Reviewer 1

The overall conclusions of this study – sulfate-dependent AOM by ANME archaea in the seep sediments, and slow organic matter remineralization at the background sites – are plausible, but the manuscript as a whole makes a somewhat improvised impression and many datasets are not presented to their best advantage. The metagenomic and transcriptomic analysis remains at a fairly generic level and does not comment on interesting results (for example the Chloroflexota dsr genes); the phylogenetic data are used to very limited effect. Much more is possible by going beyond phylum-level generalizations; it is very hard to say anything meaningful if the analysis and discussion remain stuck on this level. Phylogenies exist, and they should be used and explored.

To summarize, the manuscript needs more work and additional analyses to sharpen the conclusions.

Answer: *We would like to thank Prof. Teske for spending time and effort into revising our paper. We agreed to most of his suggestions to improve the paper. Here, we provide point-by-point responses to his comments.*

Original comments by the Reviewer are listed in regular font while our answers appear in italic font.

Manuscript comments

Unpleasant surprises in the Introduction:

Line 35 and ff. The presence of marker genes reveals potential, not activity. For the latter, you would need transcriptional data.

Answer: *Wording was changed.*

Lines 102 to 105: Chloroflexota are sulfate reducers? Sulfate reducers (Desulfobacterota) use simple and halogenated HCs as electron acceptors (not sulfate?). The involvement of the Asgards in methane and HC degradation is also highly debatable and very likely applies to specialized lineages only; generalizations across a phylum (or superphylum, see Eme et al. 2023 Nature) are not helpful. These lines need to be rewritten and disentangled, to avoid nonsensical statements.

Answer: *Thank you for catching our mistake. Indeed, sulfate reducers (Desulfobacterota) use simple and halogenated HCs as electron donors with sulfate as electron acceptor.*

Line 290: Did you obtain and analyze gene transcripts (mRNA)? Apparently yes, but this needs to be introduced more clearly.

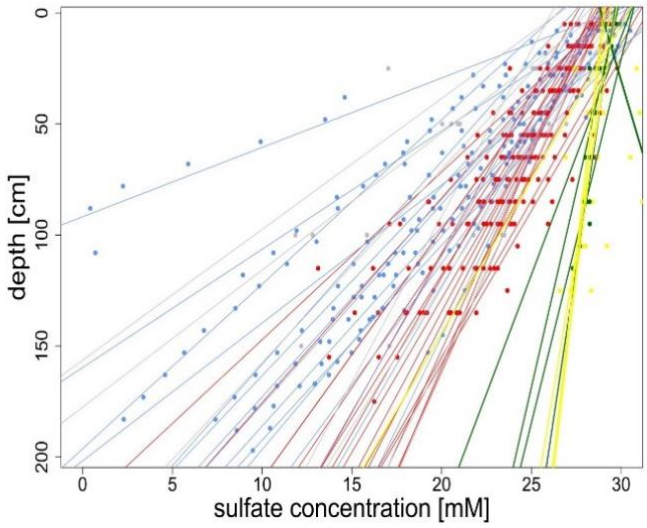
Answer: *Yes, we analyzed gene transcripts and revised the text accordingly to emphasize this.*

Lines 306/7: Why are these groups not “statistically significant”? How was this tested?

Answer: *The t-test statistics were added to the text.*

Concerning figures 2 and 3, I would bring the original porewater profiles from the supplements back into the main manuscript, to demonstrate that the profiles are in fact quite linear. Or make a suitable selection from the original profiles in a nicely designed full-page figure (“Representative profiles from …”) so that the reader can see them without having to check the supplements. There is no reason to hide the real data in the supplements. After all, the central argument of this manuscript depends on these profiles!

