

Vigderovich et al., in their revised manuscript titled “Long-term incubations provide insight into the mechanisms of anaerobic oxidation of methane in methanogenic lake sediments” made considerable changes to the previous version. I believe the results in the manuscript are still interesting and appropriate for the Biogeosciences journal, however, I still think there is major work to be done before publication.

The manuscript needs attention in its consistency, flow, redundancy and clarity. The scientific English has gotten much better since the last version, however, there are still some syntax and sentence structure issues that make it difficult for the reader to follow. The manuscript also taps into results and interpretations from previous work from the research group and needs to do a better job of clarifying which interpretations are from THIS manuscript vs. previous work (i.e. fresh batch incubations from Bar-Or et al.). I again cannot identify them all, but I will provide examples below. I highly encourage the authors to consider using an editorial service to address the English in the manuscript.

Major Comments:

The introduction provides known facts about electron acceptors known to be coupled to AOM. However, it lacks general information about AOM occurring in lakes and mainly compares the marine environment generally. I suggest adding a few sentences about AOM in lakes or freshwater systems (i.e. lower sulfate concentrations, more methanogenesis, larger role of metals and nitrogen etc...) generally would help with flow and make the introduction more robust. The manuscript also lumps ANME and methanotrophic bacteria but does not introduce aerobic oxidation of methane and bacterial facilitators very well. I suggest making two separate paragraphs to describe anaerobic and aerobic oxidation of methane. The introduction now has a statement telling the audience the knowledge gap; however, I feel that the statement could be crafted better by being more specific about which methanotrophs and what kind of sediments (i.e lake sediments). Furthermore, the last paragraph of the introduction is already in the methods section and should either be taken out or greatly shortened.

The most challenging part of the manuscript is the methods section. The experiments are difficult to reproduce based on what is written here. I acknowledge the protocols found in the supplementary are useful, but the information found in the main text takes precedent. For example, there is hardly any information about how many cores were taken and when exactly they were taken between the years 2017 and 2019. It is also not clear when sediments for experiments type B and C were taken; there is one sentence that implies sediments were collected in 2013 which is confusing. Additionally, the text very sparingly cites the supplemental to direct the reader for more information about the protocols. If the authors wish to have the reader go to the supplemental for this information rather than put it in the main text it should be cited accordingly and in order.

The results now do a much better job of simply stating the data without interpretation than the previous version. However, there are several syntax and other issues which I identified some examples below in the in-line comments.

The discussion would benefit greatly with proper headers for all sections. It would also benefit with references that backup the interpretations presented, please see below for more details. The discussion would further benefit by adding sentences that conclude their interpretations for each section. There are parts of the discussion that beautifully build up what was done to help interpret the meaning but then ends rather abruptly with no resolution or even speculation of what maybe happening (see below for more details).

In line comments and edits:

L38-41: What about AOM in lake sediments?

L42: This first sentence is rather redundant considering the last sentence of the previous paragraph. Consider joining the two paragraphs to be more concise.

L45: What other metals are you referring too?

L52-55: I think 1 or 2 more sentences introducing aerobic oxidation of methane and aerobic methanotrophs should be included here. What is their difference to their anaerobic counter parts? The paper is about how the two processes are potentially co-occurring which is unusual so it would be useful to introduce the typical conditions these processes occur in lake sediments such that the reader understands the “normal” and what is not normal.

L42-55: This paragraph could have better flow. The authors just state facts about different electron acceptors but do not connect how these electron acceptors are coupled to AOM in lake sediments. For example, L51: what does selenite reduction have to do with AOM in lake sediments? How is this a supporting fact?

L50: Syntax error: “whereas nitrite fuels AOM by *Methylomirabilis*” does not make sense.

L56: There needs to be a better introductory sentence for this paragraph. Or better a concluding sentence(s) in the previous paragraph that connects AOM in lakes and then have a good intro sentence about Lake Kinneret.

L60: Are you sure the archaea are methanogenic or are they methanotrophic, not clear in the sentence?

L63: First time that type I aerobic methanotrophic bacteria are mentioned. Please see comment above about providing more introduction to aerobic oxidation of methane.

L63: What role are they playing methane cycling? I assume they are working together to oxidize methane in the lake sediments, not clear.

