Response to referee #3:

This work by Vigderovich et al., investigated the key microbial players and electron acceptors that support anaerobic oxidation of methane, methanogenesis and possibly a sulfur cycle in in the top 20 cm of sediments collected from a lake in Northern Israel. They used a variety of sediment slurry incubations amended variety of electron acceptors, electron acceptor analogs and inhibitors along with ^{13}C labeled methane and tracked the buildup of ^{13}C-DIC over time. Their results indicate that there is methane oxidation occurring by aerobic methane oxidizing bacteria and anaerobic methane oxidizing archaea (ANME) possibly with oxygen or iron oxides. However, later in the experiments the data suggest that humic substances are the most likely culprit for the turnover of ^{13}C-methane. Their metagenomic data suggest the presence of methanogens, aerobic methanotrophic bacteria and anaerobic methanotrophic archaea and suggested that there is an interplay between the groups that cycle the carbon in their experiments.

Generally, I find the data to be very interesting and does fit well within the scope of Biogeosciences and should be eventually accepted with major revisions. My main critiques for the manuscript are in the clarity, flow in all sections and a few discussion inquiries I would like to address in this review.

We would like to thank the reviewer for the positive and very instructive review. We realized based on all reviews that the manuscript was not perfectly presented. As mentioned above, we clarified and simplified the paper throughout; we re-wrote and organized the methods section and added experimental protocols to the supplementary material. We moved discussions from the results to the discussion section. The microbial data was discussed more thoroughly and a principal component analysis biplot was added. All co-authors edited the manuscript, and we also edited the English. We believe that the paper flows better now and is much clearer for the readers. We also accepted all other comments, which we believe improved the revised version.

General comments:

The introduction lacks a clear identification of the gap in the knowledge to which scientific questions are based on. The question and hypothesis in L84-86 is rather vague and could be clearer.

We agree, and we clarified in the introduction the gap of knowledge, the aims and the hypothesis, as explained in the response to the first reviewer.

The methods unfortunately are riddled with syntax errors and missing study site and methodological details that need to be clarified. This is particularly important as methods sections should be written such that anyone could reproduce the experiment. This review cannot identify and fix all of them but will provide examples of some of the most severe below.

We totally agree and have supplied more details on the methods such that every treatment should be reproducible and clear. We accepted all comments below and edited the methods section accordingly. We also added the protocol for each experiment in the supplementary information.

The beginning portions of the results section sound more like a discussion. There is more space being used to repeat the experiments and experimental setup in this section than simply reporting the data from the experiments. The results section would be stronger if the authors would report the data without method explanations or with interpretation. Further into

the section there are more numbers that are reported but there are other sections that read crudely. Consider having introductory and conclusion sentences and sub-headers (applies to other sections) to better separate sections which will help with flow.

We accept this comment. In the revised version, we improved the results chapter by starting with a short introductory and overview of the results (as also requested by the first reviewer), by reporting the data with minimum interpretation but with some conclusion sentences to better separate sections and to guide the reader.

Figures 2 and 3 could be organized better. There is a lot of data and the scales do not match which could be misleading at a first glance. Furthermore, there is some data in the supplementary material that belongs in the main text. For example, the authors suggest that aerobic methanotrophic bacteria play a role in the overall AOM process. Their geochemical data do not definitively track aerobic methanotrophic bacteria activity (not sure you even could) but the molecular data do indicate their presence.

We reorganized the figures and added the microbial data and its indications to the main text, as was similarly requested by the second reviewer.

