Biogeosciences Discussions

Biogeosciences Discuss., referee comment RC4
https://doi.org/10.5194/bg-2021-223-RC4, 2021

Comment on bg-2021-223

Anonymous Referee #4

Referee comment on "Long-term incubations provide insight into the mechanisms of anaerobic oxidation of methane in methanogenic lake sediments" by Hanni Vigderovich et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-223-RC4, 2021

In this study, the role of AOM in Lake Kinneret sediment incubations was explored. Several incubations tested which terminal electron acceptors accounted for AOM activity. The main findings were that:
• pre-incubation with methane for 3 months significantly increased AOM
• hematite seemed the most likely iron mineral used as terminal electron acceptor (TEA) for AOM although it did not stimulate AOM and other iron minerals could have inhibited AOM
• natural humic acids and black coffee could be TEA for AOM
• sulfate-AOM was determined neglectable
• BES inhibition indicated that archaea mediated AOM, which was supported by metagenomic and 13C-lipid analyses

Major comments
It will improve the study to have its goals clarified in the introduction by L84-95. What were the specific research questions and knowledge gaps addressed here? What is this study addressing that was not known from your previous studies? This was still not clear to me after reading the entire manuscript. I think this is reflected in the title: "Modification of methane oxidation pathways during long-term incubations of methanic lake sediments" - I could not understand which modification occurred (bacterial methanotrophs did not thrive? TEA changed?). Think about a more specific title that summarizes the main key message of the study.
The materials and methods section needs major improvements for experiment reproducibility - adding amounts, concentrations, units, calculations etc. Sequencing data must be made available and an accession number must be provided. Data that has been already published and is here reproduced must be made clear.
All these incubations were done but no methane oxidation rates are provided in the manuscript, so calculating them and presenting them would add a lot of value.
Metagenomics results were barely used (same goes for lipid data). Consider doing metabolic reconstruction of the MAGs recovered here or use this data for another study that explores metabolic potential and mechanisms of potential taxa responsible for Fe-AOM.

Detailed comments
review grammar of the manuscript
L42 - ANME between parentheses
Intro: add background on the black coffee experiment - what was the hypothesis and the literature background?
2.1 add geographical coordinates of sampling site
2.2 indicate that concentrations of substrates in pre-incubated sediment experiments are provided in table S1 but bring this table to main text given that it is vital for the manuscript and experimental reproducibility. Also, add to this table similar details about the other two types of experiments (semi-bioreactor and incubation with recently collected material) which are so far missing from the methods section. Indicate if substrates were bought or synthesized (especially for minerals) with manufacturers / synthesis protocols.
2.3 Name it "Porewater and gas analyses"?
L202 can you provide methane detection limit in total amount (µmol) instead of concentration (µM)? Also, add the volume of gas injected into the GC?
Eq 1 and 2: provide units for each term, label eq (1) in L205 and (2) in L206; invert eq (1) so it will be ð□□¹ð□□·ð□□¼13ð□□¶ð□□□ = ð□□¥ × ð□□¹13ð□□¶ð□□»4 + (1 − ð□□¥) × ð□□¹ð□□·ð□□¼13ð□□¶ð□□□
Also, can you add what was the final time used to calculate rates? Were rates derived from the slope or from the difference between T0 and T-final?
L161, section 2.2.2 - add bioreactor volume and manufacturer information?
2.4 at L215 needs more details for experimental reproducibility: what was the sample exactly (sediment? how many g?), concentrations of added compounds and steps - protocol format given that a modification of Sturt et al., 2004 was used. Suggestion: release the step-by-step protocol as supplemental material or zenodo link with doi number. Deposit sequencing data and add a data availability statement.
2.5 How were counts per million reads calculated? Add formula to methods here. Also, can you briefly list all tools that produced data part of this manuscript and are part of the SqueezeMeta pipeline? For instance, what did you use for MAG taxonomic classification? And for genome annotation / gene search?
I could not fully understand if and which results presented in this manuscript are already published (i.e. L115-117, L249-253). Can you please clarify this? Also, given that a number of different incubations were performed, I suggest numbering them consistently in text, tables and figures to facilitate tracking.
L265-273 & Figure 2 = the most useful to me would be a plot of methane oxidation rates as a figure and, in the text, something like this: "treatment X or addition of X increased methane oxidation rates (in nmol/dry g sed/day to allow comparisons with other studies/settings) by X% relative to controls". Also, in Fig 2, what is the difference between blue, red and yellow? Add this information in the legend.
Fig 2, 3 and 4 = Is it possible to improve the quality? Also, it would be great to have methane oxidation rates in the text or as a figure - from all these different incubations, the only number provided is "3-8 % of the 13C-methane" in L454, which should be presented as a rate - this information I would find most valuable from this study and would allow comparisons with data from other environments, which could be added to the discussion.
3.2 I suggest showing metagenomic results in the main manuscript. My suggestion is to make a heat map with MAG coverage normalized by metagenome size (instead of RPKM values) and add to this figure the info of Table S3. Also, instead of binscore, use MAG completeness and contamination (in %). Would also be good to know how many MAGs were reconstructed and which ones represent candidate iron reducers - FeGenie could be useful for that: https://doi.org/10.3389/fmicb.2020.00037
Table S4 I was surprised that mcrA and pmoA are not in this table! I think including these and iron reduction and extracellular electron transfer genes would be better use of your metagenomic datasets, which could be extensively better explored in this study.