L64: Syntax error; how do you have aerobic methanotrophic activity in anoxic environments? This statement implies an aerobic process occurring in anoxic conditions which is contradictory. Do you mean “The combined methanotrophic activity by anaerobic archaea and aerobic bacteria in anoxic lake sediments might be supported by the presence of microlevel oxygen....”?

L66: Please provide a number for microlevel. Also there needs to be references that support the second half of the statement.

L67-68: Which methanotrophs do you mean here? Do you mean both the ANME and bacteria? I assume this trend is not well understood in lake sediments either? I see this is your knowledge gap statement and this statement could be stronger by being more specific.

L69-84: Most of this paragraph is redundant and already in the methods section. I understand the purpose of the paragraph, but it is far too long and should be brief.

L87: “in the North of Israel” implies the lake is not in Israel. Northern Israel would be more correct.

L90: From April till when?

L90-91: Are the surface water between 15-30 degrees C all year long? Or are certain months 15 or 30?

L91-94: What sediment depths is this data referring too? This sentence also seems redundant since in L94-96 reports nearly the same data in table S1.

L92: Are the references for clays in this sentence only for clays or both clays and carbonates? If the latter where is the reference for the carbonates?

L99: At what sediment depth does it get as low as 0.5 mM?

L104: Inhibitors for methanogenesis not methanogens.

L105: Is it necessary to say this is a “Long-term” incubation? All of the incubations seem to be long-term, why not just say two stage?

L105-106: Sentence is hard to follow, please restructure.

L107: The slurry was further diluted with what?

L109: How fresh is freshly? This is said a lot throughout the manuscript, but it is not clear what fresh means. Where are these sediments from? What sediment depth(s) are these sediments from?

L112: If you say several manipulations in this fashion then I would expect a description of each manipulation because this is rather vague. But since they are manipulations from previous work just say “and amended with... according to (REFERENCES)...”

L117-122: I think this paragraph needs to be its own subsection to describe how sediments were collected for all your experiments. Right now I have to assume this is how sediments were collected only for the “Long term two stage incubation”. Then the question is how were sediments collected for types B and C?

L117: Could you please specify how many sampling campaigns there were between 2017 and 2019.

L118: Was there a research vessel involved, if so which one?

L118: Syntax error: how does one use a gravity corer with 50 cm Perspex cores? Do you mean equipped with 50 cm Perspex core liners? How many cores were taken? What was the total length of sediment collected? Was it the same amount taken every time? How long did sediments sit in the core liners before processing?

L120: How many parallel cores?

L120-122: Was sediment sliced into intervals and put into 50 mL conical vials for centrifugation and then pooled later? There are some details missing as to how the sediments were processed for porewater extraction.

L121: please add a times symbol between 9300 and “g”.

L123: How much sediment? How was the sediment sampled from the core liner (i.e. slicing, cut-off syringe, bulk transfer)? Was the sediment added to the porewater or was porewater added after sediment was sampled? Were these also sealed with stoppers and crimped?

L125: Please provide product details for methane that was injected; similar to the nitrogen.

L123: I think you already said this in L119. I am confused, how many slurries are there?

L127: I think there is a syntax error. Is there a reason to have two experimental pathways when significant AOM is observed? Or do you mean that if no significant AOM is observed in the slurries then porewater is exchanged and continued to be monitored? Not clear.

L130-138: This information could be better integrated in the beginning of this section.

L130: I am still confused about the sampling in this section. There was 2 years that sediments were collected, and 10 sets were made for this experiment. When was each set collected? Did each set run in parallel with each other or did they run independently and different times?

L132: Is the pre-incubation the first stage? Please be consistent with your identifiers.

L132: Was the 1st stage bottle opened and then sampled? Not clear.

L137-138: Did your killed controls have any sediment in them? This sentence reads as if only the bottles were autoclaved and 13C-methane was added to them.

L142-144: I'd rather like to see how AQDS was prepared for your experiments and not what it has been shown to do; which was already introduced in the intro.

L139-154: This paragraph would read better if you included which experiment number or set corresponds to which electron acceptor or shuttler like you do with the inhibitors in the end of the paragraph.

L151-154: Are experiment numbers the same as "sets" in L130? Please be consistent with your identifiers.

