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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 #1

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Marlow and coworkers tested D-labeling of methane as an alternative tracer based method to determine methane oxidation rates in aerobic methanotrophic cultures, and in oxic and anoxic (AOM-active) sediments. D-labeled methane was added to a sample and the change of water deuterium isotopic composition was measured using Cavity-Ringdown Laser Absorption Spectroscopy (Los Gatos). Values were used to determine rates of methane oxidation. Alternatively the 14C-methane method was used and rates were compared. The authors found generally higher rates with the D-methane compared to 14C-methane tracer approach. As reasons for this, although not really clearly pointed, two mechanisms were discussed. Most aerobic methanotrophs use primarily methane as carbon source - hence 14C-methane is partly transferred into organic

biomass and not into CO2. Methane-H is instead almost fully transferred into water, hence seems to be promising for an accurate measurement of aerobic methanotrophy. In anaerobic methanotrophy back reaction that cause isotopic exchange were identified, which would lead to rather overestimated real rates. In general reported values have a good precision, meaning a low standard deviation – however it has not been tested if the rates are comparable to chemical measurements and as such lacks a trueness/ accuracy validation. The manuscript is full of slang, and often misses accurate terms, and lacks structure. Due to this in particular the discussion is not easy to follow. Furthermore section titles are not informative. This would need to be improved. Furthermore one has to criticize that the main, new method is insufficiently described. Why is there no formula? Instead the new approach is only described in text. From this I at least think that the rate determination is slightly incorrect. As far as I can anticipate the authors use ratios instead of (required) fractions in their calculation. Please also refer to the labeling percentage you used.

Below are my further comments, which are not complete – because sometimes I could simply not follow the discussions!

The abstract is not really productive, here a suggestion on how to improve it:

"Biological methane oxidation is a globally relevant sink of methane and it proceeds in aerobic and anaerobic pathways. However measuring rates of methane oxidation in natural samples remains challenging. Here we present a new approach for measuring rates of microbial methane oxidation that bases on the addition of labeled monodeuterated methane (CH3D) to a sample, and quantification of the label in water phase via isotope ratio mass spectrometry. As comparison we performed the well-established 14CH4 radiotracer approach the CH3D procedure. We provide measurement with cultures of I and type II aerobic methanotrophs and for sediment and carbonate rock samples incubated under anoxic and oxic incubation conditions. [results] [what is the different and why is it] We also employ this method to investigate the role of pressure on methane oxidation rates in anoxic seep sediment, revealing an 80% increase at the

equivalent of ∼900 m water depth (40 MPa). [Conclusion ; where and why should the new method be used]

L47: Often involving ? where is the real counter evidence/ or do you mean the Haroon Paper?

L50: I would cite the Mc Glynn and Wegener nature studies on the mechanism for AOM, they seem to be the most promising approaches, also considering the new Scheller study Same Paragraph: As later aerobic methanotrophy is discussed it should also be introduced here

L56: The relevance in nature was discussed above, does not need to be repeated here

L58: AOM rate measurements have traditionally. . .

The performed measurement does not discriminate aerobic or anaerobic methane oxidation – I would rephrase it: The turnover of methane to carbon dioxide in environmental samples is often traced using stable or radiocarbon labeling approaches

L61: Stable isotope 13CH4 tracers is not correct: Methane labelled with 13C or something

L62: but the natural presence of 13C in marine dissolved carbon requires accurate detection of the reactant and product concentration and isotopic compositions. (I think that is what you mean.

L66: “though low molarities make samples susceptible to exsolution. . .” what should this mean? “ I think you wanted to elaborate on concentration/ diffusion profiles. . .. But then you need to tell a full story. . . that it really not enough

L68: “carbon movement into oxidized species“ . . .. Does not sound scientific – track the oxidation of methane to CO2

L69: Labeling with tritiated methane was introduced to track aerobic methane turnover in the water column. The shorter half-life time allows higher specific activity, and the

procedures for the separation of reactant and product are less complicated and can thus be performed on sea.

