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Interactive comment on "Monodeuterated methane: an isotopic probe to measure biological methane metabolism rates and track catabolic exchange reactions" *by* Jeffrey J. Marlow et al.

Anonymous Referee #2

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General comment

The manuscript describes a novel technical approach to measure biological methane oxidation and track H-atoms through methanotrophic metabolisms. In part one of the manuscript, the authors focus on a comparison between the new CH3D method with the established 14CH4 method. For comparison of the results of the two methods a scaling was determined. To evaluate their method the authors performed measurements on methanotrophic culures and environmental sediment samples. Part two describes how the CH3D approach can be used to track H-atoms in anaerobic an aerobic methanotrophic pathways. Part three deals with a pressure experiment in which sediment samples were incubated at 9 MPa and 0.1 MPa to discuss the effect of pressure

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on methane turnover. The title of the manuscript describes the work adequately and the manuscript is well structure and written. Based on the few comments below, I suggest minor revision before the manuscript will be published.

Specific comments

Abstract

The abstract is well written and nicely reflects the outcome of the work.

Line 10. The poor precision of the established methods to determine a methane oxidation rate is mentioned (e.g. 14CH4). That is definitely true and an increase of precision is desirable. However, if precision is one of the major points that should be improved, the authors should tell the reader something about how (and in which range) the new method affect the precision of the measurements. That should be integrated in the abstract with a comparison in numbers of the precisions “14CH4 against CH3D” - derived from their comparative studies.

Line 14. The description of the central analytical device is rather weak “. . .using a water isotope analyzer”. Since this is the new basic tool that allows this new approach, a subclause about how the system works (Off-axis ICOS technology) should be already integrated in the abstract.

Line 21ff. The pressure experiment is very nice approach that shows how important incubations under in situ conditions are to determine real methane turnover rates. However, the story get lost in the abstract (just 2 lines) and appears a bit out of context (see comments below to chapter 3.4).

Line 26ff. Point 2 is difficult to understand without reading the manuscript. The phrase “scaling factor” is difficult to understand without a deeper context. I would suggest reformulating and extending the sentence.

Introduction

The introduction is well structured and gives appropriate background information. Line 46-55. AOM is introduced but no background information about aerobic methane oxidation (MOB types) are delivered. This should be completed, because the lab test presented in the manuscript are not only focused on AOM.

Line 57. “biogeochemical curiosity”, please rephrase or explain what you mean in more detail.

Line 71. References. I would also add a more recent paper, because the methods used changed a bit (e.g. I. Bussmann et al., Assessment of the radio 3H-CH4 tracer technique to measure aerobic methane oxidation in the water column, L&O Methods, 2015

Line 84ff.Why did the authors decided to test their new approach against 14CH4 and not also against tritium labeled methane with its improved specific activity that allows incubations under more realistic methane concentrations.

Line 85. Please explain in more detail what you mean with “partial versus complete methane oxidation”.

Line 91ff. This sentence is redundant and could be deleted.

Line 82ff. I cannot find any hint to the pressure experiment in this outlook. Such an outlook should cover the main aspects discussed in the following text.

Methods

The chapter is well structured and explains the different methods in an appropriate way. Line 100ff. As mentioned above, also here the pressure experiment is a bit out of context. Why did the authors decide to perform these experiments without a comparison with 14CH4 rate measurements? See also comments below for chapter 3.4.

Line 130. Is the information about “unique four-digit serial number” needed? I think the

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sentence can be deleted.

Line 131. Maybe insert: “The “active” designation in our sample description (e.g. Figure 2) refers. . .”

Line 141. That is difficult for me to follow. A few sentences before the authors say that carbonates are formed during “active” periods of seepage (line 137) and now they say that carbonates (L. Carb) can also exist under “low-activity”. Please explain the difference between L. Carb and A. Carb in more detail.

Line 147ff. I would suggest to move the entire paragraph to line 130. First describe how the samples were taken and then how the samples were named (paragraph 130ff).

Line 153. The samples were stored in Ar-flushed bags. Does this influence the methane concentration in the sample and also maybe the activity of the microorganisms? How long were the sample stored?

Line 155. The samples were maintained under 2x10^5 Pa CH4 headspace for one month. Why one month? And how does that fit to in situ conditions (methane concentration)? And if there are differences can we expect that it also influences the activity of microorganisms in the experiment? A comment on that should at least be given in the discussion somewhere.