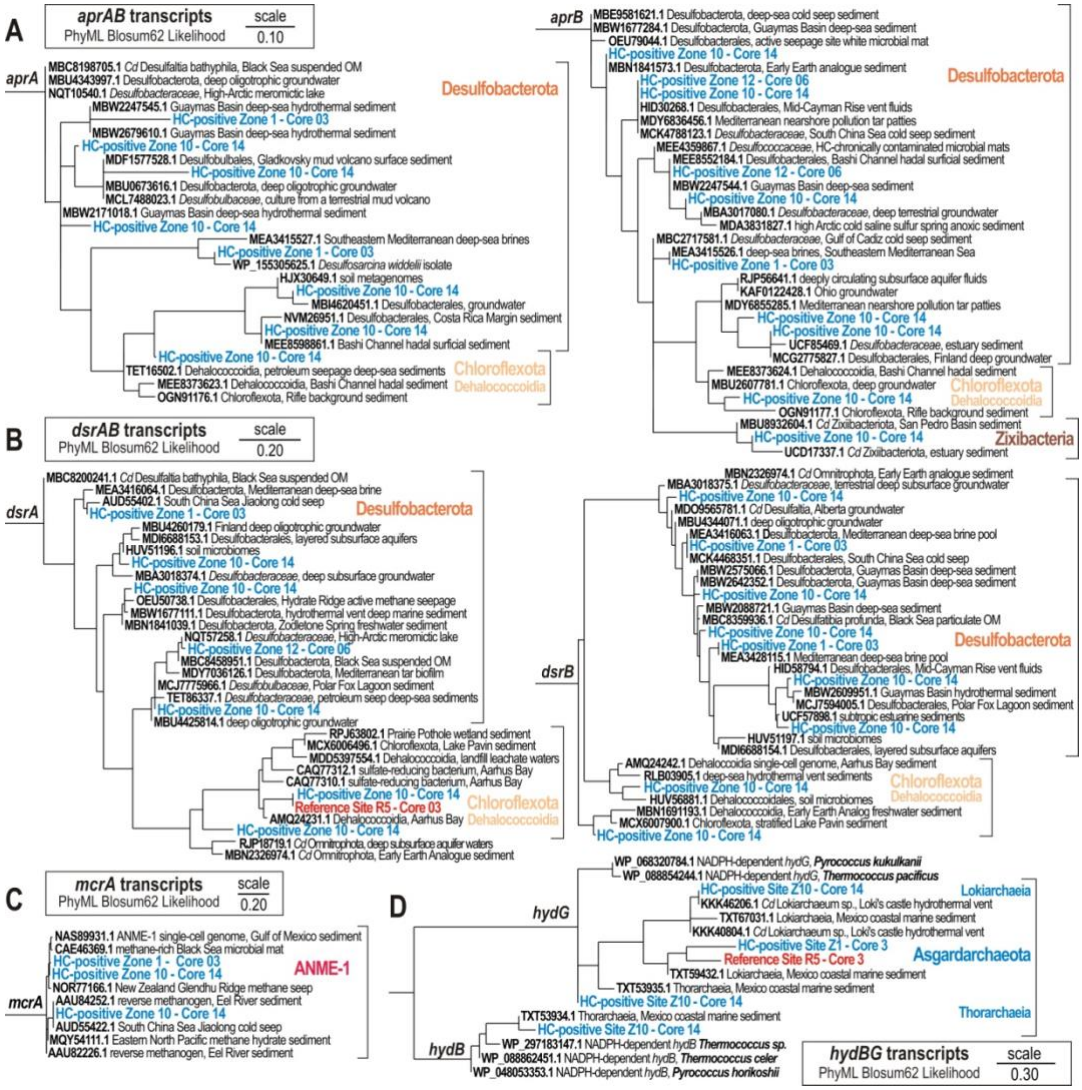
Answer: *We have combined Figures 2, 3 and 4 and added some representative profiles to show that the linear regression is justified. The new figure was implemented to the main text (Fig. 2). We do not think it to be such a good idea to simply compile representative profiles from the supplement, as we believe that the trends we report in the main text can be captured much more quickly by depicting regression lines plotted jointly instead of overwhelming the reader with a huge number of individual plots. We have also plotted the data points together with their regression lines, but the resulting plot appears too busy and one can no longer tell the individual cores apart (see plot here under).*



