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Biogeosciences Discussions

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 #3

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The authors tested a potential new method to use monodeuterated methane (CH3D) as a metabolic substrate for methane oxidation rate measurements by quantifying the change in the aqueous D/H ratio over time. In the study, two methanotrophic cultures and several environmental samples were used to compare the radio of the novel CH3D method with the existing and well established 14C isotope method. The new approach is complementary to existing radio (14C)- and stable (13C) carbon isotopic methods. Because it isn't a stand-alone technique; stressing the alleged advantage of a non-toxic, rapid, and easy-to-use method is obsolete.

The overall structure of the manuscript is clear, focusing on the different metabolic pathways, which were approved by the new method by using different biological samples.

The authors give a nice overview of the potential of the presented method, although the argumentation in parts of the manuscript isn't easy to follow. From my perspective, the principle of the method isn't sufficiently explained (see comments below as well as the statements of the other referees). Also the not informative subtitles need to be improved!

I suggest minor revision before the manuscript will be published.

The abstract is smooth to read and well structured.

The introduction gives a nice overview of the main topics of the publication and is well structured. L36 the biogeochemical cycles in BOTH natural freshwater and marine environments is mentioned, but in L39 only the estimated methane emission in marine settings is given. Can you show values of produced methane in natural freshwater and rice fields/wetlands/permafrost as well?

L47 biochemical intricacies – what is meant with that phrase?

L48 -55 AOM is described. Because aerobic methane oxidation is a major part of the experimental setup and the discussion, aerobic methane oxidation should be introduced as well.

L57 biochemical curiosity – what precisely is meant by this?

L58 AOM rate measurements have traditionally been conducted using a handful of techniques. – I understand this sentence as introduction for the following methods, which not discriminate aerobic or anaerobic methane oxidation. It should be rephrased, see RC1.

L61 Stable isotope 13CH4 tracers – 13CH4 isn't a tracer. Better: 13C-labelled methane

L70 and the procedural advantages of working with a water-phase product rather than gaseous products – this is one of the main advantages? I think RC1 makes a good point.

L107 and 109 where did you get the cultures from? Are these maintenance cultures or ordered as pure culture from a company?

L118 how did you see that the exponential growth phase was reached? Which test did you use?

L130 All samples received a unique four-digit serial number. – Unnecessary.

L151 What is “compacted sediment”? And would it be interesting to know from what depth below seafloor (which layer of the push core) the sediment comes from? Or is it the whole push core sediment, which was transferred into a bag? But still, from what depth below seafloor comes the sediment?

L152 What‘s a mylar bag? There is a reference for the glass bottle (L158) but not for the bag. In general: check references for lab equipment.

L171 1.9 days? Better use hours, if it is necessary to mention the exact time point.

L190 how did you do the leak check?

L198 the water isotope analyzer determine the D/H ration of the sample – can you explain more detailed how the analyzer works?

I am agree with both referees: a formula would be helpful to understand the method and the principle of the general measurement procedure. Maybe it is possible to visualize both in a schematic diagram.

L281 tubes #1a, #1b, and #1c – from which tubes/samples are you talking about? I assume you mean your replicates, then either delete the specification of tube labels and just talk about replicates or expand table 1 and include that kind of extra information.

Chapter 3.4. I am not sure, if the pressure experiment gives an additional value to the manuscript or rather create more confusion. The main goal is to present the method and to explain the method in a way that it’s clear and easy to understand – that is sometimes a challenge especially in Chapter 3.3.

L454 is, where you bring up by the first time in the whole article for what the abbreviation NMR stands for. Should be mentioned earlier in L256.

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