In the discussion are a lot of interpretations and claims for which are speculative and do not offer enough literature examples that support the interpretations. For example, in L364: I don't think that the addition of iron-oxides generally inhibits AOM according to your data. Where in the literature do you find an example of iron-oxides acting as an inhibitor to AOM? Yes, there seems to be less buildup of your 13C-DIC but the system still observes a buildup of 13C-DIC after ~450 days in figure 2 and in figure 3A after 450 days. If it was truly an inhibitor then the 13C-DIC would be identical to the killed control trend throughout the whole experiment, just like the BES trend in Figure 4 where you know BES is inhibiting activity. But Figure 3A lacks a killed control for hematite so how does one know that the addition of hematite is truly an inhibitor? Instead, you observe the trend to become slightly depleted in 13C before the full spike, then see a rebound in the hematite trend back to levels closer to the beginning of the experiment, in which case how do you explain that? In addition, according to Fig 3A, all 4 amendment experiments had decreasing 13C-DIC which leads me to believe that there might be something else at play perhaps the organoclastic iron reduction as the authors mentioned or something in the experimental setup that is causing all of your replicates to all have decreasing 13C-DIC.

It seems that our statements were not as accurate as we thought. We thus clarified them in the revised version: We are discussing "inhibition" here not as direct damage to the pathway of AOM, but rather making another process more favorable than the AOM. Perhaps a more accurate phrasing would be "an inhibition in the AOM signal". We are considering the ^{13}C-enrichment in the DIC in the treatments without adding an electron acceptor as the baseline of these slurries. The methane oxidized in those treatments should be about the same in every other bottle in the experiment unless the addition of the electron acceptor discouraged the AOM (except for the killed controls). If the $\delta^{13}C_{DIC}$ values are lower than the "baseline" when an electron acceptor is added, it could be from the following reasons: 1. there is another microbial process converting organic carbon to inorganic carbon in addition to the methane oxidation. This means that the DIC pool (which consists of all the products of organic matter oxidation in the slurry) in those treatments is diluted with ^{12}C compared to the treatments without an additional electron acceptor, which comes from another source other than methane (since the same amount of labeled methane was added to all the experiment bottles in each experiment). In most experiments, we only added ^{13}C-labeled methane to begin with so a decrease in isotopic value due to methane oxidation is unlikely. 2. It directly interferes with the microbial pathway of the AOM. As the reviewer mentioned, there is still an increase of $\delta^{13}C$-DIC in the incubations containing different electron acceptors, and therefore

we believe that the former option is the correct one. When we added an electron acceptor and saw that the $\delta^{13}C_{DIC}$ values were lower than without it, we concluded that either there is an inhibition of the methane oxidation (less methane is being oxidized now) or that there is much more oxidation of ^{12}C- organic carbon than without the electron acceptor addition, which results in a dilution of the final DIC pool.

Specific inline comments and edits:

L44: AOM coupled to other electron acceptors is not a theory anymore. The word "theoretically" was deleted.

L49-50: Consider adding equations of the AOM reactions. We did not add the equations as they can be found in the literature, however can add them to the supplementary if needed.

L47-L54: Consider joining L47 into next paragraph at L50. The lines were joined.

L74-75: Move citation to the end of sentence. The citation was moved.

L111-112: "1) Two stage slurry incubations with 1:1 sediment - pore water ratio for three months, followed by a 1:3 ratio and the addition of different manipulations for up to 18 months." This sentence is far too long and could be split up to be clearer about the analysis. The sentence was split.

L113-114: "Semi-continuous bioreactor experiments with freshly collected methanic sediments and porewater with 1:4 ratio, where porewater was exchanged regularly." Syntax error, do you mean 1:4 sediment to porewater ratio or do you mean the porewater has a 1:4 ratio, in which case what is the 1:4 ratio in the porewater? Same can be said for L115-117. We meant 1:4 sediment to porewater ratio. This was clarified in the text.

L97: The methods would also greatly benefit with a couple sentences that explain how the sediments were retrieved from the Lake (i.e. ship/small boat, instruments (mulitcorer, pushcores, gravity cores etc…). The text was edited to include information about how the sediments were retrieved from the lake.

L98-106: This section is rather vague, I think it would be stronger with more details about the lake such as; approximate temperature and size and perhaps a nearby city for reference. A map would also make this section stronger. The details were added to the text and a map was added to the supplementary.

L109: Here you mention you are going to assess the different electron acceptors for AOM. It may be wise to have a sentence somewhere either in the methods or in the intro that you are lumping all methane oxidation by archaea (ANME's) and bacteria (Methylococcales). Many in the community may just be thinking AOM process is being conducted by the ANME's but it appears (not clear) you are referring to both, correct? Yes, we wanted to see if the aerobic methanotrophs are still active and involved in the two-stage incubations. We thank the reviewer for pointing this out. A sentence was added to the introduction clarifying that we were investigating whether both archaea and bacteria are still responsible for methane oxidation in the long-term anaerobic incubations.

L118: This section would be also stronger with one sentence explaining what the purpose of the two-stage incubation is. The purpose of the two-stage incubation was clarified in the text. It was done in order to enrich the microbial population that is involved in the methane oxidation process in the natural Lake Kinneret sediments fast by slight dilution with

porewater, and then to explore the long-term incubation effect on AOM process with larger dilution. Sediments were therefore pre-incubated in a 1:1 sediment-porewater ratio with total of 20% methane in the headspace (18% natural methane (98.9 % 12C, 1.1 % 13C) and 2% 13C- labeled methane (99% 13C)). When we observed an AOM signal (13C-DIC enrichment) we diluted the slurry once more (1:3 sediment-porewater ratio), added different treatments and started the experiment.

L119-120: Add more information of where you sampled perhaps on a map. I do not know where "Station A" is. Additionally, do you know precisely when the sediments were collected between 2017 and 2019? How long did it take for the sediments to be processed into sediment slurries? Were they stored and reactivated? If sediment samples that were collected in 2017 and processed in 2019 how do you know those samples are still viable for this study? Station A is located at the center of the lake, which is also the deepest point of the lake (water depth of 42 m), and it is shown on the added map in the supplementary. From 2017 until 2019, we undertook multiple cruises to Station A to collect the sediments for our slurry experiments using a gravity core with Perspex liners (60 cm long, inner diameter 5 cm). The cores were kept at 4℃ until further processing (up to 48 hours). Targeting solely the zone below 15 cm, sediments were transferred into 250 ml bottles, then diluted with porewater at a 1:1 ratio. The latter was extracted from the same depth using parallel cores. After three months of incubation, the slurry was divided into new bottles and diluted again to reach 1:3 sediment to porewater ratio. To these bottles, different electron acceptors/inhibitors were added. The sediments in all the experiments were set up immediately (within a day of collection), but they were spread along the two years. The methods section was edited to include and clarify these details.

L120: What was the container that the sediments were pooled into? The sediments were collected using a gravity corer with 50 cm Perspex cores and sediments from the methanogenic depth (below 20 cm) were incubated in 250 ml bottles.

L121: Please add the speed (rcf x g) for the centrifugation. The speed (9300 g) for the centrifugation was added.

L133-134: What do you mean "already running experiment" is this separate from the two-stage experiment? If so, this was never introduced. What we meant was that the inhibitor was during the second stage to the specific bottles. This was clarified in the text.

L138-139: Syntax; this sentence makes it seem like there is a separate experiment within one. Was there a reason not to add acetylene in the beginning like BES? The sentence was corrected to clarify that BES and acetylene were added to two different experiments other than the one with the addition of molybdate. Acetylene was not added at the beginning of the experiment because this was the first time we used it, and we wanted to observe first the ^{13}C enrichment in the DIC in the specific bottle before the inhibition. Since the addition was relatively easy by injecting gas to the headspace, we decided that this would be the way to see that acetylene really stopped the AOM.

L145-147: It was mentioned that ^{13}C-label was added after the pre-incubation, please add the time you added the label. The ^{13}C-labeled methane was added to the bottles immediately when we set the incubations (within 48 hr from sampling).

L150: Never begin a sentence with numerical, instead spell out two. Thanks for this comment. It was corrected

L150- What method and instrument was used to measure the Fe(II)? To measure Fe(II) we fixed the filtered porewater immediately after sampling with Ferrozine (a chelator that creates

a strong complex with Fe(II)) according to Stooky (1970). The absorbance of the complex is measured by spectrophotometer at 562 nm wavelength. It is described and clarified better in the analytical methods (the former porewater analyses section).

L152- How was the methane measured? The methane was measured from the headspace after shaking (to allow the methane to transfer from the porewater to the headspace) on a focus gas chromatograph (GC) equipped with a flame ionization detector (FID). It is described in the analytical methods section (former porewater analyses section).

L153- Is there an equation used to calculate methane? Methane amount in the headspace was measured using a calibration curve. The concentrations in the porewater were calculated using the known bottle volume and slurry volume. We assume that every change in the concentration is due to methanotrophs/methanogens activity in the slurry. This was clarified better in the text.

L154-155: This is the first time the “Black Coffee experiments” was introduced. Please add why you included this. The black coffee means coffee grinds as an organic source, and was added to test whether another type of complex organic compound influences the oxidation of methane. However, we removed this treatment from the manuscript, as the other reviewers suggested.

L154: Is there a reason why there is inconsistencies between duplicates or triplicate samples (i.e. not enough sample or prioritized sample for certain treatments of interest?). Please elaborate. Yes, due to the limited availability of porewater and sediment, samples were prioritized, and we needed to dictate how many replicates would be set up.

L156-157: This sentence is contradicting and with parentheticals is incomplete. The sentence was rewritten.

L156-157: This is the first mention of any killed controls in this part of the experiments. How many were there? How were they prepared? (etc). Killed controls (triplicates or duplicates) were part of each set of sediments. Killed control bottles with sediment and porewater were autoclaved twice after they were flushed with N_2 together with the rest of the experiment bottles. After they cooled down, the relevant additions (i.e. electron acceptors and ^{13}C-labeled methane) were added. This was clarified in the text. Given the quantity of experiments presented, a detailed description of how many replicates for each treatment was included in table S2 in the supplementary, including the killed controls.

L158- What is so special about the lake in Alaska that humic substances had to be extracted from. Why not get them from Lake Kinneret? We are working on extracting humic subtances from Lake Kinneret, which would be the best option. However, it is not a trivial procedure. We decided, therefore, to add natural humic substances that we already had available from a lake in Alaska when we set up that specific experiment. This allowed us to compare our results from the synthesized analogue with a natural one.

L164-165: What was the other bioreactor amended with? Or is it a control? The second bioreactor was set up as a control, without iron oxides. This was clarified in the text.

L165-166: Syntax issues, had to read it several times over to understand that you are trying to describe how 13C methane was added to the headspace free bioreactor. The sentence was changed to: “To dissolve ^{13}C-labeled methane in the porewater, 15 ml of porewater were replaced with 15 ml of methane gas to produce methane-only headspace for 24 hours.”

L170-172: How many total weeks did the bioreactor run for? The bioreactor ran for 67 weeks. The duration of the reactor was added to the text.

L175: This is the first time that a duration of the experiment has been introduced. Consider adding the actual experiment duration somewhere in the method. The duration of the experiments was added to the supplementary information for each experiment.

L175-177: Good introductory sentence. Please move this sentence to the beginning of the section.The sentence was moved as suggested.

L185: Figure 1 caption should be moved into the text. Particularly you did not describe how you set up the third experiment till the caption. Details from Figure caption 1 were moved to the text. The third experiment was set up and described in detail in the Bar-Or et al., 2017 paper. However, we added also details in the text.

L192: What kind of autosampler? Was this done at the home lab? Yes, this was done at Sivan's lab at Ben Gurion University. The autosampler is a headspace autosampler (CTC analytics. Type PC PAL) which is connected to the IRMS.

L197-199: should be moved to L150.We preferred to keep all the analytical methods in the same section.

L199-202: Which bottle is now being sampled? This is a new section and don't know which experiment is being sampled. The sentence would be stronger if you indicate the reason why you track methane and ethylene (i.e. tracking methanogenesis and acetylene turnover). Methane was measured in the experiment without any electron acceptor in order to assess the methanogenesis rate in the natural sediments that went through the two-stage incubation. This was clarified in the text.

L207 - 208: You list the same variable "x" as two different parameters. This was corrected.

L216: Please indicate which set of experiments the samples come from. The text now specifies from which experiments the samples originated.

