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Interactive comment on "Metagenomic insights into the metabolism of microbial communities that mediate iron and methane cycling in Lake Kinneret sediments" *by* Michal Elul et al.

Anonymous Referee #4

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Summary:

The manuscript by Elul et al reports the results of 16s amplicon and shotgun metagenomic analysis of a narrow sediment horizon from Lake Kinneret. These DNA analyses were conducted on freshly sampled sediment and sediment that had undergone the incubations characterized in detail in Bar-Or et al 2017. The authors focus their attention on enzyme systems that may be associated with iron or methane cycling. The authors provide information on the phylogenetic composition of the microbial community in general, as well as assign phylogenetic composition to specific enzymes by BLASTing the metagenome reads against the refseq database.

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Major concerns:

1) Insufficient information is given about the incubations which is needed to fully evaluate the likelihood of the conclusions presented in the current work (most crucially, these incubations are methanogenic).

2) The suggestion that Methanothrix may carry out a methane oxidizing metabolism breaks with everything that is known about this group, and the claim is not supported by any experimental data. This suggestion should be removed.

3) The authors do not carry out any calculations to support their claim that traditional ANME are not abundant enough to carry out the trace AOM they claim to observe, and no effort is made to engage with the thermodynamic feasibility of the processes they are proposing, which is fairly straightforward and should be done.

Concerns 1&2:

This manuscript is framed as a study that will draw significant insight from incubations. Incubations with specific substrates or inhibitors can be very powerful tools in environmental microbiology, particularly when the microbial community responds to the incubation conditions, and when care is taken to clearly describe the bulk geochemical processes that have occurred in the incubations. Unfortunately, this is not the case in this study, while I understand that the bulk of the description of the incubations was previously published, a few key pieces of information have been left out of the current manuscript.

It would likely appear to a reader that these are incubations in which methane oxidation is the dominant process since so much emphasis is put on AOM as compared to methanogenesis. AOM is the most discussed metabolism in the abstract, and a major conclusion is the surprising attribution of AOM metabolism to Methanothrix. However, these incubations are NOT carrying out the net oxidation of methane, they are net methanogenic (see Figure 2b of Bar-Or 2017 “Positive methane concentrations reflect

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net methanogenesis during iron-coupled AOM.").

To put the results more plainly: sequencing of methanogenic incubations reveals a dominant archaeon that is a well-known methanogen. When stated in this way, I cannot support the publication of such a speculative assignment of AOM activity to Methanothrix. The simplest explanation is that the dominant methanogen is growing via the dominant methane cycling process, i.e. methanogenesis.

The justification for any discussion of AOM relies heavily on the previous publication that found 13C methane was converted into 13C CO2, and this activity was inhibited by BES. Methanogens carry out backflux of isotopic label from methane to CO2, and the authors have cited the classic paper that shows this (Zehnder and Brock, 1979). Methanothrix could indeed be responsible for the conversion of 13C methane into 13C CO2, but this observation does not constitute evidence that they carry out net AOM in the environment or in these incubations. It is crucially important for metabolisms that are so close to equilibrium for the authors to be very clear about whether they are suggesting an organisms is making energy for growth by carrying out AOM, or whether the organism may simply be responsible for the equilibration of isotope labels in the opposite direction of the process they are using for energy generation.

Another line of evidence for AOM is reaction-diffusion modeling that was carried out on Lake Kinneret sediments (Adler et al 2011), which concluded that there was peak methanogenesis 5-12cm below the sediment surface, and there was deeper AOM region under that. But microbial 16s profiling carried out in Bar-Or et al 2015, did not show a significant change of methanothrix (there referred to as methanosaeta) between the methanogenic and the methane oxidation zones.

This is a big claim the authors are trying to make, and it would require some sort of direct evidence like: 1) if there was an incubation where AOM was the dominant processes and the authors were able to show that methanothrix was the only organism present with the seven step methanogenesis pathway; 2) or better yet that methanoth-

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rix was enriched under these conditions vs. conditions without methane/Fe addition; 3) or, upon the addition of methane (and Fe?) there was a positive reaction of methanothrix based on metatranscriptome analyses, 4) or, at the very least that in nature there was a correlation between methanothrix abundance and the horizons where methane oxidation is occurring.

Unfortunately, the community did not significantly change under any incubation condition (line 45), and there is no correlation presented from the natural distribution of species, so there is no valid justification for assigning a novel role to an organism that could just be making methane. Unless stronger evidence exists, all claims like the one in line 375: "Our data hints that Methanothrix, which has not been considered to be involved in Fe-AOM previously, has the potential to be involved in methane oxidation, as presented in figure 5" should be removed.

Concern 3:

If the authors reject the isotope backflux idea (there is not a clear quantitative argument against this, even in Bar-Or et al 2017), and insist that there must be an organism subsisting on AOM in their incubations, then it is unclear why the minor, traditional ANME organisms will not suffice.

In the abstract the authors write (lines 23-24) that “bonafide [sic] anaerobic oxidizers of methane (ANME) and denitrifying methanotrophs Methylomirabilia (NC10) were scarce”, discounting their role in AOM in these sediments. But then they highlight on line 25-26 “We show that putative aerobes, such as methane-oxidizing bacteria Methylomonas and their methylotrophic syntrophs methylotenera... can be involved in the oxidation of methane...”.

