

This is an interesting study. However, I side with both reviewers, and particularly reviewer #1 that the manuscript is not well organized, although it has potential of being published. The manuscript cannot be published in the current form. However, the re-submission is encouraged.

The writing style is poor and the manuscript needs to be completely re-structured including tables and figures.

If the authors want to make a re-submission, he/she may first revise the tables and figures and send it to me for comments before starting the writing of main text.

#### Specific comments

##### 1. Title

- (1) The title might be improved because it does not specify the role of archaeal communities. Is it methan-metabolizing or something else? There are lots of functions of methane-related archaeal communities. Please get it more focused.
- (2) It might be rephrased as "biogeochemical evidence for anaerobic methane oxidation at active submarine mud volcanoes on the Canadian Beaufort Sea slope". The term "Biogeochemical" already contains the meaning of microbiology. In addition, this study is mainly focused on ANME and it is not necessarily extended to "methane-related". Biogeochemical evidence is a mere evidence which does not preclude the importance of other organisms.

##### 2. The abstract needs to be re-organized.

- (1) For example, the authors summarized the key findings as the following. "In this study, we provide first evidence of lipid biomarker patterns and phylogenetic identities of key microbes mediating anaerobic oxidation of methane (AOM) communities in active mud volcanos (MVs) on the continental slope of the Canadian Beaufort Sea. Our lipid and 16S rRNA results indicate that archaea of the ANME-2c and ANME-3 clades are involved in AOM in the MVs investigated."  
In the abstract, the authors need to present the first evidence of lipid biomarker for anaerobic methane oxidation, and explain why these biomarker can be used. Then the phylogenetic identities of key microbes can be followed. The implication could be then presented.
- (2) L31. The enriched  $^{13}\text{C}$ . The value cannot be enriched.
- (3) L31 to 34. It is a bit unusual to show the data in this way. This is not an important point. The authors claimed that contribution of AOM-related biomass to sedimentary TOC was in general negligible. This might be important, but it is not the key point as specified in the significance section. The number 1 priority is to show the evidence for the presence of AOM, and then microbial identity, and then finish the ms by concluding the importance of AOM biomass contribution.
- (4) L35-36. It is a bit unusual to show the evidence in this manner by claiming that "However, the  $\delta^{13}\text{C}$  values of sn-2- and sn-3-hydroxyarchaeol were more negative

than CH<sub>4</sub>, indicating the presence of AOM communities, albeit in a small amount”.

Firstly, why n-2- and sn-3-hydroxyarchaeol can be used as a biomarker. Secondly, how negative it is, how small the amount it is. This is the key information of this study.

- (5) L36-38. This sentence is just the conclusion. The reader need to know the data and evidence, i.e., what is the specific evidence, how the ratio is changing, and why the author feel that the ratio of sn-2- hydroxyarchaeol to archaeol and the 16S rRNA results indeed indicated that archaea of the ANME-2c and ANME-3 clades were involved in AOM.
- (6) L38-40. This study already revealed the phylogenetic diversity of AOM, and why future studies are still needed? In addition, why uppermost surface sediments is mentioned, and what is the point?

### 3. The Introduction

- (7) L47. Please delete e.g.
- (8) L48. Pls delete the following. by hydrographers aboard the C.C.G.S. John A. MacDonald, a Canadian Coast Guard icebreaker.
- (9) L61. Delete e.g.
- (10)L69. Delete e.g.,
- (11)L74. It needs to be specified why  $\delta^{13}C$  CH<sub>4</sub> values of  $-64$  ‰, indicating a microbial source
- (12)L75. What is the connection between the L74-75 sentence and L76 sentence? It seems rather descriptive
- (13)L77. How well it is investigated, what is the key findings about the methane-rich fluid dynamics ?
- (14)L78. Please state why this investigation is important, instead of saying that it have not yet been investigated
- (15)In summary. Significant revision needs to be made including (a) why AOM could be important in the samples tested in this study; (b) what is the key biomarkers of AOM, and its applicability in this study. For example, the use of GDGT and other archaeol as biomarker for AOM and other archaeal. Maybe the difference in GDGT between ammonia-oxidizing archaea and AOM should be specified; (3) What is the phylogeny of AOM, and what is the expected output of AOM in this study; (4) What is significance if the AOM metabolism is deciphered and so on

### 4. Materials and Methods

- (16)What does the term “methanomicrobial operational taxonomic units” mean?
- (17)The authors need to specify how AOM sequences were selected, aligned and analyzed. Of particular concern is the robustness of the phylogenetic identity of AOM

### 5. Results

- (18)L227. Delete the start sentence.
- (19)L227-231. Please start the result section with the most important data. It is unusual that

the starting evidence can be placed within the supplementary materials and methods. This TOC is placed in the abstract as the starting point, but why the key data is in supplementary table S1? In case that the author feel TOC is not the most important data, then the most important one should be described first, instead of TOC which can still be used in supplementary table S1.