The Metagenomics section bumps into some very interesting questions, and more or less ignores them. Line 410 ff: Are the Chloroflexota dsr genes functioning in the oxidative or reductive direction? Do they function in assimilatory or dissimilatory sulfate reduction? To which group of the Chloroflexota (a huge and highly diverse phylum!) do they belong? Assimilatory sulfate reduction is documented for Chloroflexota: Zheng R, Wang C, Sun C. 2024. Deep-sea *in situ* and laboratory multi-omics provide insights into the sulfur assimilation of a deep-sea *Chloroflexota*bacterium. mBio 15:e00004-24. https://doi.org/10.1128/mbio.00004-24

Answer: *We decided to combine the phylogenetic trees plotted for aprAB, dsrAB and mcrA genes into one single Figure and implement it to the main text (Figure 7, also see here under). To address a further comment by the Reviewer, we also implemented a phylogenetic tree for hydB-G gene transcripts. The aprAB and dsrAB trees clearly show that none of these genes correspond to their reverse function. Moreover, the sediment being fully anoxic, this would be quite surprising to detect oxidation of sulfur via reverse aprAB or dsrAB (mostly known in aerobic Gammaproteobacteria) and the genes annotated as aprAB and dsrAB are predicted to function in dissimilatory sulfate reduction.*

Concerning taxonomic assignment to putative sulfate-reducing Chloroflexota, we could reach down to the order level using the NCBI database, i.e. Dehalococcoidales. Otherwise, anaerobic oxidation of methane is actively performed by ANME-1 archaea. With regards to the hydB-G genes, we can show that the class Lokiarchaeia and Thorarchaeia express genes involved in sulfur/polysulfide reduction (i.e. sulfhydrogenase).



The Figure 6. Carbon dioxide per se is not abundant in normally buffered seawater; DIC occurs mostly as bicarbonate and some carbonate anions, and also microbially produced CO2 will enter the carbonate equilibrium (and thus magically disappear if you look for CO2 only). This is the likely reason why the HC-rich samples and the background sediments do not show a significant difference in CO2 concentrations.

Answer: *This is a good argument which has been added to the results section as there is no corresponding section in the discussion.*

Figure 7. Are you using “alkalinity flux” in the sense of total DIC flux (CO2 plus bicarbonate plus carbonate), or are you thinking of seawater alkalinity (which includes buffering contributions from other seawater compounds as well)?

Answer: *We measured total alkalinity. We have added an explanatory sentence to the manuscript in chapter 3.4.*

Figure 8 is unreadable. It is impossible to tell which colors belong to archaea and to bacteria (for example, ANME has the same shade of red as Nitrospirota). Try another solution, either by providing contrasting colors to bacteria and archaea (blue, green and yellow for bacteria; red and purple for archaea), or design separate plots for bacteria and archaea.

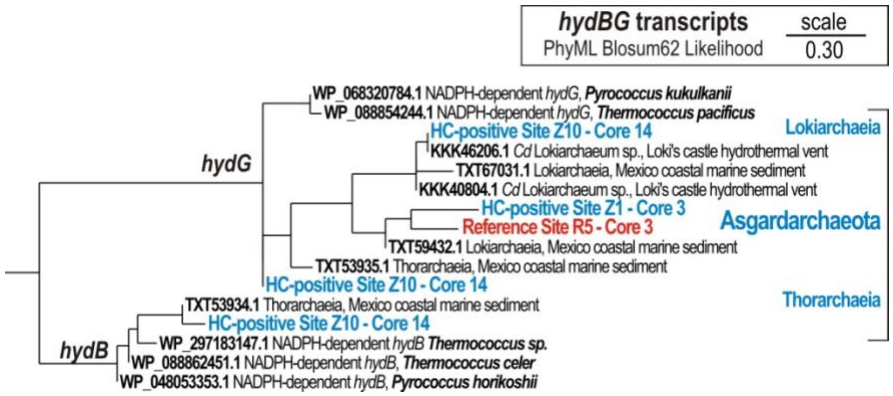
Answer: *Taxonomic assignments to Bacteria start with Acidobacteriota (signified in bright yellow). Most of the functional genes are assigned to Desulfobacterota and Chloroflexota whose color coding does not overlap with the one used for Archaea.*

Line 470: Do you have 13C data for methane to distinguish biogenic from deeply-sourced (thermogenic) methane? Is there anything usable about Barents Sea methane in the literature?