L254: I think it would be better to break 3.1 into sub sections to have better flow. It is difficult for the brain to switch between experimental setups. We agree with the reviewer. The section has been divided into sub-sections.

L255: Is the pre-incubated long term experiments the same as the two stage experiments? Please be more consistent with the names of experiments. Yes, they are the same. We made sure that the experiments will be referred as "two-stage experiments" throughout the manuscript.

L255-256: Here is an example of discussion text in the results. How much 13C methane exactly was converted? The sentence was changed, and it now includes the amount of ^{13}C-methane converted.

L257-258: This sentence sounds like it should be in the discussion. Consider instead to report the actual permit value and leave the microbial population statement for when you report the microbial ecology. The part of the microbial population was deleted, and the rest of the sentence was changed.

L261-263: This sentence is very confusing and how does this relate to the statement you had about AOM in the previous sentence? Please reorganize. This sentence was moved to the methods section.

L260-273: The whole paragraph sounds like it belongs in the discussion. Perhaps move to discussion or add more details about the data. The paragraph was re-edited and now includes specific details about the experimental data without a discussion.

L278-280: Move to methods. The sentence was moved as suggested.

L274: Was sulfate ever measured in your experiments? The natural sediments for these slurries were taken from below 20 cm depth, where sulfate was not observed in depth profiles. Sulfate was also not detected during the fresh slurry experiments (Bar-Or et al. 2017). However, there could be theoretically a cryptic cycle that would produce very low concentrations of sulfate (and consume it fast), and therefore we tested this possibility by inhibiting sulfate reduction with molybdate.

L288: Is the 308 days the end of the experiment? Yes, the duration of the nitrate experiment was 306 days.

L284: End of what? How many days was that? The end of the nitrate experiment (306 days). This was clarified in the text.

L292: What is PCA? Please spell out acronym. What was the result of the AQDS addition? Not clear. Phenazine-1-carboxylic acid (PCA) is an analogue for methanophenazines that are found on the membrane of some methanogens and used to shuttle electrons. The acronym is spelled in the methods section. The addition of AQDS slightly decreased the δ^{13}C-DIC values. This was clarified in the text.

L296 By how much did the Fe(II) and delta 13C increase? Please report. There was an increase of 90 µM in Fe(II), and of ~200‰ in the δ^{13}C-DIC. The text now reads: “The results show that first, the $\delta^{13}C_{DIC}$ values did not change (Fig. 3F), while a steep increase of ~90 µM in their Fe(II) concentrations was observed (Fig. S3). However, after 20 days, the $\delta^{13}C_{DIC}$ values of these slurries started to increase dramatically from 150‰ to 340‰…”

L297: What was the slope? The slope was 2.2 ‰ day^{-1}. This was added to the text.

L289-305: The results are very vague and sound more discussion are. Please add in the decreasing and increasing permil and concentrations values for this section. The paragraph was edited to include the concentrations values.

L301: A lot going on in Figure 2 and legend could be better organized. The legend was re-written with new phrasing. The different pre-incubation exp was changed to “two-stage experiment” with numbering of the different experiments (which are now included in the experiments details table) to clarify what is presented.

L311-312: Figure 3. Consider making all y-axis scales the same. We considered this, however in one of the experiments the values reach 2000 permils and it would mask the rest of the trends. We decided to make the y-axis scales of all the graphs the same except for the one with these high values, and we added a line in the caption: “note the different scale of the y-axis in panel E.”

Consider moving the Fig 3 F next to Fig 3 A since they both seem to be the experiments that indicate when 13C label was added. Also I do not recall an exact time when the label was added in the methods. We moved the figures as suggested. The labeling time is mentioned in Table S2, which was moved to the main text. It is also clarified in the detailed protocol of each experiment in the supplementary methods section.

Fig 3C: Are the NO3 (grey circles) and the Hematite + NO3 1 mM (green triangles) data on top of each other? Please check your graphs. Yes, they are on top of each other. This was now mentioned in the text.

L319: Please report in text how high of an abundance and which species. We added a supplementary information table that shows the coverage of all the taxon represented by metagenome-assembled genomes.

