

Point-by-point response to referee's comments (our answers in blue):

In the latest iteration of this manuscript by Vigderovich et al., the authors put considerable effort to the language, flow, and clarity through the whole document. The methods are much easier to follow and are much more reproduceable. The results are properly reported and discussed. However, I have only one minor comment which I will point to below. Otherwise, the remaining minor comments and edits are rather small and mostly cosmetic (see inline comments).

We thank the referee for the kind words and the thoughtful and thorough review. We addressed all the comments and revised the conclusions as suggested. We believe that the manuscript is now ready for publication.

Minor comment:

The results from the metagenomic and lipid analysis clearly show evidence of aerobic methane oxidizing bacteria. The discussion does clearly state that if aerobic methane oxidizing bacteria do play a role in turning over methane in these experiments is quite low. However, the conclusion reads as if they play a much bigger role in the turnover with methane which is hugely speculative. The problem I have with this portion of the manuscript is that aerobic methane oxidizing bacteria were not directly tested in any of the experimental setups. Oxygen concentrations were not determined, and the experiments were all set up anaerobically. Thus, the incubations were not setup to directly test for aerobic oxidation of methane activity. I still think though the metagenomic and lipid findings are a real bonus dataset and are very interesting and should be investigated further. However, I do not think the results presented in this paper warrant the statement in the conclusion that aerobic methane oxidizing bacteria play a role since it wasn't directly tested. I therefore, suggest that the paper would be much stronger by really highlighting the coupling of hematite to AOM by anaerobic archaea. Following this conclusion, provide examples of how aerobic oxidation of methane by the bacteria maybe involved in Lake Kinneret sediments, but the results presented here show a need for further direct testing of this potential.

We understand the points of this comment and the conclusions section was revised carefully accordingly.

Inline comments:

Overall, the manuscript reads much better. Below are some inline edits and comments I caught while reading. It is likely I did not catch all and I suggest the authors go back and thoroughly fix minor grammatical errors.

We addressed all the comments and fixed grammatical errors throughout the manuscript.

L54-55: There are other types of aerobic methane oxidizing bacteria. This sentence reads as if there is only 1 in existence. I suggest just adding in parentheses type II and type X.

Type II and X were added in the parentheses as suggested.

L52: get rid of "Thus"

The word was removed.

L79: Are you saying Fe-AOM is oxidizing methane in the methanogenic zone or Fe-AOM removes methane produced from methanogenic zone in the top 20 cm?

In Lake Kinneret sediments methane is produced from the top centimeter to at least 40 cm depth. Suggesting that this entire sediment column is the methanogenic zone. Methane concentrations peak at approximately 15 cm and then decrease. We consider the sediments below 15 cm to be the “deep methanogenic zone” where methane concentrations decrease due to Fe-AOM, which removes 10-15% of the produced methane at those depths (16-40 cm). We clarified the text: “iron coupled-AOM (Fe-AOM) removes 10-15% of the produced methane in the deeper part of the methanogenic zone (> 20 cm below the water-sediment interface).”

L88: Include “conditions” after “hypoxia”

The word “conditions” was added as suggested.

L94: Delete “are available”

The phrase was deleted.

L100: Add “of” before “two stages”

The word “of” was added.

L127: Please add how much ¹³C-methane you added.

The details were added. We refer to Table 1 for specific information regarding the amount of ¹³C-methane added.

L137-139: This would probably read better as two sentences.

The sentence was divided into two: “Semi-continuous bioreactor experiments in which sediments were collected up to three days before the experiment was set up (freshly sampled sediments). The sediment to porewater ratio was 1:4 and porewater was exchanged regularly.”

L150: Cores were sliced at what cm intervals?

We used the bottom part (as a bulk) of each core (below 20 cm).

L151: Please provide details on the dedicated container. Also why not directly into falcon tubes?

We used a 5 L plastic container. We collect the bottom part of at least 10 cores. We transfer the sediment from the core straight to the container. The text was revised and clarified; it now reads: “The bottom part of the sediment cores (the deeper methanogenic zone, i.e., below 20 cm) was transferred, as a bulk, to a dedicated 5 L plastic container onboard.”