L75: measurements of methane turnover remain

L77: delete: "Nearly all of the aforementioned approaches are carbon-based; a hydrogen-based tracer offers an additional dimension to investigations of methane biochemical dynamics. “

L83: that aqueous D/H values are consistently proportional to 14C-based rate measurements for given laboratory treatments tested in this study

Of course it is the rates derived from the comparison of . . .. D/H are consistently proportional to those measured. . .

But better remove 82-92 : those are your results

Methods

2.1.1. Aerobic Methanotroph Cultures it is : Experiments with cultured aerobic methanotrophic bacteria After several successful transfers – how is a transfer successful – how did you see that cells were growing

L116: Parallel incubations incorporated 14CH4 to allow for three time points of destructive sampling, and killed and radiolabel-free controls. In parallel incubation we tested the turnover of 14CH4. . .. And then go on with a clear description as above

L121: Here you could have compared it with purely chemical measurements

2.1.2. Environmental Samples: Methane Seep Sediments and Carbonates Again that is not an informative headline: Measurement of methane turnover in seep sediments and seep carbonates

L137-46: Unnecessary

L192: Analytical procedures 2.2.1 Rename – that is not a CH3D rate measurement

Determination of rates based on deuterium oxide formation? Or something similar Bring also the D-Methane concentration first – or was this not done at all?

Methods: I don't see a formula how D-values are transformed into rates – even though it a new method which is describe. As a quick remark, to be mathematically correct and fully comparable, mathematically you cannot use ratios – you need to use fractions instead FD=R(DH)/(R(DH)+1) And please revise all your downstream calculations

How much of the methane was used up in total in your approach how much methane was added. Did you do any control measurement????

L234: Name in "Labeled 14C-inorganic carbon or define it once- it was before at pH14, so only CO3–.

L255: Your main new method is misplaced and only briefly described – you need to mention how you go from the spectra (which is the input of labeled methane) to the D2O

3. Results and discussion 3.1. "Aerobic methanotrophic cultures" is not a title that can refer to a result – name it Comparison of rate measurements in aerobic methanotrophic cultures 3.2. Similar as above 3.2 needs a complete revision – I don't understand it Since 3.1 and 3.2 are only results one could make them results, 3.3. could be discussion

3.3. It is not the "H:C tracer ratio" that needs understanding here, but the rates derived from 13CH4 and CDH3 incubations

"The CH3D and 14CH4 approaches quantify distinct aspects of methanotrophy,"??? I thought you mention both quantify turnover of methane?

L313: Change to: Because methane is an inert molecule abiotic exchange between methane and water protons is not expected, and indeed the D/H ratios in controls remained stable. Use term significantly/ significant should be reserved for statistical analyses. Not here. Please show those results and any measurement somewhere –

the reference to Fig.1 is inappropriate- I don't see isotope values there

L323: If you refer to backfluxes such as in Holler et al., you should allow the backfluxes throughout the model (figure 4). And based on this and further studies (i.e. Yoshinaga et al. ,2014) relative backfluxes in AOM should increase with lower / no methane

3.3.1. The H:C Tracer Ratio in Anaerobic Methanotrophy (Sorry, again this title is less than sloppy). Relation methane turnover rates derived from D and C labeling ? or something similar

L327-360 I cannot follow this discussion, especially from L332, I have a vague idea what the author wants to tell. The reversibility of specific steps, in particular of the activation step will lead to higher apparent rates in D compared to C labeling.

379: Quantifying anaerobic methane oxidation at different methane pressures

3.5 I would put the monodeuterated methane experimental investigations further up to the results. The interpretation of these experiments is really not well judged.

L421: Why do you think one would have less isotope fractionation effects in D than in C?

L422: The precision to meet a chemically measured value was unfortunately not tested at all – so the results might be highly reproducible but we don't know yet what they show.

L423ff: Please state what both methods achieve: In aerobic methanotrophy D labeling might be more realistic to reproduce total complete oxidation rates of methane than the methane carbon labeling, because methane carbon is partly assimilated. In AOM C-labeling should be better to track methane oxidation, as a backflux would be small. D-labeling of methane likely overestimates methane oxidation rates, as rate – determining backflux can occur at all steps.

4. Conclusion. You might have been precise with your measurement but accuracy or

even trueness of your measurement was not tested

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