Response to the reviewer (in blue):

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.
We would like to thank the reviewer for the positive, constructive, thorough and thoughtful review. We addressed all of the comments and sent the manuscript to be professionally edited for English. We believe that the manuscript is now ready for publication.

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.
All of the reviewer’s comments about the introduction were addressed: We restructured the introduction, we added the general requested information (about aerobic and anaerobic methanotrophy, about AOM in freshwater compared to the marine environment and about the involvement of aerobic bacteria in AOM), and we focused our statement about the knowledge gap. We also shortened the last paragraph.

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.
All of the reviewer's comments about the methods were addressed. The main text of the revised version of the article contains more detailed explanations about the experiments, and more references to the supplementary material were added throughout to aid the reader.

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).
We addressed all of the relevant comments as detailed below.

In line comments and edits:

L38-41: What about AOM in lake sediments?
We restructured the introduction, and we added organized information about the phenomena of aerobic and anaerobic methanotrophy in lakes compared to marine environments.
L42: This first sentence is rather redundant considering the last sentence of the previous paragraph. Consider joining the two paragraphs to be more concise.
We revised the text.
L45: What other metals are you referring too?
We are referring to metals such as chromium, and that detail has been added to the text.
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.
We agree with the reviewer and added two paragraphs to explain aerobic and anaerobic methanotrophy processes in different environments.
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?
We improved the paragraph's flow by specifically describing each process vis-à-vis the different environments.
L50: Syntax error: "whereas nitrite fuels AOM by Methylomirabilis" does not make sense.
The sentence was changed.
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.
As suggested, we added an introductory sentence for the paragraph to contextualize our work in the frame of the AOM in general and in the frame of AOM in lakes.
L60: Are you sure the archaea are methanogenic or are they methanotrophic, not clear in the sentence?
Though they are known methanogens, they might perform AOM via reverse methanogenesis. This is now clarified in the revised text.
L63: First time that type I aerobic methanotrophic bacteria are mentioned. Please see comment above about providing more introduction to aerobic oxidation of methane.
They are now introduced earlier as suggested.
L63: What role are the playing methane cycling? I assume they are working together to oxidize methane in the lake sediments, not clear.
The role played by these aerobic bacteria is better explained, with greater detail, in the revised text.
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…."?
Such aerobic activity is observed in many environments that are considered anoxic. It is possible that there may still be trace amounts of undetected oxygen in the sediment or that it is somehow being generated in small amounts. This is better explained in the revised introduction and discussion.
L66: Please provide a number for microlevel. Also there needs to be references that support the second half of the statement.
We added the reference that describes that undetected trace amounts of oxygen (nanomolar levels) may be trapped in "anoxic" environments.
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.
We clarified this detail, referring to aerobic methanotrophs in the text.
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.
We substantially shortened this paragraph in the revised version.
L87: "in the North of Israel" implies the lake is not in Israel. Northern Israel would be more correct.
This detail was changed as suggested.
L90: From April till when?
Until December. This was added to the text.
L90-91: Are the surface water between 15-30 degrees C all year long? Or are certain months 15 or 30?
In the years that were studied, surface water temperatures ranged from 15°C in the winter (January) to 32°C in the summer (August).
L91-94: What sediment depths is this data referring to? This sentence also seems redundant since in L94-96 reports nearly the same data in table S1.

The sentence was removed, and only the previous data that we collected for the methanogenesis zone were cited.

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

The references were for both clays and carbonates, but the sentence was removed.

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

At sediment depths 30 cm below the water-sediment interface, the value is as low as 0.5 mM. This detail was added to the text.

L104: Inhibitors for methanogenesis not methanogens.

The term was corrected.

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?

Though the reviewer is correct, we wanted to emphasize the lengths of the incubations, which is needed due to the methanogen turnover time of several months.

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

The sentence was restructured.

L107: The slurry was further diluted with what?

The slurry was further diluted with porewater extracted from the same depth; this detail was added to the text.

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?

