Review of ‘Global geographic variability in freshwater methane hydrogen isotope ratios and its implications for emissions source apportionment and microbial biogeochemistry’ by Douglas et al.

Reviewer: Susan Waldron, University of Glasgow susan.waldron@glasgow.ac.uk

Review questions:

1. Does the paper address relevant scientific questions within the scope of BG?

Yes

2. