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Biogeosciences Discussions

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.

Dong-Hun Lee et al.

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Associated editor 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

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 completed 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.

Reply: We thank the editor for providing us an opportunity to revise this manuscript. We will revise the manuscript according to the comments made by both the reviewers and the associate editor as indicated in the rebuttals.

Specific comments

1. Title 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. 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.

Reply: We will revise the title as suggested by the editor: "Biogeochemical evidence for anaerobic methane oxidation at active submarine mud volcanoes on the Canadian Beaufort Sea slope".

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

Reply: We will revise the abstract as suggested by the editor highlighting that the presence and abundance of dignostic lipids allowed this conclusion

L31. The enriched 13C. The value cannot be enriched. Reply: We will correct it as "high values". But we should mention that the isotopic composition can be enriched in 13C.

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.

Reply: This is a good point and we will re-structure the abstract following the suggestion made by the editor.

L35-36. It is a bit unusual to show the evidence in this manner by claiming that "However, the δ 13 C values of sn-2- and sn-3-hydroxyarchaeol were more negative than CH4, 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.

Reply: We will revise it as suggested by the editor.

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.

Reply: We will add information on the ratio of sn-2- hydroxyarchaeol to archaeol and the 16S rRNA results to draw the conclusion.

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?

Reply: Our study provides the first biogeochemical and microbial data from active mud volcanoes in the Canadian Beaufort Sea. However we could not investigate the oxic-related methanotrophs in this study. That is why further studies are necessary. We will clarify this aspect in the revised version.

The Introduction

L47. Please delete e.g.

Reply: We will delete it.

L48. Pls delete the following. by hydrographers aboard the C.C.G.S. John A. MacDonald, a Canadian Coast Guard icebreaker.

Reply: We will delete it.

L61. Delete e.g.

Reply: We will delete it.

L69. Delete e.g.,

Reply: We will delete it.

L74. It needs to be specified why δ13C CH4 values of –64 ‰ indicating a microbial source

Reply: We will add the aspect of biogenic methane production and migration associ-

ated with the microbial signature of d13C value of CH4 (Whiticar, 1999) in the revised version.

L75. What is the connection between the L74-75 sentence and L76 sentence? It seems rather descriptive

Reply: We will clarify the connection between the chemosynthetic communities and the ascending methane source in the Beaufort Sea based on the previous studies.

L77. How well it is investigated, what is the key findings about the methane-rich fluid dynamicsïij§

Reply: Paull et al. (2015) reported that the ascending methane sources might be related to the dissociation of permafrost and/or gas hydrates in subsurface, confirmed by a detailed bathymetric mapping with AUV and seismic survey. Furthermore, previous studies with ROV showed that Beaufort MVs were active edifices characterized by ongoing eruptions. Particularly, the ascending fluid sources can provide essential energy for inhabited microbial organisms's nutritional metabolism. We will clarify this point to strengthen our objectives in the revised vision.

L78. Please state why this investigation is important, instead of saying that it have not yet been investigated

Reply: We will state importancy of AOM reaction regarded as the major barrier against methane efflux from marine sediments into the ocean. We will clearly address it in the revised version.

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

Reply: We agree with the editor for this structure. Thus, we will clarify these parts into the discussion in the revised version.

Materials and Methods

What does the term "methanomicrobial operational taxonomic units" mean?

Reply: "methanomicrobial operational taxonomic units" means the operational taxonomic units of the class Methanomicrobia. We will add more details in the revised manuscript to avoid any confusion.

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

Reply: Sequences of Methanomicrobia which include the archaeal group involved in AOM were selected for the phylogenetic analysis based on their proportion and robusteness of tree topology assesed. Although we already described it, we will clarify it in the revised version.

Results

L227. Delete the start sentence.

Reply: We will delete it as recommended by the editor.

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.

Reply: As also suggested by the reviewer1, we will move the Fig. S1 (in submitted

version) will be shown as a main figure in the revised version. We will also move Table S1 into the main text in the revised version. Instead Fig. 2 (in submitted version) will be moved into the Supplemenatry information as Fig. S1.

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

Reply: We will delete it.

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?

Reply: We agree with the editor's comments since there is no diversity information provided in the submitted verison. The comparison of archaeal diversity is not the main focus of this study and thus we will delete this sentence (L259-260) in the revised version. Instead, the statistics of the sequnces including the diversity indices will be added as a new supplementary table.

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)

Reply: We agree with the editor 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. This may hint how the other

archaeal groups develop with depth.

Discussion

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

Reply: We will revise it as recommended.

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

Reply: Unfortunately, the methane concentration data was not available for this study due to active the gas expansion during core recovery. This manuscript focuses on the AOM-related methanotrophs occurring in three mud volcanoes in the Canadian Beaufort Sea. Particularly, these sites are closely interacting with ascending methane for fueling specific microbial communities (i. e. methanotrophs). Thus, the evidence on gas fluid sources in the previous study (Paull et al., 2015) is important for understanding AOM process by methanotroph.

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

Reply: In our opinion, this point is somewhat out of focus of our manuscript. However, we will briefly explain it, as suggested.

L279-281. What is the relationship of AOM to the fact conveyed by these sentences?
L283-285. What is the point of the interstitial gas?

Reply: The problem of free gas is always that it is not accessible by microbes. And, in most cases, mousse/foamy appearance of sediments is a post sampling artefacet because of gas expansion during recovery. The part of gas description within core sediments will move to the sample collection part in order to clarify AOM evidence in

the discussion.

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.

Reply: AOM had comparably little impact on the TOC content which 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 parameter at this part of the manuscript. So the point can be mentioned as discussed in our opinion.

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

Reply: As mentioned above, the methane data are not available for this study.

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.

Reply: As suggested by the editor, we will move Fig. S1 and Table S1 into the main text in the revised version, but Fig. 2 into the Supplemenatry 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. We will mention that other electron acceptors such as nitrate/nitrite and oxidised Fe/Mn are typically not available at this depth, because the penetration depth of those ones is thermodynamically very limited.

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.

Reply: As mentioned above, we will first emphasize evidence for sulfate-dependent AOM, which is typically the dominant methane oxidation process in marine sediment. On the other hand, AOM with alternative electron acceptors (e.g. Mn and Fe oxides)

in marine settings is probably mediated by specialised ANMEs, but it remains unclear how far potential partner bacteria are involved in these processes. Accordingly, we will address this point in the revised version.

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

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

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

Reply: This compound was previously identified as a diagnostic marker molecule for a sulfate-reducing bacterium in sediment where AOM was an important microbial process (Pancost et al., 2001). Moreover, we not only detected this compound but also measured its stable carbon isotopic composition, suggesting some degree of methane and other organic sources. As suggested by the editor, we will further explain the SRB-related DGD in the revised version.

L319. Do the author mean the contribution to GDGD, and so what?

Reply: We will discuss in more detail the contribution of archaea to the GDGT pool in the revised version. For example, we will incorporate a new aspect relating the relatively low δ13C values of archaeol and biphytanes-derived from GDGTs with the activity of anaerobic methanotrophs. We will also address the potential carbon assimilation (e.g. chemoorganoautotrophs) of AOM-related archaea along the sharply depleted sulfate profiles.

L327-328. There is no solid evidence in support of this statement.

Reply: We will moderate our statement in the revised version and will emphasize the steeply depleted-sulfate profiles to strengthen our discussion with respect to the near-sedimentsurface S-cycle in the revised version.

L327-338. Much of these discussion appears more appropriate as the results

Reply: In our opinion, the significant AOM evidence could be shown from the detected biomarkers with a 13C depleted signature as the results of methane assimilation. As suggested by the editor, we do think that this paragraph can go into the results and then only the highlight should be kept here.

L339-341. What is the logic between the sulfate profiles and siboglinid tubeworms??? L344. What does the constrained mean?

Reply: This section aims at describing the indirect AOM influence on the surrounding ecosystem. For example that sulfide, an end product of sulfate dependent AOM can be utilized by thiotrophs such as symbiotic megafauna and free-living bacterial mats. We will strengthen this discussion in the revised version

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.

Reply: We agree with the editor for this point. We will revised this part of the discussion in the revised version, as recommended by the editor.

L354-356. This should be placed in the introduction

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

L354-375. Please clearly specify the lipids that are representative of different archaea. L380. What is ANME2-specific lipid?

Reply: As mentioned above, archaeol, sn-2 and -3-hydroxyarchaeol and to a lesser degree GDGTs are synthesized in diagnostic ratios by the different ANME groups. We will add a paragraph highlighting this and will then relate our findings to the previously detected ratios. In brief, Niemann and Elvert (2008) found that a sn2-archaeol:archaeol ratio of >1 is typical for ANME 2 archaea. We found a ratio of 1.3 to 1.8 and indeed, our 16S rRNA analyses showed an abundance of ANME2c and ANME3 in respect to

the corresponding values.

L407. To clarify

Reply: We will correct it.

L407-412. It can be described in the materials and method section

Reply: The mentioned chemotaxanomy/lipid data were used to identify different ANME groups. Hence, we would prefer to leave this as part of the discussion.

L412-424. These sentences are rather descriptive, and it might be more appropriate in the section of Results.

Reply: While the overall archaeal communities is indeed described in the results, we would prefer to leave this part on the methanomicrobia clade in the discussion. Particularly, the methanomicrobia OTUs c116, C1698 and C1784 forme a cluster with ANME-2c and -3. Without an introduction to the phylogeny, this part of the discussion would become tedious for the non-specialist in methanotrophic diversity. Thus, we also think that it is a suitable arrangement in this part of the discussion.

L430-437. What is your conclusion about AOM in this study, when compared to other studies?

Reply: Although we currently think that the distribution of ANME-2c and ANME-3 is probably controlled by the methane flux, it might be possible that other geochemical factors (e.g. oxgen and sulfide) influence these groups too. We will address this point in the revised version.

L437. Does this mean that in this study methane concentration is low?

Reply: Often (though not neccesarily), high fluxes are related to high pore fluid mathen cocnetrations. However, when comparing the different ANME2 subclusters (a versus c) then ANME2c appears to prefer niches with lower methane fluxes

L444 and L450. Where is the measured data of methane flux

Reply: We cannot present flux data becuase we have not CH4 concentraton data. However the preferences to differential flux regimes shown in the literature indicate differential flux regimes. We inferred differences in ANME groups through the thermal gradients (calculated from heat flow in core sediments) as mentioned above, although methane data are not available for this study.

L454-464. Part of this discussion should be made in the result section.

Reply: For this point, we do not agree with the editor. In our opinion, the distribution of confirmed ANMEs was closely related to methane flux, like the Haakon Mosby Mud Volcano located in the Barents Sea. Thus, this discussion is important to identify different distrubitions of ANMEs along with the variation of methane flux.

L476-477. The authors need to specify that these sn-2- and sn-3-hydroxyarchaeol are representative of AOM at the very beginning.

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

L480. There is no evidence of methane concentration. How could the author claim that methane was oxidized?

Reply: Our lipid and microbial data clearly showed the presence of AOM communities in our study sites. Most importantly, the depleted ïĄd'13C values show clear signs of methane derived carbon incorporation, suggesting an active ANME-2 and -3 community.

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

Reply: We revised the tables and figures as recommended by the editor.

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.

Reply: We revised it as suggested.

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.

Reply: We replaced it in the supplementary.

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.

Reply: We revised it as suggested.

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.

Reply: We revised it as suggested.

The most important data that are related to AOM in Table S1 should be made as a figure and placed in the main text.

Reply: The figures related Table S1 are illustrated as Fig. 3 and 4.

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.

Reply: In our opinion, it is good to show the data as figures to see the pattern although the exact data are presented as a table.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2018-91/bg-2018-91-AC2-supplement.pdf

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

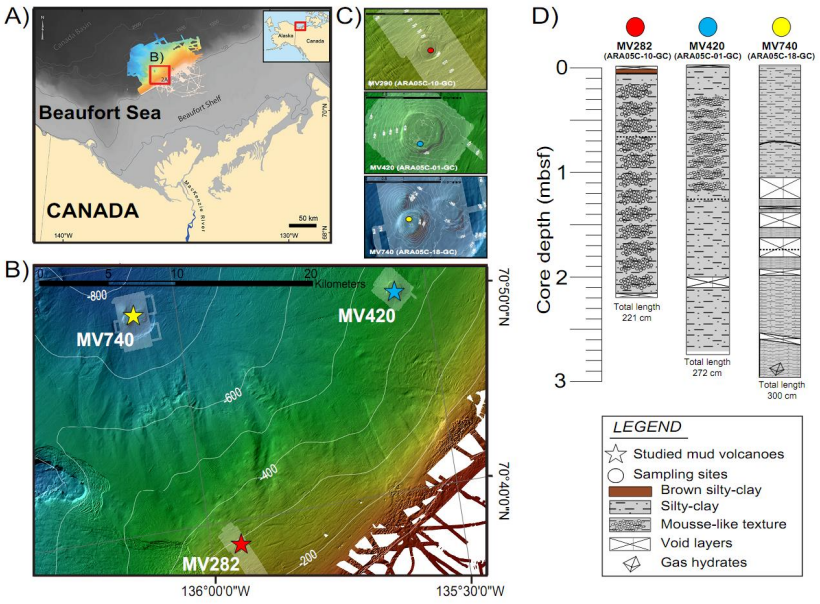
A)
Beaufort Sea
CANADA
Beaufort Shelf
Alaska
Canada
50 km
140°W
130°W
70°N
68°N
B)
C)
MV282 (ARA05C-10-GC)
MV420 (ARA05C-01-GC)
MV740 (ARA05C-18-GC)
D)
MV282
(ARA05C-10-GC)
MV420
(ARA05C-01-GC)
MV740
(ARA05C-18-GC)
Core depth (mbsf)
0
1
2
3
Total length
221 cm
Total length
272 cm
Total length
300 cm
LEGEND
Studied mud volcanoes
Sampling sites
Brown silty-clay
Silty-clay
Mousse-like texture
Void layers
Gas hydrates
B)
0
5
10
20
Kilometers
-800
-600
-400
-200
MV740
MV420
MV282
70°50'0"N
70°40'0"N
136°00'0"W
135°30'0"W

Fig. 1.

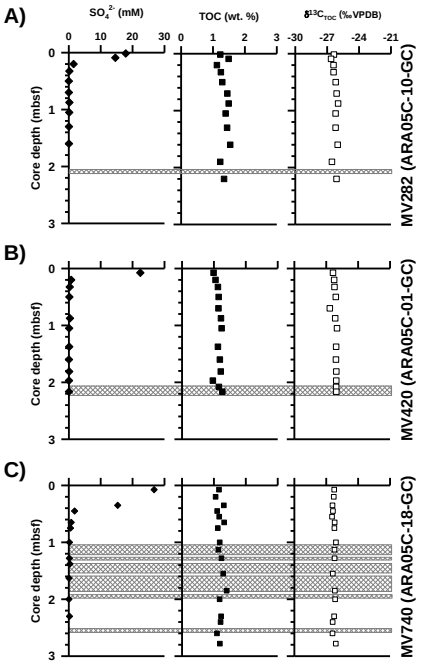
A)
SO_4^{2-} (mM)
TOC (wt. %)
$\delta^{13}C_{TOC}$ (‰VPDB)
0
10
20
30
0
1
2
3
-30
-27
-24
-21
Core depth (mbsf)
0
1
2
3
MV282 (ARA05C-10-GC)
B)
Core depth (mbsf)
MV420 (ARA05C-01-GC)
C)
Core depth (mbsf)
MV740 (ARA05C-18-GC)

Fig. 2.

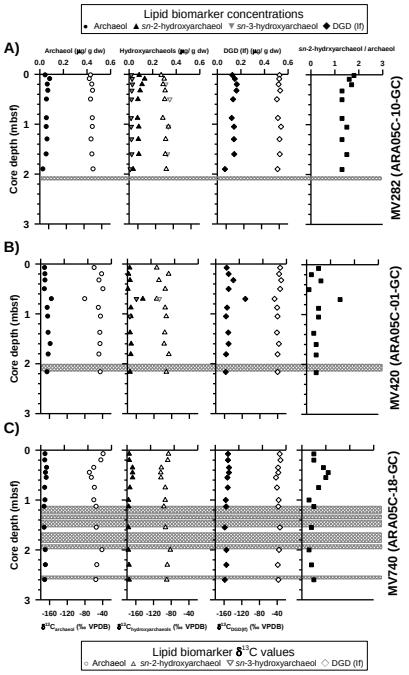
Lipid biomarker concentrations
Archaeol
sn-2-hydroxyarchaeol
sn-3-hydroxyarchaeol
DGD (If)
A)
B)
C)
Archaeol (µg/ g dw)
Hydroxyarchaeols (µg/ g dw)
DGD (If) (µg/ g dw)
sn-2-hydroxyarchaeol / archaeol
Core depth (mbsf)
MV282 (ARA05C-10-GC)
MV420 (ARA05C-01-GC)
MV740 (ARA05C-18-GC)
Lipid biomarker δ13C values

Fig. 3.

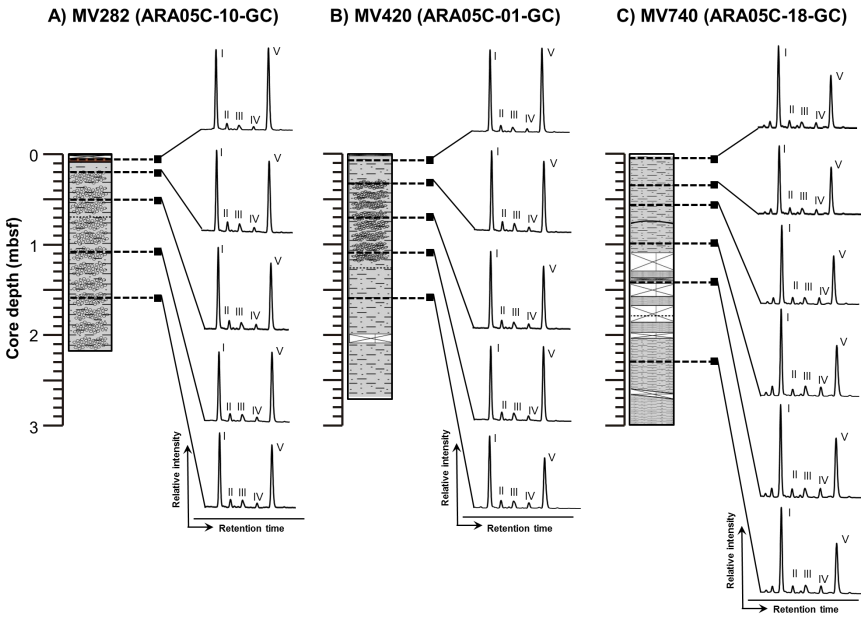
A) MV282 (ARA05C-10-GC)
B) MV420 (ARA05C-01-GC)
C) MV740 (ARA05C-18-GC)
Core depth (mbsf)
Relative intensity
Retention time

Fig. 4.