Freshly means up to three days from sediment sampling. This was clarified in the text that now reads: "Semi-continuous bioreactor experiments in which sediments were collected up to three days before the experiment was set up (freshly sampled sediments) …". All of the experiments were performed with sediments from the methanogenic zone (depths > 20 cm).

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)…"

The sentence was changed as suggested, and it now reads: "Batch incubation experiments with freshly sampled sediments and porewater at a 1:5 ratio, respectively, and amended with hematite. This experimental set-up was described in our previous studies (Bar-Or et al., 2017; Elul et al., 2021)."

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?

We agree with the reviewer and the text was modified as suggested.

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

There were 7 sampling campaigns. This detail was added to the text.

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

Yes, a research vessel, *Lillian*. This detail was added to the text.

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?

The text was corrected as suggested and the missing details were added. The text now reads: "The sediments for the slurries conducted in the current work were collected during seven sampling campaigns aboard the research vessel *Lillian* between 2017 and 2019 from the center of the lake (Station A, Fig. S1) using a gravity corer with a 50-cm Perspex core liner. The length of the sediment in each core was 35-45 cm. During each sampling campaign, 1-2 sediment cores were collected for the incubations and 10 cores were collected for the porewater extraction. Sediments from the methanogenic zone (sediment depths > 20 cm) were diluted with porewater from the methanogenic zone of parallel cores sampled on the same day. The porewater was extracted on the day of sampling. The sediment cores were sliced while onboard, and sediment samples from the methanogenic zone (> 20 cm) were transferred to a dedicated container. In the lab, sediments were collected with 20-ml cutoff syringes and moved to 50-ml falcon tubes. The porewater was extracted by centrifugation at 9300 × g for 15 min at 4℃, filtered by 0.22-µM filters into 250-ml pre-autoclaved glass bottles, crimp-sealed with rubber stoppers, and flushed for 30 min with N_2. The extracted porewater was kept under anaerobic conditions at 4 until its use. The sediments for the incubations were subsamples from the liners and diluted no later than three days after their collection from the lake and treated further according to the experimental strategies described above (setup A or B)."

L120: How many parallel cores?

Ten parallel cores. This detail was added to the text. Please see the response to the comment above.

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.

The sediment cores for the porewater extraction were sliced onboard. Sediment from depths below 20 cm was transferred to a container. In the lab, we transferred the sediment from the container (using cutoff 20-ml syringes) to 50-ml falcon tubes that were then centrifuged. The extracted porewater was kept under anaerobic conditions at 4°C in pre-autoclaved bottles until its use. These details were added to the revised text.

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

The symbol was added.

L123: How much sediment? How was the sediment sampled from the core liner (i.e. slicing, cutoff 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?

The relevant data were added to the text, which now reads: "In the first stage (pre-incubation slurry), the sediment core was sliced under a nitrogen atmosphere and sediments from depths > 20 cm were collected into zipper bags. The sediment was homogenized, and between 80-100 gr transferred into 250-ml glass bottles under continuous N_2 flushing. The sediments were diluted with the extracted porewater to create a 1:1 sediment to porewater slurry with a headspace of 70-90 ml (Fig. 1). The slurries were sealed with rubber stoppers and crimped caps and were flushed with N_2 (99.999%, MAXIMA, Israel) for 30 min."

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

The details (99.99%, MAXIMA, Israel) were added.

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

We clarified this point in the revised text. We added a subsection to explain the sampling procedures at the lake and in the lab, after which we referred to each experiment type for specific dilution details and slurry amounts.

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.

We produced 1:1 slurry to porewater ratio and monitored the pre-incubations. We exchanged the anoxic porewater to supply the natural dissolved nutrients and organics in addition to methane and enrich the community with high sediment to porewater ratio (as in natural sediment, where their abundance is very low). Then we further diluted the samples to enable more microbial enrichment and the extensive geochemical investigation. We also tested the aerobic methanotrophic activity at each stage by collecting samples for the metagenome and lipid analyses.

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

We rearranged the information according to the reviewer's suggestion.

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?

The sediments were collected during the 7 campaigns between the years 2017-2019 (including 2019). Some of the two-stage experiments ran simultaneously and some independently at different times. The details of the dates and the numbers of treatments are clarified in the revised main text and supplementary.

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

Yes; we clarified this detail in the text.

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

At the end of the first stage (i.e., pre-incubation), the bottles were opened under anaerobic conditions and transferred for the second stage using a syringe and a Tygon tube. These details were added to the text: "…the pre-incubation bottle was opened and subsamples (~18 g each) were transferred with a syringe and a Tygon® tube under a laminar hood and continuous flushing of N_2 gas into 60-ml glass bottles. The subsamples were then diluted with fresh anoxic porewater …"

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.

Yes, the killed controls contained slurry that was autoclaved twice. This was clarified in the text by switching the word "bottle" to "slurry": "The "killed" control slurries in each experiment…"

L142-144: Id 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.

The sentence was changed as suggested to: "The synthetic analog for humic substances, i.e., 9,10-anthraquinone-2,6-disulfonate (AQDS), was dissolved in DDW (detailed in the supplementary information) and added to the bottles of experiment no. 6 until a final concentration of 5 mM was achieved in each bottle."

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.

The experiment numbers were added as suggested.

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

Yes. The text was corrected: “This study presents ten two-stage incubation experiments (experiment numbers 1-10) with different treatments…”

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

Molybdate was added to one of the natural slurries, to one of the magnetite treatments, and to the slurries with the amorphous iron. This detail was clarified in the revised version.

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

We attempted to use triplicates whenever it was possible. In some cases, due to limitations associated with the slurries, we had to use duplicates. This was explained in the revised version: “All live treatments were set up in duplicate or triplicate, depending on the amount of the pre-incubated slurry aimed for each experiment and the results are presented as the average with an error bar.”

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

A thermokarst lake near Fairbanks, Alaska. Unfortunately, natural humic substances were not available from Lake Kinneret, and thus we used humic substances that were available for us from another freshwater environment with profiles that exhibited similar methanogenic zone with iron reduction. These details were added to the text.

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.

Yes, there is a section for the geochemistry measurements (2.3.1) that is now indicated as suggested with the respective heading in the Analytical methods (2.3).

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.

We agree, and therefore, a new subsection with the relevant sediment collection information was added as the referee suggested in a previous comment.

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.

Yes, the porewater was extracted from the same methanogenic depth as described in the new subsection. This was clarified in the text.

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

We added 13 ml of ^{12}C and 2 ml of ^{13}C methane (total of 15 ml) to the bioreactors.

L174: What batch experiments?

The two-stage experiments and the fresh batch experiment. We clarified this in the revised version.

L175: Using an electrode not by electrode.

This was corrected.

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.

The sediments for all of the pre-incubations and for the bioreactors were collected between 2017-2019. Only the sediments for the fresh batch experiment were collected in 2013. We clarified this in the revised version in the new subsection, 2.2.1 General.

L185: Please provide the material of the experimental bottles.

These were glass bottles. This was added to the text.

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

The information was removed from the caption. It can be found in sub-section 2.2.2 (experiment type A set-up) of the revised version.

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.

We agree and sub-headers were added.

L206: Determined not "measured". You measured on a spectrophotometer.

The word measured was changed to "determined" as suggested.

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

The two sentences were combined as suggested: "A 100-µL headspace sample was taken for methane measurements with a gas-tight syringe and was analyzed by gas chromatograph (Focus GC, Thermo) equipped with a flame ionization detector (FID) and a packed column (Shincarbon ST) with a helium carrier gas (UHP) and a detection limit of 1 nmol CH_4."

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

The following details for the GC were added to the text: gas chromatograph (Focus GC, Thermo) equipped with flame ionization detector (FID) and packed column (Shincarbon ST) with a helium carrier gas (UHP).

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

Yes. This was clarified in the text.

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

This refers to the duplicate samples that were taken from the semi-bioreactor at that time point. They are called "a" and "b" in the supplementary coverage table. This was clarified in the text.

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

The text was changed as suggested to: "Between 19 and 40 million…"

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

The word was corrected.

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?

Experiments type A (two stages) and B (fresh bioreactor) were performed during this study. We meant that these results were compared also to the fresh batch slurry incubations

presented by Bar-Or et al. and Elul et al. (type C), which were also set up by our group and co-authors. We clarified these details in the text.

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.

We meant all the non-killed treatments which were amended with $^{13}CH_4$. This was clarified in the text: "At the same time there was a conversion of ^{13}C-methane to ^{13}C-DIC in all the non-killed slurries amended with ^{13}C-methane, indicating AOM (Figs. 3 and 4)."

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

The word was removed.

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

We rewrote the sentence: "The $\delta^{13}C_{DIC}$ values of the "methane-only" control slurries reached as high values as 743‰."

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 nmol gr^-1 Day^-1. I suggest making it all one unit type as it is very confusing.

The figure was moved to the main text (Fig. 2) and we changed the units to nmol/gr dry sediment for concentrations and nmol/gr dry sediment*day for rates.

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

The long-term two-stage experiments are called "two-stage" experiments as a shorter version throughout most of the manuscript. This is clarified in subsection 2.2.2 Experiment A setup.

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

We meant to say "experiments" and not "treatments", we added hematite to the three two-stage experiments presented in Figure 2. This detail was corrected in the revised version.

L274-276: Sentence could be structured better.

The sentence was restructured as suggested: "The addition of hematite to three different experiments increased the $\delta^{13}C_{DIC}$ values over time to 694‰ (Fig. 3) similar to the behavior of the methane-only controls."

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

The citation was moved.

L286: AOM is not "of" the incubation it is "in"

The word "of" was changed as suggested.

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

Sulfate was absent (BDL) in the natural sediments at the beginning of the experiments and thus was not measured later (it was also absent at our previous published experiments). However, we still tested its potential involvement as a short-lived intermediate with undetected concentrations by using a sulfate reduction inhibitor (molybdate) and did not find any evidence for that. We clarified this in the revised version (also in the discussion): "The involvement of sulfate in the AOM in the two-stage incubations was tested, even in the absence of detectable sulfate in the methanogenic sediments. This is as sulfate could theoretically still be a short living intermediate for the AOM process in an active cryptic sulfur cycle (Holmkvist et al., 2011)."

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.

The repetitive text was removed.

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

The sentence was restructured, and now reads: "This addition did not affect the increasing trend of $\delta^{13}C_{DIC}$ with time, and therefore, the AOM rates remained unchanged, similar to the observation in the fresh batch incubations (Bar-Or et al., 2017)."

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?

As mentioned above, sulfur species were tested for their possible involvement in the Fe-AOM in a cryptic cycle, even though the levels of dissolved sulfide and sulfate were negligible. This cryptic cycling occurs via oxidation of pyrite or FeS by iron oxides. We added molybdate to the methane-only treatment and to the magnetite treatment at the same time point. The addition of molybdate to the fresh batch experiment showed that sulfur species are not intermediates in the Fe-AOM process. Instead, competitive sulfur metabolism over the iron oxide occurs. In our two-stage experiment, we also observed that sulfate is not involved in the AOM. These details were clarified in the revised text.

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

As for the sulfate, nitrate and nitrite were not detected in the natural methanic sediments over the years (Nüsslein et al., 2001; Sivan et al., 2011; Bar-Or et al., 2017; Elul et al., 2021). This was clarified, including the specific references.

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

The sentence was reorganized to read: "Following the addition of 0.5 mM of nitrite, we observed no increase in $\delta^{13}C_{DIC}$ values during the first 222 days (Fig. 4D), after which they increased from 34‰ to 54‰ by the end of the experiment."

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

This is the AOM rate for nitrite coupling, as explained in the revised text.

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

This was corrected and the references were added.

L325: Methanogenesis not methanogens'

This was corrected.

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 aren't the rest of the 10 sets of the long term two stage results in the graph. Isn't the point to compare all three experiments in its entirety?

Each serial number (SN) refers to a specific experiment detailed in Table 1. This was clarified in the text. We use the SN to enable the reader to know to which experiment we are referring. We present only two treatments from each experiment presented in figure 3 (methane-only and hematite) and matching the experiments with serial numbers was the easiest way to identify which experiment we are referring to. The graph shows that there is no difference between the results of our two-stage methane-only and hematite addition

treatments, which is in contrast to what was observed previously in a fresh-batch experiment at Lake Kinneret (i.e. Bar-Or et al. 2017). For that, we could only use experiments that had methane-only and hematite addition treatments, and therefore, not all of the two-stage experiments are presented in Figure 2.

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

The SN refers to a specific two-stage experiment, as explained in the comment above. Each experiment consists of several treatments. We put the serial numbers of the experiments in table 2, so the reader can easily understand from which experiment the treatments were taken.

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

Figure 6 (previously Fig.5) was moved as suggested.

L399: Get rid of "our"

The word was removed.

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?

The citations were moved to their respective analysis as suggested. The discussion there refers to what is known about Lake Kinneret sediments. This was clarified in the text: "The *in-situ* geochemical and microbial diversity profiles (Bar-Or et al., 2015) and the geochemical (Sivan et al., 2011; Bar-Or et al., 2017; Fig. 3) and metagenomic (Elul et al., 2021) analyses of batch incubations with fresh sediments provided strong support for the occurrence of Fe-AOM in sediments of the methanogenic zone below 20 cm."

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

We are talking indeed about previous work. The references were added.

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.

We reduced the amount of introduction/background in that part of the discussion, keeping mainly what was done in this study. That paragraph now reads: "The *in-situ* geochemical and microbial diversity profiles (Bar-Or et al., 2015) and the geochemical (Sivan et al., 2011; Bar-Or et al., 2017; Fig. 3) and metagenomic (Elul et al., 2021) analyses of batch incubations with fresh sediments provided strong support for the occurrence of Fe-AOM in sediments of the methanogenic zone below 20 cm. Such profiles and alongside incubations showed an unexpected presence of aerobic bacterial methanotrophs together with anaerobic microorganisms, such as methanogens and iron reducers (Adler et al., 2011; Sivan et al., 2011; Bar-Or et al., 2015; Bar-Or et al., 2017; Elul et al., 2021). These findings suggested that both *mcr* gene-bearing archaea and aerobic bacterial methanotrophs mediate methane oxidation. In the current study, we have supportive evidence of considerable AOM in the long-term incubations, even after the two treatment stages and considering the low abundance of the microbial populations."

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.,.

We added the comparison of the results here with that of Bar-Or et al.. 2017.

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.

We moved the title "Potential electron acceptors for AOM in the long-term two-stage incubation experiments" to the part of the discussion that, from there on, the text discusses the electron acceptors that were added to the long-term two-stage experiments.

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.

We agree with the reviewer, this is indeed the main challenge in trying to mimic the original natural environment while working with cultures that are not pure. We, therefore, attempted to conduct several types of experiments that are close to natural systems and to learn from them about life in these sediments. We added a statement about this challenge in the revised version: "We assume that the observed difference in the bioreactors would have been more pronounced if methane concentrations had been higher, but it is still a significant finding. We also note that the difference between the bioreactors results may also be due to the fact that each bioreactor community developed separately."

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.

That is correct, but since the data for the hematite addition are close to those of the methane-only treatment, we cannot conclusively state that the hematite addition stimulated the AOM.

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

The phrase "significant difference" was changed to "significant finding".

L425-428: Any lit to support that explanation?

It is merely a statement that if labeled (enriched) ^{13}C methane is oxidized, ^{13}C-DIC will be enriched, while alternatively, if natural organic carbon, which is isotopically light, is oxidized, ^{13}C-DIC will be depleted.

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

The sentence was edited. Now reads: "Using mass-balance estimations in the methane-only and in the amorphous iron treatments and considering the DIC concentrations and $\delta^{13}C_{DIC}$ values of the methane-only treatments at the beginning of the experiment (6 mM and 60‰, respectively) and the values at the end (6.5 mM and 360‰, respectively), about 0.5 mM of the DIC was added by the AOM of methane with $\delta^{13}C$ of ~4000‰."

