Biogeosciences Discussions

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Comment on bg-2021-223

Anonymous Referee #3

Referee comment on "Modification of methane oxidation pathways during long-term incubations of methanic lake sediments" by Hanni Vigderovich et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-223-RC3, 2021

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.

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.

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.

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.

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.

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.

Specific inline comments and edits:

L44: AOM coupled to other electron acceptors is not a theory anymore.

L49-50: Consider adding equations of the AOM reactions.

L47-L54: Consider joining L47 into next paragraph at L50.

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

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.

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.

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…).

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.

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?

L118: This section would be also stronger with one sentence explaining what the purpose of the two-stage incubation is.

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?

L120: What was the container that the sediments were pooled into?

L121: Please add the speed (rcf x g) for the centrifugation.

L133-134: What do you mean "already running experiment" is this separate from the two-stage experiment? If so, this was never introduced.

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?

L145-147: It was mentioned that ^{13}C-label was added after the pre-incubation, please add the time you added the label.

L150: Never begin a sentence with numerical, instead spell out two.

L150- What method and instrument was used to measure the Fe(II)?

L152- How was the methane measured?

L153- Is there an equation used to calculate methane?

L154-155: This is the first time the "Black Coffee experiments" was introduced. Please add why you included this

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.

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

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).

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?

L164-165: What was the other bioreactor amended with? Or is it a control?

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.

L170-172: How many total weeks did the bioreactor run for?

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.

L175-177: Good introductory sentence. Please move this sentence to the beginning of the section.

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.

L192: What kind of autosampler? Was this done at the home lab?

L197-199: should be moved to L150.

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).

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

L216: Please indicate which set of experiments the samples come from.

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.

L255: Is the pre-incubated long term experiments the same as the two stage experiments? Please be more consistent with the names of experiments.

L255-256: Here is an example of discussion text in the results. How much 13C methane exactly was 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.

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.

L260-273: The whole paragraph sounds like it belongs in the discussion. Perhaps move to discussion or add more details about the data.

L278-280: Move to methods.

L274: Was sulfate ever measured in your experiments?

L288: Is the 308 days the end of the experiment?

L284: End of what? How many days was that?

L292: What is PCA? Please spell out acronym. What was the result of the AQDS addition? Not clear.

L296 By how much did the Fe(II) and delta 13C increase? Please report.

L297: What was the slope?

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.

L301: A lot going on in Figure 2 and legend could be better organized.

L311-312: Figure 3. Consider making all y-axis scales the same

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.

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.

L319: Please report in text how high of an abundance and which species.

L320 and 321: Hyphen between "sulfate reducing"

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

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

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

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.

L342-344: Is the mechanic zone where Fe-AOM the same sediment regions that you obtained for this study?

L344-348: Please clarify, I can't tell if you are referring to the current study or other works.

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

L357-359: But then how do you explain the sharp increase in the 13C DIC in Line 354?

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?

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 1^{st}order mass balance to explain that?

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.

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.

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.

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)?

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.

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.

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.

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.

L488: You mean aerobic methane oxidation is decoupled from iron reduction right?