

Reply: As mentioned above, we will show Table S1 in the main text which includes the concentration and isotopic values of specific lipid biomarkers. As the reviewer notified above, our bulk data showed relatively little impacton of AOM on total organic carbon contents of the sediment and the total carbon isotopic composition. But lipid and microbial data showed the presence of AOM in our study sites. Although diether lipids (e.g. archaeol) are less specific and likely produced by all ANMEs, we will try

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to more in detail discuss the contribution of archaea to the GDGT pool in the revised version as suggested by the Reviewer.

Line 320 ff. very important to notice: sulfate is only present in surface sediments, all sediments below (20 to 50 cm) are methanogenic at present time. Lipids particular of archaeal origin are preserved for long. I miss the discussion of data Line 334 ff:

Reply: It is a good point that the archaeal lipid can be preserved for longer period and thus can be present after the present SMTZ, as also mentioned below by the reviewer. This point will be discussed in the revised version.

Chemotaxonomy: It should not be stated it is strictly ANME-1 or ANME 2, both organisms can exist next to each other. Furthermore, ANME lipids may remain also after in zones without AOM activity. This becomes evident when analyzing the microbial compositions in 4.2.2. Potential AOM zones have the highest sequence numbers of ANME archaea. This should be clearly discussed

Reply: We will revise it as recommended by the reviewer.

4.3. Albeit in the title of this sections, mechanisms controlling the microbial community compositions were not discussed at all. However, the zones of highest ANME sequence numbers are in agreement with AOM zone. Other than this it should be discussed how the other archaeal groups develop with depth.

Reply: We agree with the reviewer that the 16S rRNA sequence covers only ANME and thus other archaeal groups are not fully considered. In the revised version, we will incorporate the distribution of other archaeal groups, inferred from predominant GDGTs profiles (e.g. GDGT-0 and crenarchaeol) as well as 16S rRNA sequences. This may hint how the other archaeal groups develop with depth.

Conclusions: Nothing new, I am not sure if those conclusions are needed.

Reply: Our study provides the first biogeochemical and microbial data of active mud volcanoes in the Canadian Beaufort Sea, which enhance our understanding to better

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understand methane cycles in this system. Hence, we do think that what stated in the conclusions is valid. In future, a follow-up study will be conducted to examine in more detail distinct methane oxidation processes (e.g. AOM and MOx) near surface using ROV push cores. Nevertheless, we agree that the conclusions also contain a wrapup/summary style of data and will thus rename the section to summary and conclusions.

Some detailed comments along reading. Abstract: Please find another start: That sounds very technical (Line 31 to 34): AOM related biomass mainly derives from inorganic carbon (i.e. Kellermann et al., 2012, Wegener et al., 2016 Front. Microbiol), hence this discussion point is rather weak and of course most biphytanes do not come from methane

Reply: Several studies including the ones stated by the reviewer could show that AOM communities assimilate DIC, however, in AOM systems, this derives from methane. Thus, AOM-derived lipids (and other biomass) is typically ^{13}C -depleted. As mentioned above, we will emphasize the additionally carbon assimilation of AOM-related archaea along the sharply depleted sulfate profiles.

Line 31 A value cannot be enriched, but is either high or low

Reply: We will correct it (as high values).

Line 49: Why not simpler: The following mapping of the southern Beaufort Sea revealed numerous

Reply: As recommended by review, we will correct it (line 51-53) like “The following mapping of the southern Beaufort Sea revealed numerous PLFs which had the various shaped-features may indicate localized unstable seabed conditions.”

Line 54: Based on their formation processes PLFs can be classified into five categories; please also state how they are formed

Reply: In our opinion, it is somewhat out of focus of our manuscript. However, we will

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briefly mention the formation processes of PLFs in the revised version.

Line 55ff: “The PLFs on the Beaufort Sea shelf appear to be geographically controlled by the presence of submerged permafrost” The appearance of PLFs on the Beaufort Sea is connected / seems to be connected with the presence of permafrost –or something similar?

Reply: As recommended by the reviewer, we will correct it as follows: “The appearance of PLFs in the Beaufort Sea seems to be connected with the presence of submerged permafrost.

Line 56ff: If PLFs have different origins, please make clear which one you discuss now. Are these the true pingos now, and do you stop discussing the other ones from here on?

Reply: The PLFs investigated are the true pingos which are designated as active mud volcanos in the previous studies (e.g. Paull et al., 2007).

Line 72 indicating microbial production

Reply: We will correct this sentence as recommended.

Line 76: The PLFs of the Beaufort Sea are mapped and fluid dynamics have been reasonable well understood, but the biogeochemistry of processes related to the anaerobic oxidation of methane (AOM) were not investigated.

Reply: We will change it as recommended.

Line 82: but the microbial communities involved in the anaerobic oxidation of methane

Reply: We will revise it as follows: “Bulk chemical compounds, specific archaeal lipids and microbial communities were investigated in order to characterize the anaerobic oxidation of methane (AOM) communities in the MV sediments.”

Line 96: “Upon recovery, all three sediment cores were observed to expand and bubble

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profusely” – rewrite . Upon recovery, in all three sediment cores . . . was observed.

Reply: We will correct this sentence as follows: "Upon recovery, in all three sediment cores, the formation of bubbles was observed in the sediment matrix."

Line 97: Start sentence with on board – because you likely sampled on board but did not do the analyses.

Reply: We will correct it as suggested.

Line 108: Revise sentence The isotope ratios of TOC were reported in as deviations against the Vienna Pee Dee Belemnite (VPDB)

Reply: We will correct it as suggested.

Line 189: how much DNA have you used for PCR, what is the specificity of these primers Guess it is a primer for the amplification for partial 16S sequences of archaea. Please also reference these primers if you have not developed them

Reply: We will add the quantity of gDNAs used for PCR. Regarding the archaeal primers used in this study, we have developed archaeal primers and that's why only the primer sequences were mentioned without reference. In the revised version of the MS, we will state this more clearly.

Line 205 – 208: please reference tools used for these operations Fig.1 is only later discussed; it should be mentioned earlier, i.e. in Methods, the results introduction to be Fig. 1

Reply: We will mention the Fig. 1 earlier (e.g. in the methods).

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2018-91>, 2018.

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