(20)L299. What does the systematically mean, it can be deleted.

(21)

(22)L258-259. There is no diversity information in Table S2 and Fig. S2. The diversity index is missing. In addition, the majority of sequences in Table S2 and Fig. S2 are from archaea, why it is low?

(23)L260-271. The result section needs to be improved significantly. The current version is somehow pointless. This study is aimed to anaerobic methane oxidation. But only a very small fraction of archaeal communities can be classified as ANME. Whatever, the authors first of all need to emphasize the ANME sequences, then sulfate-reducing sequence, then other sequences. Pls stay focused on your main theme of this study. Among 25 profile sample detected, apparently Marine Crenarchaeota Group (MCG) predominate archaeal communities in this study except for MV420-0.08. In addition, the authors need to specify the relationship of archaeal lipids to archaea, i.e., what is the specific archaeal lipid for each dominant group of archaeal communities (at phylogenetical level)

## 6. Discussion

(24)L274. The evidence of AOM in Beaufort Sea mud volcanoes. This title is more appropriate as the result section

(25)L275-277. The authors need to provide the concentration of methane, which is the core data of this study. The data cannot be found in the Fig.1. In addition, simply judged from the title of Paull et al., 2015, it appears that this paper is not closely related to methane

(26)L279. The authors need to specify how the gas is charged in Fig. 1D. please specify

(27)L279-281. What is the relationship of AOM to the fact conveyed by these sentences?

(28)L283-285. What is the point of the interstitial gas?

(29)L285-290. This is not the key point in this study. This conclusion is of minor concern for this study. The contribution of AOM to TOC apparently is out of the scope of this study. In addition, It is also very hard to conclude that the contribution of AOM-related biomass to sedimentary TOC is rather low at the MVs investigated.

(30)L291. The authors first of all need to show methane data

(31)L304. How do the authors know these are sulfate-dependent AOM. If the abundance of sulfate-dependent AOM is elevated, it should be placed in the main text.

(32)L291-305. These sentences are mostly pointless. The evidence of AOM IN THIS STUDY should be first emphasized, and then discussed in the context of other studies.

(33)L308-309. This can be placed in the introduction section.

(34)L311-312. To what extent, the author are certain that these DGD can represent sulfate-reducing bacteria?

- (35)L313-314. If it is not supportive of SRB, it may suggest that other electronic acceptor such as Mn/iron/nitrate might be involved? Whatever, it cannot be stopped here and further discussion should be made.
- (36)L319. Do the author mean the contribution to GDGD, and so what?
- (37)L327-328. There is no solid evidence in support of this statement.
- (38)L327-338. Much of these discussion appears more appropriate as the results
- (39)L339-341. What is the logic between the sulfate profiles and siboglinid tubeworms???
- (40)L344. What does the constrained mean?
- (41)L344-350. Maybe the author want to emphasize how sulfate is generated, and then used in support of methane oxidation. The paragraph needs to be re-organized.
- (42)L354-356. This should be placed in the introduction
- (43)L354-375. Please clearly specify the lipids that are representative of different archaea.
- (44)L380. What is ANME2-specific lipid?
- (45)L407. To clarify
- (46)L407-412. It can be described in the materials and method section
- (47)L412-424. These sentences are rather descriptive, and it might be more appropriate in the section of Results.
- (48)L430-437. What is your conclusion about AOM in this study, when compared to other studies?
- (49)L437. Does this mean that in this study methane concentration is low?
- (50)L444 and L450. Where is the measured data of methane flux
- (51)L454-464. Part of this discussion should be made in the result section.
- (52)L476-477. The authors need to specify that these sn-2- and sn-3-hydroxyarchaeol are representative of AOM at the very beginning.
- (53)L480. There is no evidence of methane concentration. How could the author claim that methane was oxidized?

As for the Tables and Figures, they need to be significantly re-structured.

- (1) Fig. 1 should be re-organized. Fig. 1B could be placed at the bottom left. Fig. C could be place at top right. Fig. 1D appears to be the most important one which could have more space like the current Fig. 1B, and place in the middle right.
- (2) Fig. 2. In a scientific paper it is unusual to show an example figure. This figure tells the readers very little information, and it should be placed in the supplementary, or it should be placed side by side with the data measurements.
- (3) The title of this ms is about biogeochemical. Therefore, in the main text, the BIO and the Geochemical data should be included. But all figures are about the BIO evidence.
- (4) Table S1 should be placed in the main text as the figure 2. Methane concentration is of particular concern, and should be placed together with sulfate gradient.
- (5) The most important data that are related to AOM in Table S1 should be made as a figure and placed in the main text.
- (6) What are the key information of Fig. 3 and Fig .4. these data appear to be from Table S1. Please stay focused on the AOM as much as possible.