L328-331 The numbers here do not match Table 1, which shows more data than discussed here. Maybe this table is not so important and could go to supplemental materials?
Table 1 = Can you clarify what exactly each incubation is and what are killed controls potentially present here?
MAG coverages indicate Bathyarchaeota could be mediating Fe-AOM or play an indirect important role given that they are more abundant than ANME-1 - here the metabolic reconstruction of these MAGs would be fundamental! No mcrA was found in Bathyarchaeota - did you use an HMM that could find divergent sequences? what about other genes in reverse methanogenesis? what is Bathyarchaeota's metabolic potential in your incubations?
From table S1 I assume hematite is the dominant iron mineral in lake sediments, is it? Then I find curious that this most promising terminal electron acceptor did not stimulate Fe-AOM while other iron minerals could have even inhibited AOM. Can these results alone be taken as evidence for Fe-AOM? I find them insufficient. More discussion is needed to hypothesize about what is happening and how to improve experimental conditions.
In the semi-bioreactor experiment, why was little methane provided (when the methane headspace was replaced by anoxic liquid)? For how long were these semi-bioreactors operated? ~600 days? Also, any particular reason for calling them "semi" and not simply "bioreactors"? Finally, know that from our experience shaking biomass/sediments disrupts AOM activity (related to L166-7). So, shaken and with little methane, I am not surprised to see in Fig 2 that there was no AOM detected in the bioreactor. In this manuscript, there is no discussion of bioreactor results, so I suggest to add something.
L423 To enrich the discussion on 13C assimilation into lipid, I suggest addressing your results in the context of these findings and potentially more:
Wegener G, Niemann H, Elvert M et al. . Assimilation of methane and inorganic carbon by microbial communities mediating the anaerobic oxidation of methane. Environ Microbiol. 2008;10:2287–98.
Kellermann MY, Wegener G, Elvert M et al. . Autotrophy as a predominant mode of carbon fixation in anaerobic methane-oxidizing microbial communities. Proc Natl Acad Sci. 2012;109:19321–6.
Julia M Kurth, Nadine T Smit, Stefanie Berger, Stefan Schouten, Mike S M Jetten, Cornelia U Welte, Anaerobic methanotrophic archaea of the ANME-2d clade feature lipid composition that differs from other ANME archaea, FEMS Microbiology Ecology, Volume 95, Issue 7, July 2019, fiz082.
L426 move to results
L426 Just because ANME are not very abundant it does not mean they are not (very) active. Here abundance is expressed as "< 1.5 %" - specify what this number refers to (relative abundance? how was this calculated? add to methods)
L443 I think it's appropriate to tune this down: "we hypothesize Methanothrix could be involved in Fe-AOM". High potential when ANME-1 is present and other archaea are more abundant is a bit stretching; but it would be nice to see some actual physiological evidence for the involvement of Methanothrix in Fe-AOM in the future. Here your back flux inferences also support ANME-1's role being much larger than Methanothrix.
L469 Table S6 is for the first time mentioned here in the discussion. It presents qPCR results that have not been mentioned in the methods, so these must be added and the mention must be moved to results. Methanogenesis rates are expressed in μM/day, which I found cryptic and does not allow comparisons to other studies - please convert to n or μmol/dry g sed/day
L470 I am missing and thus suggest adding a sentence hypothesizing about the key microorganisms (ANME-1) accounting for 3-8 % of 13C-methane oxidation to CO2 in these incubations. Also, what is this number referring to? Hematite-AOM? Humic acid-AOM? I would love to see rate comparisons between those!
L481-8 I find this insufficient to explain why putative bacterial methanotrophs disappeared in long-term incubations if oxygen could be generated via methanobactins. However, this must be stated at hypothesis level, we don't know if iron reduction and methane oxidation were coupled via methanobactin-produced oxygen. I think it's better to offer other

explanations or simply say it’s unclear why bacterial methanotrophs disappeared.