L320 and 321: Hyphen between “sulfate reducing”

The whole section was re-written, the term “sulfate reducing” was deleted here.

L320-321: Please rewrite sentence. Just report which SRB were present.

The whole section was re-written, and which now includes the SRB that are prominent: Desulfobacterota and Thermodesulfovibrionales (Nitrospirota).

L322: Please report the number of reads to NC-10.

The whole section was re-written, and now includes this data as coverage of the metagenome-assembled genomes.

L339-342: Where are the profiles? Or are you referring to profiles in previous studies? Please clarify.

We are referring to previous studies. This was clarified.

L342-344: Is this really your previous work, or the current work or is this the work from the citations at the end of the sentence? I think you are trying to compare the three different experiments but it makes it sound as if there are three papers in one. The whole paragraph summarizes our group’s previous studies on AOM in Lake Kinneret sediments.

L342-344: Is the mechanic zone where Fe-AOM the same sediment regions that you obtained for this study? Yes, they are the same. Previous experiments on Fe-AOM used sediments from the center of the lake and below 20 cm sediment depth. The sediments (and extracted porewater) used for this study were taken from the same spot and depth.

L344-348: Please clarify, I can’t tell if you are referring to the current study or other works. We are referring to the current study. This was clarified.

L354-359: Add figure references since you have two figures that compare the three setups. The references were added.

L357-359: But then how do you explain the sharp increase in the 13C DIC in Line 354? As we see the same sharp increase in the incubations without any additions, we cannot state clearly that Fe(III) (as hematite) stimulates AOM as the electron acceptor. It’s either that the high amount of Fe(III) in the sediments (3%) is enough to sustain the long term AOM by

reverse methanogenesis or that the long term AOM is stimulated by another electron acceptor.

L364: The methods indicate that the preincubation called for a full methane headspace that was half 12C and half 13C, is it not conceivable that the mass balance would lead to a slight depletion of the 13C in a closed system like this? I would argue that Figure 3A and F shows that before the addition of the 13C label the 13C DIC was similar to the control but after the addition, all trends become heavier. In which case how do you interpret that as an inhibitory response to the addition of label and iron?

Just to clarify, the headspace was never full of methane. In the pre-incubations, 18% was 12C-CH4 and 2% was 13C-CH4. In most of the two-stage experiments, 5% of the headspace was methane (only 13C-CH4). As we mentioned above, we refer to "inhibition" here not as direct damage to the pathway of AOM, but rather making another process more favorable than the AOM. We believe that there is another microbial process converting organic carbon to inorganic carbon in addition to the methane oxidation that is occurring in these slurries. In the experiment presented in figure 3A, the methane added at the start of the experiment was not labeled (a mistake); that is why we do not see an increase, but we see a slight decrease. When ^{13}C-methane was added, we see an increase in all treatments, but the highest increase was observed in the baseline treatments without any electron acceptor addition.

L367-368: I think it is conceivable to claim that organoclastic iron reduction could dilute the 13C-DIC signal in these experiments, especially over time but do you have any evidence to support that either by isotopioc analysis of organic matter in this study or previous study that could allow you to make some 1storder mass balance to explain that? Yes, we have the δ^{13}C value of the organic compounds and did some rough mass balance calculations to show it can lower the signal. This was added to the text.

L372: I agree with your statement of manganese oxide but what do you have to say about the Magnetite additions in Fig. 3A? Those were the most similar to the no electron acceptor control experiment. We believe that the hematite and magnetite additions showed a similar pattern to the natural controls probably as their natural abundance in the sediment promoted the maximum potential of the AOM, as written above.

L379: I think the result of the addition of molybdate is not super surprising. It appears that the molybdate was added rather late in the experiment when the trends are already supporting magnetite as a potential AOM electron acceptor. Sulfate reduction would be naturally inhibited since metal oxides yield much higher free energy than sulfate does in the redox cascade. Yes, we agree. However, molybdate was also added to the treatment without an electron acceptor. If any kind of SO_4^{2-}-dependent AOM component existed, the increase in the δ^{13}C-DIC values would have been stopped by molybdate addition.