It is not at all clear why the authors feel that ANME should be discounted while aerobic methanotrophs should be accepted as being responsible for methane oxidation. On line 176 the authors say that 0.3-0.8% of their reads map to ANME-1. And the very next paragraph the authors discuss the type I methanotrophs which are found to be 0.4-

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1.8% of the community. There is no meaningful difference between 0.3-0.8% and 0.4-1.8% in terms of abundance, so why do they feel comfortable highlighting the possible role of aerobic methanotrophs at this abundance and not the anaerobic ones? Why have the aerobic methane oxidizers made it into Fig 5 but the bona fide ANME have not?

AOM is not the dominant process, so its seems reasonable that if there is a small methane oxidizing community that it could be carried out by normal methane oxidizers that are in low abundance. The only way to rule this out is to determine the rate of AOM, try to estimate what 0.3-0.8% read mapping may correspond to in terms of cell numbers, and then calculate a cell specific rate and show that this rate seems far too high when compared to values present in the literature for ANME rates. None of this work is done.

When discussing possible metabolisms and their putative relative importance, it is very helpful to discuss the thermodynamic feasibility of these reactions. But in the summary line 380-381 the authors write ". . .whether this process [methanothrix AOM] is justified from the thermodynamic and kinetic perspectives, remains to be elucidated.". Doing the thermodynamic analysis should be a bare minimum requirement when suggesting a remarkable new metabolism for an organism. What are the relative free energies associated with acetoclastic methanogenesis and then Fe-AOM vs. acetate oxidizing iron reduction? For a study that is essentially just a single metagenomic analysis (since there is no noteworthy difference between any of the samples), the authors should at least attempt to supplement their discussion with thermodynamic discussions.

Minor comments:

"Consortium" should not be used interchangeably with "community" especially in the context of AOM research where "consortium" is very commonly used to refers to a physical, presumably syntrophic association between two microorganisms. Since no evidence is provided about actually association between any organisms described in

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this study “consortium” should be replaced throughout with “community”.

Line 361: “Our results show that in general, the phylogenetic diversity is a good predictor of the functional diversity in these samples”. This is too broad of a statement for a paper that has a fairly narrow focus on iron and methane cycling.

Line 20: not clear what “intrinsic” means in this context. Are any organisms in this sample not intrinsic?

Line 63: Assigning Thermodesulfovibrio to a carbon oxidizing, iron reducing metabolism is wildly speculative and should be removed unless more work is done to support the claim. The authors cite Spring et al 1993 (indirectly, by way of Bar-Or et al 2015) for this claim. Spring et al does not make this claim, they suggest as a throw-away hypothetical in the discussion section that it could be possible that Magnetobacterium could gain energy from sulfide oxidation coupled to iron reduction. They had no evidence for that claim, just suggested it was possible because Magnetobacterium has magnetosomes and lives in sulfidic environments. If the authors want to follow up this speculation with analysis, then they could look for the magnetosome genes in their metagenomes and see if they are phylogenetically aligned with Magnetobacterium (see Lin et al 2014 for the genes in magnetobacterium, https://www.nature.com/articles/ismej201494). If these thermodesulfovibrio have magnetosomes then maybe its worth mentioning this, but even then, it is probably worth noting that there is no actual evidence that these organisms can grow in this way.

Line 143-145: Here the use of “limiting nutrient” is confusing. This term often refers to something that is a growth requirement because it is needed for the production of biomolecules or cofactors, P, N, Fe, etc. This is a different concept than iron being used for the purpose of an electron acceptor, which seems to be the focus of this study. Clarification is needed.

Line 151: three groups are listed and then “3-6% read abundance, respectively”. Incorrect usage of respectively, not clear what each groups abundance is.

Line 158: class-level phylogenetic information should not be taken as evidence for the pH optimal for a group (the authors actually site a paper that describes how a different species of thermodesulfovibrio is alkaliphilic as compared to other species in that genera). This is definitely is not evidence for acidic/basic microenvironments.

Line 378: “positive correlation between Methanosarcinales abundance and concentrations of reduced iron in the deep sediment sections (Bar-Or et al 2017)”. This is a very strange claim and I cannot find any significant data that supports it. Bar-Or 2017 does not include pore water profiles or depth profiles of Methanosarcinales, so maybe this reference is supposed to be Bar-Or et al 2015? Even so, the data presented in Bar-Or et al 2015 Figure 4 is single replicate from three depth points. It looks like the difference between 6-9cm and 29-32cm for methanosarcinales is 50% -> 55% at most? With this level of replication this is not a significant correlation that should be taken as evidence supporting methanosarcinales being responsible for iron reduction.

Figure 4: something is wrong with the description, or the data presented. For OmcS LK-2017 the number next to the bar is 4, which the caption says corresponds to the number of total reads mapped to a gene. That bar shows very fine delineations, “Deltaproteobacteria” is maybe 1/20th of the total area of the bar? How can you get 1/20th with only 4 reads mapped? This comment applies to other bars in the OmcS figure. Maybe worth revisiting how these were calculated?

Line 389: “Another possible explanation for the methylated compound leakage is the reversibility of the enzymes involved in AOM, in particular methyl-CoM reductase”. Mcr does not may methylated compounds like the ones the authors are referring to in the forward or reverse direction, so the reversibility of this enzyme has nothing to do with this discussion.

Figure 5. The schematic in the top left shows iron reduction (Fe(III) -> Fe(II)) producing electrons

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