L151: Is there a reason why molybdate was only added to the magnetite samples?

L155: A sentence after this one describing why duplicate or triplicates were made would be useful.

L159: Please provide which lake and why did you pick a different lake to get humic substances.

L161: So far I have not seen how your geochemistry (iron (II) and methane) are measured. I assume there is a section for that but perhaps add a parenthetical to indicate that.

L167-168: When were these sediments collected and by what method. Were they collected by gravity corers like experiment A? Please elaborate. If all sediments for all experiments were collected between 2017 and 2019 by gravity corers etc... then that should be stated before all the experiments are described.

L169: Similarly, with the porewater. Is this porewater from the same methanogenic depth? Please add details or blanket the information before all the experiments are described.

L171: What is the relative proportion of the 12C and 13C in the mixture?

L174: What batch experiments?

L175: Using an electrode not by electrode.

L182-183: Are you saying the pre-incubation sediments were collected in 2013 instead of between 2017 and 2019? The timing of sample collection for the sediment samples for any of these experiments is not clear at all. I suggest that there should be a new section in the beginning of the methods describing when sampling occurred for which experimentation.

L185: Please provide the material of the experimental bottles.

L190-195: There is information in the figure caption that should be in the main part of the methods. Please move them accordingly.

L198: I suggest this section needs to have subsections to disambiguate between geochemistry and molecular analysis. It is confusing to read about geochemical analysis and then jump to molecular analysis and then back to stable isotope analysis. Having sub headers would help the reader not only know when you are switching gears but also, for example, if someone wanted to find quickly a detail about the molecular analysis without reading the whole manuscript, they could easily find it using the sub headers.

L206: Determined not “measured”. You measured on a spectrophotometer.

L207-210: These two sentences do not read well. One could easily put them together for better flow.

L208-210: Please provide the GC model, column type and carrier gas.

L210-211: Ethylene was similarly determined to what? You mean that ethylene from acetylene reduction was measured by GC right?

L231: Not clear what is meant by duplicates a and b in the semi-bioreactor experiment.

L235: Never good to start a sentence with a numerical. Consider starting with “A range of ...” or spelling out nineteen.

L245: Rate should be plural. Please check tenses throughout.

L262-263: Syntax error. This sentence reads as if the results from type A and B were compared to the batch slurry incubation presented by Bar-Or et al., and Elul et al.,. You mean that you are comparing YOUR type C experiments with Bar-Or et al., and Elul et al., type C experiments, correct?

L266: Which samples do you mean when you say natural non-killed slurries? Figure 2 and 3 do not have any identifiers called “Natural non-killed slurries”. Do you mean all the slurries with different amendments? If so then I would not consider them natural. This is not clear and very confusing.

L266: How “significant” is the AOM and relative to what? Did you do any statistics? I would be careful using this word.

L266-268: Sentence does not read well, please restructure.

L270: I think that it is interesting that you have such a methanogenesis rate and Figure S3 should be moved into the main text. Also Figure S3 reports a rate in μM in the figure caption, mM in PW on the S3 y-axis and in $\text{nmol gr}^{-1} \text{Day}^{-1}$. I suggest making it all one unit type as it is very confusing.

L271: Now it is a two-stage incubation and not long term please be consistent.

L275: What treatments? Do you mean replicates? Figure 2 suggests that they are replicates.

L274-276: Sentence could be structured better.

L282: Please move the Figure 3B citation to just after the describing the increase from the nontrite.

L286: AOM is not “of” the incubation it is “in”

L287: Was sulfate measured in this study? I do not recall it in the methods.

L289: I do not think that molybdate inhibits the sulfate reducing bacteria, it rather inhibits sulfate reduction by acting as an analog to sulfate and bind to APS enzyme. Please be careful with distinguishing organism with metabolism. This has also already been stated elsewhere, consider removing.

L290-291: This sentence does not read well. Please restructure.

L290-291: This is not so surprising since magnetite was added and iron reduction is not inhibited by molybdate and it outcompetes with sulfate reduction. If there is no sulfate/sulfide concentrations reported nor any rates, then does this have a place in this manuscript?

L294-297: First time nitrate/nitrite measurements were mentioned. How did you measure it? This should rather be in the methods.

L301-303: This sentence does not read well please reorganize.