L385-386: This is interesting but also not super surprising because I believe many sulfate reducers are also iron reducers. Dig into the literature and see if any of the sulfate reducers you detect have been shown to conduct iron reduction. We are aware of this, and and in fact, we are currently working on a project with a sulfate reducer that can reduce iron as well. We believe that at least some of the Fe(III) in the natural sediments are being reduced by sulfate-reducing bacteria. Nevertheless, this does not contradict the fact that at first impression it would be expected that when sulfate reducers are present, they will reduce sulfate. Therefore, we are mentioning their presence in our slurries here while discussing the possibility of SO_4-AOM. We added a sentence to the text regarding the potential of the sulfate-reducing bacteria to reduce iron as well.

L392-393: I don't think you can totally confirm that nitrate and nitrite is inhibitory. Fig 3C shows some buildup of the 13C-DIC and again I would be more convinced that there is a true inhibitory effect of the nitrite, if the trends were identical to the killed control. But even then, what evidence is there that nitrate or nitrite inhibits the enzymatic pathway in AOM, like BES does? Does the literature have any suggestions? In addition, you also added hematite to those samples with nitrate in Fig. 3C so how do you know that the buildup of 13C-DIC that you do see is from denitrification coupled to AOM or iron reduction coupled to AOM? Did you measure a buildup of N2 in parallel? Were you able to somehow inhibit iron reduction (if you did that would be cool and would love to know)? As we answered above, we believe that we witnessed the microbes favoring a different process than methane oxidation. Methane oxidation is still occurring in these treatments that show lower enrichments than the treatments without an electron acceptor, only there is also another process that is now more favorable - so either there is less methane oxidation than the "baseline" treatment because of different microbial populations, or there is much more oxidation of organic matter that dilutes the isotopic AOM signal. We understand the confusion over the term "inhibition", and we have clarified it in the text.

We did not measure N_2 buildup (the headspace was mostly N_2), and it is very challenging to inhibit the iron reduction, at least not the actual iron reduction pathway.

L399: If AQDS has a high electron shuttling capability leading to higher organ clastic turnover then in a closed system like this wouldn't your 13C-DIC become very deplete (Rayleigh distillation) over time and not just plateau like your data suggests? I rather think AQDS just doesn't support anything since it looks just like your killed control or else it would have looked like some kinetic process if any biology was involved. The addition of AQDS did result in a slight depletion of the $\delta^{13}C_{DIC}$ values (it is not visual because of the scaling). It's a depletion of about 17 ‰, and we see an increase of about 70 µM in the iron concentrations while without AQDS there is an increase of only 30 µM (not presented). This suggests that the AQDS supports organoclastic iron reduction. This is now discussed in the text, and a graph of the Fe(II) concentrations was added (to the supplementary information).

L 406: I really think your Fe(II) data belongs in the main text especially here where Figure 3F really needs S3 to support your claim. We accept the referee's suggestion and have moved figure S3 to the main text.

L412: I think it would be worthwhile to have spent a bit more time on magnetite as another potential electron acceptor since Figure 3A is convincing enough, though the scaling in the y-axis is deceiving. We discussed this potential further in the revised version.

L446-448: This was left open ended. What does trace methane oxidation have to do with your study? Plus your experiments are in slurries over long periods of time with amendments that are probably much different that the natural environment so would trace methane oxidation be a likely occurring thing in a slurry. In our two-stage experiments, the net methane-related process is methanogenesis. However, we still observe an enrichment in ^{13}C-DIC when we add ^{13}C-labeled methane. Because of that, we cannot say that this is standard AOM. Other studies that observed similar methane oxidation when the net is methane production called it "trace methane oxidation" (Moran et al., 2005; 2007).

L488: You mean aerobic methane oxidation is decoupled from iron reduction right? That is what we meant, but we have rephrased it better in the text, which now reads: "It appears that methanotrophic bacteria cannot survive the long-term slurry incubations and thus iron reduction and aerobic methane oxidation are decoupled"