Answer: *In principle, it is possible to distinguish isotopically between thermogenic and biogenic methane on the basis of the isotopes, but in our case this is not possible because the methane concentrations in the sediment samples were too low for isotopic measurements (see also results in chapter 3.3).*

Line 480: How does sulfide production stimulate the Asgards? This is again an example of overly generic statements that really mean nothing. Remember that the Asgards consist of multiple phylum-level lineages (Eme et al. 2023 Nature); is it likely that they will all behave in the same way when tickled by sulfide?

Answer: *We identified sulfhydrogenase (hydB and hydG), aka sulfur reductase (EC 1.12.98.4), which is typically involved in respiratory sulfur and polysulfide reduction. The reduced sulfur species targeted by this metabolic process are inferred to derive from dissimilatory sulfate reduction. The corresponding ORFs were taxonomically assigned to the phylum Asgardarchaeota, i.e. class Lokiarchaeia and Thorarchaeia. To support this statement, we provide a phylogenomic tree (see here under and Figure 7D in the main manuscript).*



Line 485: What are the geochemical features that can be used to tell apart seep and background sites? The discussion always returns to sulfate in its various guises (penetration depth, SMTZ …). Low organic carbon content is discussed, not as a diagnostic feature but as a factor that helps HC detection by keeping the heterotrophic background down.

Answer: *The sites were selected by our industry partner Aker BP, based on their extensive exploration activities in the area, including exploratory drilling. From the data presented in our study, none of the datasets alone can be used to differentiate between HC and Reference sites. Only through a combination of various parameters, including the geochemical data can we make a distinction.*

Line 500 ff: the enormous variability of SMTZ depth in continental margin sediments argues against using this criterion for identifying slow seep areas

Answer: *We tend to disagree with the Reviewer's comment. Although the high variability of the SMTZ at the continental margin means that the SMTZ depth cannot generally be used as a criterion for the identification of seeps, a depth shift on the local scale (our HC and Reference sites are just a few km apart) can certainly serve as a criterion. We clarified this in the text.*

Lines 510 ff: these discussion paragraphs come across as somewhat generic. For example, a more careful analysis of the metagenomic data could help to identify whether DIC removal or sulfate recycling via sulfide oxidation are more likely. For example, do you have dsr genes that function in the oxidative direction? These can be told apart from their reductive cousins (Dahl et al. 2005. J Bacteriol. 187(4):1392-404). Also, what is known about the redox status of these Barents Sea sediments? Oxygen, nitrate, metals – anything that could serve as an electron sink for sulfur oxidation?

Answer: *From previous work in the area we know that the sediment is fully anoxic below the uppermost few cm. Also, the aprAB and dsrAB trees clearly show that none of these genes correspond to their reverse function. We did not identify dsrC, which potentially works in sulfur oxidation. We assume that carbonate ions are originating from AOM at greater depths, diffusing upward and eventually precipitate as calcium carbonate. However, given the sulfate concentration profiles and the scarce methane data, it becomes clear that AOM is not a quantitatively important process in the cored depth interval. Also, the low level of detection of transcripts related to AOM suggest that the imprint on the geochemistry is minimal to non-detectable.*

