Associated editor
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Biogeochemical evidence of anaerobic methane oxidation on active submarine mud volcanoes on the continental slope of the Canadian Beaufort Sea

The key concern I would like to point out is that the writing of abstract. Please remove the statements that might be controversial, and that are not widely accepted. For example, phylogenetic analysis of 16S rRNA gene could be considered as the ultimate evidence for the presence of ANME, but the ratio of of sn-2-hydroxyarchaeol to archaeol (>1) might not be that conclusive. In addition, the general term ANME-specific lipid could be used rather than sn-2-hydroxyarchaeol in the abstract, unless these lipids are solely detected in ANME. In the introduction and discussion, the authors can then discuss the advantage and disadvantages of these lipids as biomarkers of ANMEs. In a word, one has to bear it in mind that sequencing analysis of 16S rRNA genes is more conclusive evidence than the lipid-based chemotaxonomy.
Reply: We thank the associated editor for the constructive comments. We revised the abstract according to the comments made by the associate editor.

Minor comments
(1) L34-36. How confident the authors are by pointing out that these sn-2 and sn-3 hydroxyarchaeol are ANME-specific biomarker. If it was also detected in some of methanogens, the authors might have to rephrase these sentences.
Reply: We clarified this sentence as follows: The carbon isotopic compositions ($\delta^{13}C$) of sn-2- and sn-3-hydroxyarchaeol showed the highly ^{13}C-depleted values (–114 ‰ to –82 ‰) associated with a steep depletion in sulfate concentrations within 0.7 m of sediment depths. This suggested the presence of methanotrophic archaea involved in sulfate dependent–AOM, albeit in a small amount.

(2) L36-37. I guess phylogentic analysis alone is sufficient to claim that ANME-2c and 3 are the predominant methane oxidizers. The ratio of sn-2-hydroxyarchaeol to archaeol may not be that conclusive.
Reply: Although the ratio of sn-2-hydroxyarchaeol to archaeol is not that conclusive compared to the phylogenic analyses, it can still provide the first clue for their presence.

(3) L42. Please conclude the abstract by stating the importance and/or implication of this study, but not the perspective for future study. For example, these results suggest that niche diversification of active mud volcanoes has shaped distinct archaeal communities that play important roles in anaerobic methane oxidation and organic matter turnover in Beaufort Sea.
Reply: We revised this sentence as suggested: Consequently, our results suggest that the niche diversification of active mud volcanoes has shaped distinct archaeal communities that play important roles in AOM in the Beaufort Sea.

(4) The redundant description? i.e., The content of L53-56 appears to be similar to that of Line 60-63.
Reply: We agree with the associated editor. Thus, we deleted the content of L53-56.

(5) L66-67. There is no necessary to cite 6 different references for a simple statement. Please delete some

Reply: we deleted some references (i.e. Steele et al., 2008; Thatcher et al., 2013, Somavilla 2013).

(6) L107. by using a combination suite of lipid and nucleic acid analyses...
Reply: We revised this sentence as suggested.

(7) L213. Please specify the samples used for DNA extraction
Reply: We specified it as suggested.

(8) L257, Please indicate that these archaeol can be considered as the biomarker for ANME.
Reply: We clarified specific biomarkers (i.e. archaeol and sn-2-hyroxyarchaeol).

(9) L281. Rephrase as "Depth profile of archaeal communities"
Reply: We revised it.

(10) L282. It can be rephrased as following: Archaeal communities were phylogenetically classified as the taxonomic level of class
Reply: We revised it as recommended.

(11) L326. Do the authors determine the concentration of others electronic acceptors such as Mn and Nitrate and Fe?
Reply: Unfortunately, other electronic acceptors are not available for this study. However, we discussed potential possibilities for AOM coupled with other electron acceptors in the previously submitted version. Consequently, by using a combined suite of lipid and nucleic acid analyses, we could provide the evidence of sulfate-dependent AOM in our study sites.

(12) L331. One cannot get a clear idea of this section,. i.e., it the AOB biomass contribution to TOC is low, so what?
Reply: In the case of mud volcanoes where methane is actively venting as free gases, methanotrophs are not easy to assimilate methane as their carbon sources. Indeed, with respect to narrow ranges of $\delta^{13}C_{TOC}$ values, we infer that the biomass of methanotrophic communities inhabiting the Beaufort mud volcanoes comprises a minor portion in the total organic carbon pool. Thus, this seems to be linked to the relatively low abundances of AOM biomass in accordance with active methane fluxes in the Beaufort mud volcanoes. Nevertheless, by using a suite of lipid and nucleic acid analyses, our results suggest that distinct archaeal communities (ANME-2c and ANME-3) inhabit the Beaufort mud volcanoes playing an important role in AOM in the Beaufort Sea, albeit in a small amount.

(13) L347. In the result section, please briefly mention that these non-isoprenoid DGD could be considered as a potential marker of sulfate-reducing bacteria
Reply: Based on previous studies, we already mentioned that in the submitted version previously. But, we clarified this sentence in the revised version

(14) L385. Delete and referneces therein. It has been mentioned
Reply: We deleted it.

(15) L391-396. It seems contradictory to some extent. For example, In the abstract, the authors claimed that both sn-2-hydroxyarchaeol and sn-3-hydroxyarchaeol are representative of ANMEs, But in this paragraph, it appears that these archaeol could be found also in methanogens. The question is how specific these biomarkers for ANMEs

Reply: The methanotrophic evidence inferred from these biomarkers can be obtained from the ^{13}C-depleted values as the result of their carbon assimilations by mentioned organisms. Actually, previous studies reported the predominance of methanotrophs which is related to the methanogenic orders *Methanomicrobiales* and *Methanosarcinales* (Hinrich et al., 1999). Thus, considering δ^{13}C values of methane in our study sites, the occurrence of ^{13}C-depleted lipids suggests the presence of methanotrophic archaea rather than methanogens. This highlights the importance of the combination of biomarkers works with the isotopic analyses.

(16) L399. Does this mean that sn-2-hydroxyarchaeol is a better indicator for ANME-2, while archaeol can represent ANME-1 much better?

Reply: In accordance with the currently available literature data, each archaeal biomarker showed differences in abundance among methanotrophs (ANME-1, -2 and -3) (Niemann and Elvert, 2008). In this regard, the ratio of these compounds was proposed as a specific fingerprint for different ANMEs. Particularly, ^{13}C-depleted hydroxyarhcaeol have been regarded as specific ANME markers (mostly ANME-2). Consequently, together with archaeal taxanomic results, we discussed different distributions of ANMEs inhabiting each mud volcano where fluid fluxes were varied.

(17) L453. MCG group now refers to Bathyarchaeota ?

Reply: We revised it.

(18) L466. at a site?

Reply: We corrected it.

(19) Fig.1. please provide the full name of mbsf

Reply: We added the information.

(20) Fig.5. if flexible, please add the bootstrap value of >70 on the tree

Reply: We revised it.