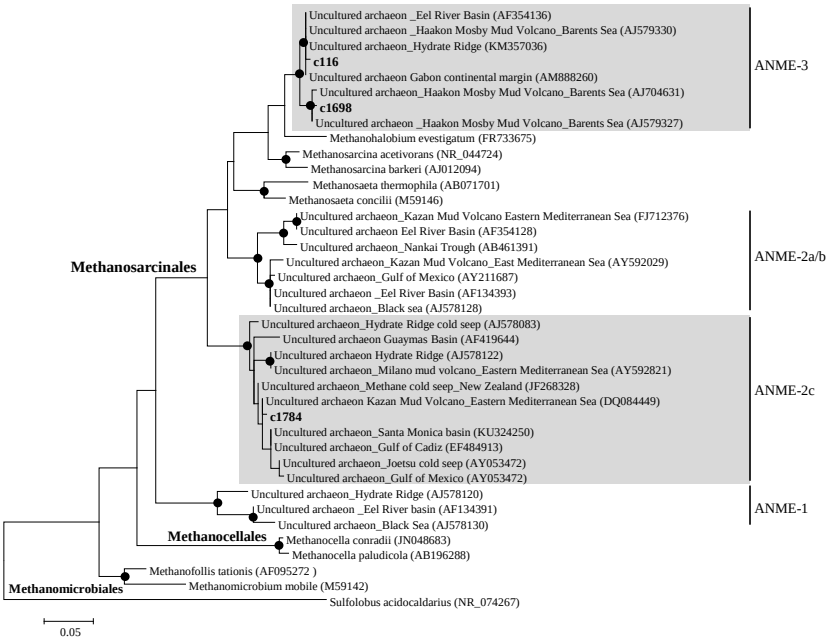
Uncultured archaeon _Eel River Basin (AF354136)
Uncultured archaeon _Haakon Mosby Mud Volcano_Barents Sea (AJ579330)
Uncultured archaeon_Hydrate Ridge (KM357036)
c116
Uncultured archaeon Gabon continental margin (AM888260)
Uncultured archaeon_Haakon Mosby Mud Volcano_Barents Sea (AJ704631)
c1698
Uncultured archaeon _Haakon Mosby Mud Volcano_Barents Sea (AJ579327)
ANME-3
Methanohalobium evestigatum (FR733675)
Methanosarcina acetivorans (NR_044724)
Methanosarcina barkeri (AJ012094)
Methanosaeta thermophila (AB071701)
Methanosaeta concilii (M59146)
Uncultured archaeon_Kazan Mud Volcano Eastern Mediterranean Sea (FJ712376)
Uncultured archaeon Eel River Basin (AF354128)
Uncultured archaeon_Nankai Trough (AB461391)
Uncultured archaeon_Kazan Mud Volcano_East Mediterranean Sea (AY592029)
Uncultured archaeon_Gulf of Mexico (AY211687)
Uncultured archaeon _Eel River Basin (AF134393)
Uncultured archaeon_Black sea (AJ578128)
ANME-2a/b
Methanosarcinales
Uncultured archaeon_Hydrate Ridge cold seep (AJ578083)
Uncultured archaeon Guaymas Basin (AF419644)
Uncultured archaeon Hydrate Ridge (AJ578122)
Uncultured archaeon_Milano mud volcano_Eastern Mediterranean Sea (AY592821)
Uncultured archaeon_Methane cold seep_New Zealand (JF268328)
Uncultured archaeon Kazan Mud Volcano_Eastern Mediterranean Sea (DQ084449)
c1784
Uncultured archaeon_Santa Monica basin (KU324250)
Uncultured archaeon_Gulf of Cadiz (EF484913)
Uncultured archaeon_Joetsu cold seep (AY053472)
Uncultured archaeon_Gulf of Mexico (AY053472)
ANME-2c
Uncultured archaeon_Hydrate Ridge (AJ578120)
Uncultured archaeon _Eel River basin (AF134391)
Uncultured archaeon_Black Sea (AJ578130)
ANME-1
Methanocellales
Methanocella conradii (JN048683)
Methanocella paludicola (AB196288)
Methanofollis tationis (AF095272)
Methanomicrobiales
Methanomicrobium mobile (M59142)
Sulfolobus acidocaldarius (NR_074267)
0.05

Fig. 5.