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.

That text was revised, and now reads: "This means that adding amorphous iron to the system encouraged iron reduction that was coupled to the oxidation of organic compounds other than methane."

L437-441: You need references to back these statements up.
References were added.
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.
Please see our response to comment L290-291. We believe that our addition of molybdate to the two-stage experimental system allowed us to observe whether the cryptic sulfur cycle is somehow involved in the AOM in the two-stage slurries. This is explained in the revised version.
L453-454: Ammonium oxidation with iron reduction needs a citation.
References were added
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 13C DIC build up after adding nitrate but potentially, the addition of nitrate resulted in more organics to be oxidized a dilute the 13C 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.
The text was adjusted as the referee suggested. It now reads: "Our results indicate that the addition of nitrate did not promote AOM, likely due to the absence of ANME-2d, which is known to use nitrate (Arshad et al., 2015; Haroon et al., 2013). In the case of nitrite, even low concentrations appeared to delay the increase in $\delta^{13}C_{DIC}$ values, suggesting that organoclastic denitrification outcompetes AOM, and despite the occurrence of Methylomirabilia, the role of nitrite-AOM is not prominent in the two-stage incubations (Figs. 4C, D). "
There was a typo in Figure 3, wherein panel D should present nitrite treatments (NO_2 and not NO_3). This was corrected in the revised version.
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…
This was corrected. It now reads: "Though humic substances were not measured directly in Lake Kinneret sediments, the DOC concentrations in the methanogenic-depth porewater were previously found to be high (~1.5 mM, Adler et al., 2011), suggesting that they may play a role in AOM."
L465-467: References
References were added.
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.,
The figure was cited. We elaborated on this point and compared our results to those of Valenzuela et al. as suggested. The text now reads: "Yet, as was done by Valenzuela et al. (2017), the addition of natural humic substances did promote AOM, compared to the rest of the electron acceptors tested, and may thus support AOM (Fig. 4B)."
L479-482: Run-on sentence. Reads poorly.

The sentence was edited, now reads: “Methane oxidation in the pre-incubated Lake Kinneret sediments is likely mediated by either ANMEs or methanogens, as the addition of BES and acetylene immediately stopped the AOM (Fig. 6), similar to the results of the killed bottles and the BES treatment in the fresh batch experiment (Bar-Or et a., 2017).”

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 a it is eluding to who is responsible for oxidizing methane. Consider structuring the paragraph to better nail who is overall responsible.

If there were micro levels of oxygen trapped in the natural sediments, they were likely used already during the first incubation period, but if not, they were exhausted by the beginning of the second stage of the incubation. The lack of oxygen damages the aerobic methanotroph population in the slurries, and therefore, we believe that they play only a minor role in the AOM observed during the two-stage experiments. The text now reads: “Using the isotopic compositions of specific lipids and metagenomics, we identified a considerable abundance of aerobic methanotrophs and methylotrophs in the fresh sediments, but not in the long-term slurries (Table 3, Fig. 7). In the natural sediments, micro levels (nano molar) of oxygen could be trapped in clays and slowly released to the porewater (Wang et al., 2018). However, if such micro levels of oxygen still existed during the time of the pre-incubation, they were probably already exhausted. Indeed, the results of our specific lipids and metagenomics analyses suggest that the aerobic methanotrophs lineages play only a minor role in the long-term slurries, probably due to complete depletion of the oxygen.” The paragraph was restructured as suggested by the referee.

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.

We believe it to be AOM by ANME-1 or by methanogens by reverse methanogenesis with, to some extent, an external electron acceptor. This was clarified in the text, which now reads: “It, therefore, seems less likely that the observed DIC values in our study were sustained by methanogenesis back flux alone (without an external electron acceptor) than by active AOM, which, in this case, is probably performed by ANME-1 or by methanogens that perform reverse methanogenesis to some extent.”

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

We added this information to the discussion as suggested by the reviewer. Please see the response to comment L487-497.