L149-154: I am a bit confused here. You collected cores and extracted porewater on the same day. However, porewater was extracted in the lab while core collection and slicing happened on board. Were these just day long excursions and you were able to bring the sediments back to the lab for extraction? If so I suggest making this more clear that field sampling and laboratory processing happened on the same day of collection.

We understand the confusion, yes, these are day-long excursions. The cores were collected and the sediments for the porewater extraction were cut and transferred to a container on board in the morning. Then, in the lab, we transfer the sediments for the porewater extraction with cutoff syringes to 50 ml falcon tubes and centrifuge them to separate the porewater. All of this is done on the same day. This was clarified in the text: “Sediments from the deeper methanogenic zone (sediment depths > 20 cm) for the experiments were diluted with porewater from the methanogenic zone of parallel cores sampled on the same day. The bottom part of the sediment cores (below 20 cm) was transferred, as a bulk, to a dedicated 5 L plastic container while onboard. The cores and the container were brought back to the lab, and the porewater was extracted on the same day of sampling. In the lab, sediments were collected from the container with 20-ml cutoff syringes and moved to 50-ml falcon tubes.”

L153: Syringe filtered or filter tower?

Syringe filtered. This was added to the text.

L155: Missing temperature units.

The units were added.

L155: Subsampled not subsamples

This was corrected.

L162: Was the N₂ atmosphere maintained in a glove bag or continuous flushing? Please add.

Continuous flushing. This was added to the text.

L163: Please add how you homogenized the sediment.

We shook the sediments in the zipper bag. This was added to the text.

L163: Add the word “was” between “gr” and “transferred”

The word was added.

L258: Add spectrophotometer model

The spectrophotometer details were added.

L263-264: For ethylene determinations did you use the same column and carrier gas for these measurements? If so consider consolidating into the previous sentence with methane.

Yes, it was in the same GC-FID, this was added.

L326: How “significant” did you do statistics. I would consider removing.

The word was removed.

L350-352: Sentence does not read well.

The sentence was revised. It now reads: “It was quantified directly by adding Na-molybdate to the methane-only controls and to the magnetite amended treatments in the second stage long-term incubations (Fig. 4A).”

L352-354: The addition of what had no increasing effect to the ¹³C DIC pool? Molybdate or magnetite?

The addition of Na-molybdate. This was clarified in the revised version.

L479: How significant of a finding is this. Where are the statistics behind this claim? I would consider removing the word “significant” in the document when statistics was not done.

The word “significant” was replaced with the word “relevant”.

L485: I think the flow of the manuscript would be better to have subsections to 4.2 for each of the electron acceptors (i.e. 4.2.1 various metal oxides as electron acceptors etc...).

We accept the referee’s suggestion, and we added subsections for 4.2 section in the revised manuscript.

L603: You mention in the discussion that Aerobic methane oxidizing bacteria play a minor role in turning over methane. This was only drawn from lipid and fatty acid determinations and genomic evidence which doesn’t necessarily mean they were active. Nor were any of these results connected to the ¹³C-DIC increases seen in the experiments. I therefore, suggest to adjust this sentence to say that the exact role of aerobic methane oxidizing bacteria in Lake Kinneret sediments needs further examination. Because as is, the text reads that they are more involved than what is reported in the data.

We agree with the referee and the whole conclusions section was revised. It now reads: “Previous results of geochemical and microbial profiles as well as incubations with fresh sediments from Lake Kinneret constitute evidence of the occurrence of Fe-AOM in the methanogenic zone. The process is performed by anaerobic archaeal methanogens and aerobic bacterial methanotrophs, which remove about 10-15% of the methane produced in the lake’s sediment. In the current study, we found that after two incubation stages and intensive purging for a prolonged duration, AOM was still significant, consuming 3-8% of the

methane produced. However, the abundance of aerobic methanotrophs decreased and anaerobic archaea (ANME-1 or specific methanogens) appeared to be solely responsible for methane turnover. AOM could be a result of carbon back flux, as the methanogenic/AOM pathway is reversible, however, the high $\delta^{13}\text{C}_{\text{DIC}}$ signal points to a metabolic reaction. Terminal electron acceptors or electron shuttles stimulating Fe-AOM are either hematite and/or humic substances. The role of the aerobic methanotrophs of the order *Methylococcales*, which were found in the freshly collected sediment experiments, remains to be examined.”