Line 166. Which gas was injected – CH4? And why does it end in a desirable headspace composition?

Line 168. What was the reason to choose this specific gas composition? Does it reflect environmental conditions?

Line 180. What are “mylar” bags. Are they gas tight? Maybe a short comment on that in the text.

Line 180. Actually, I could not find Table S2 in my documents and therefore cannot comment on that.

Line 188. Why did the authors choose 9.0 MPa. Where does the sample come from (water depth, temperature). Some more comments on the sample are needed.

Line 189. The authors tested visually the bags for leaks. I think a better method to test for leaks would be the analyses of CH4 (or CH3D) in the water of the pressure vessel at the start point and end point of the experiment.That would also deliver information about diffusion of methane through the bag into the surrounding water. Can diffusion be excluded?

Line 194. How was the volume of the water sample replaced in the culture?

Line 196. The only information about the main analytical device is the name of the model and the company. Since this tool represents something that is really new in the context of methane rate measurements, I would like to have some more details about the main analytical principle of the system (Off-axis ICOS technology). Can the authors deliver any additional references to the system (other studies)?

Line 205ff. What does it mean "sub-optimal“. Is a statistical test behind that?

Line 211. I am not sure if I understood this part correctly. Is the assumption of a linear scaling factor only based on two standards? Is the LGR system linear over the measurement range? Was this tested?

Results and Discussion

Figure 1. It would be easier for the reader to follow the discussion, if Fig. 1a and 1b would be tilted with the name of the two MOB. I would also add a legend into the figures to explain the different symbols. The axis labels and the numbering on the axis do not look very accurate: the positions of the axis labels at the y-axis is not centered in both figures; 0 on the x-axis cuts the y-axis.

Line 271. "Using data points...“ please list the data points used to derive the ratio in the text. Not only time also the methane oxidation rates.

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Line 281. To which table or figure do these numbers (e.g. #1b) belong to?

Line 284. Does the 14CH4 method yield the "full-oxidation methanotrophy“? I think the correction of the CH3H oxidation rates using the H:C tracer ratio can just deliver oxidation rates, which can be better compared with the rates obtained with 14CH4 rate measurements.

Line 291. Please specify what you mean with "second time point“? IN which table or figure can I find the numbers 4d or 8d?

Line 371. Is any data available from the experiments to determine the cell density?

Chapter 3.4 As mentioned before, I have the feeling that this part of the manuscript is a bit out of the main focus. It is for sure an interesting approach but if this approach would be extended (more samples, different simulations (e.g. pressure),...), it could stay for itself. My main question are: What is the goal of these pressure studies? To show that pressure influences methane turnover? What is the advantage of the CH3D method compared with the 14CH4 method for these kind of pressure experiments? I am sure that the influence of in situ pressure is more important for the outcome of the experiment than the use of the new CH3D rat measurement approach (e.g. higher precision?). I think it must be explained in more detailed why exactly such an experiment can help to evaluate the now CH3D approach (without having data from a parallel 14CH4 approach). Line 392. Isotopically labeled glycine and ammonium chloride was not mentioned before in the manuscript. Please give detailed information about this experiment already in the first part of the manuscript (e.g. paragraph 82ff). For what is good for? What is the goal of that labeling experiment?

Line 417. Please explain why the pressure experiment is a proof-of-concept. Pressure makes the difference in this experiment not the method that was used for methane oxidation rate measurements.

Line 437. One advantage of the CH3D method is that "it does not require the logisti-

cal, safety, and administrative hurdles associated with radiotracers such as 14CH4..." (line88ff). But to obtain absolute rates of full methane oxidation, parallel incubations with CH3D and 14CH4 must be performed. That means that we still have to take radiotracer on ships (together with the CH3D lable and analytical equipment) with all the administrative hurdles. That means no advantage for expeditions?

Figure 1 See comment above Colors are difficult (e.g. I cannot see brown on my printout). Would suggest to change the colors. Capture:

Figure 3 and 4 Please give the figure titles like "anaerobic methanotrophy pathway" and "aerobic methanotrophy pathway".

Figure 5 A legend (and also a title) in the figure would be helpful. Capture: That the data comes from the pressure experiment should be mentioned.

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