L303-304: Is this AOM rate for nitrate or nitrite coupling?

L320: Again BES is not an inhibitor of the methogen archaea it is an inhibitor of methanogenesis. You also need references.

L325: Methanogenesis not methanogens'

L327: What does the (SN-#) in figure 2 mean? I think that part should be taken out since it has no meaning for the reader and could lead to some confusion. Also why arnt the rest of the 10 sets of the long term two stage results in the graph. Isnt the point to compare all three experiments in its entirety?

L345-348: Table 2 is a bit confusing. What is the serial number? How is there multiple treatments per serial number?

L375: Figure 5 is oddly placed after the results section of the molecular analysis. I suggest moving this as to not confuse the reader.

L399: Get rid of "our"

L401-405: I think you should put the respective citation to the respective analysis in this sentence. For example, Figure 2 is not a representative of in-situ geochemical or microbial diversity profiles. Also this statement is rather generalized, are you saying this is happening everywhere or at Lake Kinneret?

L405-407: Are you talking about previous work with profiles or your results, not clear. If it is others please provide references.

L399-411: I am having a tough time distinguishing what is just a rereporting of findings from older publications and that of the interpretations of the present study. The point of the discussion is to interpret the meaning of results of the present study and use previous lit to support the argument.

L416-417: It is weird to cite a different publication to describe your result. Instead of citing your figure along with a different reference, why not use the space to compare the findings in the present study with that of Bar-Or et al.,.

L416-422: Why is the B and C experiment being discussed here when the header suggests that the Long-term two stage incubations will be discussed? It seems like here you are comparing all three experiments rather than focusing on interpreting "Potential electron acceptors for AOM in the Long-term two-stage incubation experiments". This header is misleading as one of your experimental types (A) is called Long-term two-stage incubation.

L419-420: I think that the differences in the bioreactors are interesting, but I also think that it is not super surprising as slurries in replicate can act as independent communities, because they are removed from the original environment, amended and sit for such a long time.

L420-422: I also don't understand this statement. According to your data AOM was stimulated with hematite better than all the other amendments that were done.

L420: Again, did you do any statistics to suggest that it is significant?

L425-428: Any lit to support that explanation?

L428-432: Run-on sentence, hard to follow and confusing please trim and reword.

L436-437: I am not sure your data suggest that adding iron decreases AOM activity directly. It is rather better to say iron is just being used for organoclastic iron reduction rather than to oxidize methane.

L437-441: You need references to back these statements up.

L442-451: See comment to L290-291. I think this should be omitted or greatly reduced and stated in the beginning that you believe sulfur cycling does not play a role in your experiments.

L453-454: Ammonium oxidation with iron reduction needs a citation.

L455-459: I understand your results don't indicate nitrate supports AOM but I don't understand from your interpretations how nitrate delays AOM. Why would organoclastic denitrification outcompete AOM? There is no citation in the text that supports that claim. Denitrification would of course be outcompeting other organisms for organic material but AOM is only oxidizing methane, which you added in abundance. I would agree that it is strange to have no ^{13}C DIC build up after adding nitrate but potentially, the addition of nitrate resulted in more organics to be oxidized a dilute the ^{13}C signal. Furthermore, the text clearly stated ANME-2d was not found. So I think that it is more accurate to say that nitrate does not support AOM as an electron acceptor during your experiments because the known ANME group that uses nitrate was not present. Also figure 3 C and D do not show any trends with sediment amended with nitrite.

L461-463: This sentence is contradicting. You said they weren't directly measured but then they were high. It should rather say previous work has shown DOC concentrations to be high in Lake Kinneret...

L465-467: References

L467: Cite your figure here. This is the one hit you got out of all the experiments and therefore, the crux. Please elaborate and compare to the Valenzuela et al.,

L479-482: Run-on sentence. Reads poorly.

L487-497: Do the authors have any interpretations as to why the aerobic methanotrophs play a minor role? I think this paragraph could be much stronger as it is eluding to who is responsible for oxidizing methane. Consider structuring the paragraph to better nail who is overall responsible.

L520-521: The authors built this paragraph up nicely but if it is not back flux by methanogens then what could it be? This kind of ended abruptly.

L529-531: This info could be useful in the discussion.