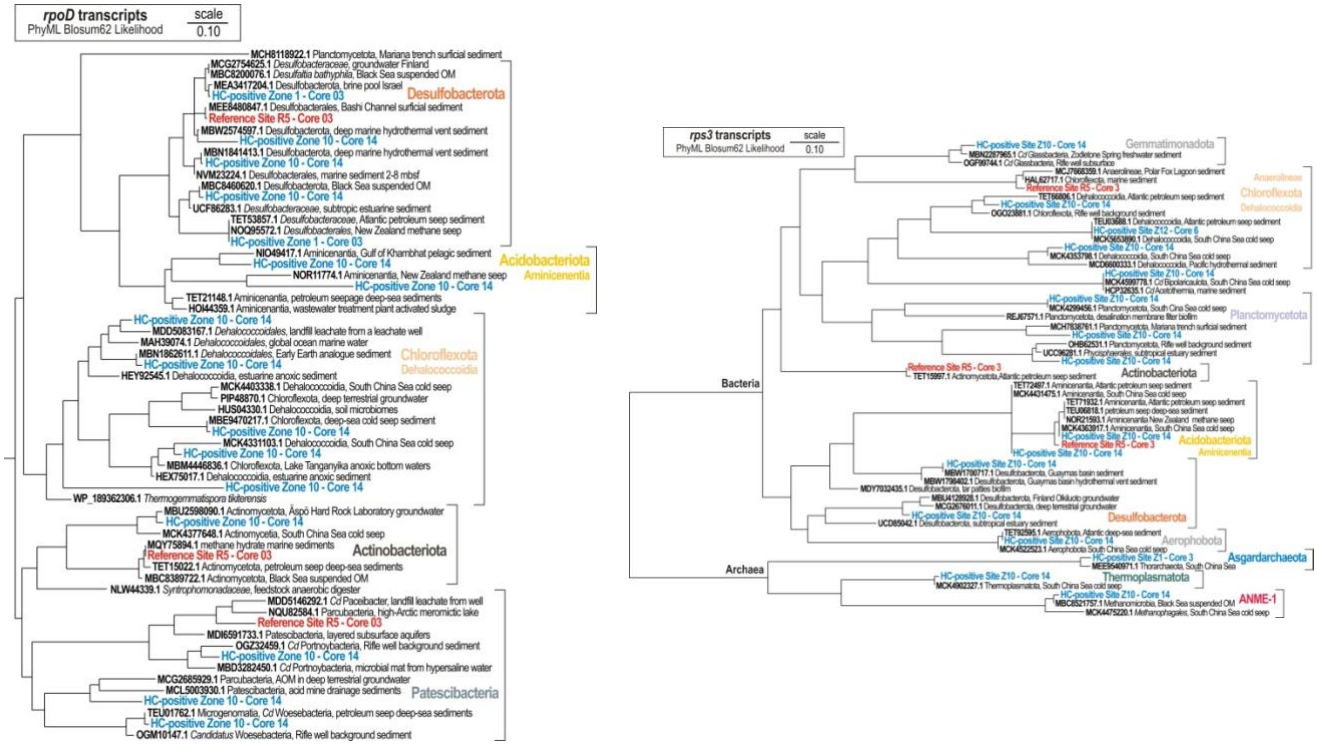
The low level of transcripts related to AOM suggest that the imprint on the geochemistry is minimal to non-detectable.

We are somewhat surprised that the Reviewer considers the analysis of our dataset to be quite generic since, to our knowledge, there is no other paper that presents data for such a great number of sampling sites, and such a wide range of geochemical and molecular biological parameter, with all samples being processed to identical standards, thereby allowing for unprecedented compatibility of the individual datasets. Therefore, the paper not only explains the influence of HC seepage, but also shows the spatial variability of various parameters.

A concluding note about the phylogenetic gene trees in the supplements – they are barely mentioned in the manuscript and not really used for anything. However, they could demonstrate phylogenetic affinity for particular groups and lineages within major phyla, and thus they could sharpen the discussion beyond generic phylum-only generalizations. Check carefully where exactly you are in phylospace, and do not rely on Genbank-only annotation.

Answer: *We combined the aprAB, dsrAB, mcrA and hydB-G phylogenetic trees into a new Figure (Fig. 7) that we implemented in the main text. We also provide an additional taxonomic tree based on rps3 transcripts as Supplementary Figure S4.*

We also like to clarify that we did not sequence 16S rRNA gene amplicons. Further, the assignment of rps3 gene sequences at a deep taxonomic level requires the compilation of reference sequences into one's own database and to perform a HMM analysis. With regards to GTDB taxonomy, we consider that it is beyond the scope of the present manuscript to produce a detailed taxonomy from the MAGs obtained by our collaborators.



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