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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.

Anonymous Referee #1

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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 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

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than exemplary GC runs. Hence these data should be displayed in the main text. Although all data seem to be correctly assessed, the discussion is shallow and lacks expertise, which is surprising based on the long and partly well-known author list. 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. In summary the authors investigate the imprint of methane in the biochemistry of one of the most remote seas on Earth. Although certainly quiet active - AOM has relatively little impacton 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.

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 δ13CTOC values of the three sediment cores investigated spanned a narrow ranges of 1.2$\pm$0.1 wt.% and –26.4$\pm$0.6‰ respectively (see Table S1 and Fig. S1), without the negative isotopic excursion that has often been associated with methane-282 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 GDGTs are always very abundant in sediments but they derive with very distinct isotopic compositions around $\sim$-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.

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: 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

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.

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

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 Lien 31 A value cannot be enriched, but is either high or low Line 49: Why not simpler: The following mapping of the southern Beaufort Sea revealed numerous Lne 54: Based on their formation processes PLFs can be classified into five categories; please also state how they are formed 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? Line 56ff: If PLFs have different origins, please make clear which

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one you discuss now... are these the true pingos now, and do you stop discussing the other ones from here on? Line 72 indicating microbial production Line 76: The PFAs 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. Line 82: but the microbial communities involved in the anaerobic oxidation of methane Line 96: "Upon recovery, all three sediment cores were observed to expand and bubble profusely" – rewrite . Upon recovery, in all three sediment cores ... was observed. Line 97: Start sentence with on board – because you likely sampled on board but did not do the analyses. Line 108: Revise sentence The isotope ratios of TOC were reported in as deviations against the Vienna Pee Dee Belemnite (VPDB) 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 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

There are for sure more details to discuss, but first the MS needs to be revised.

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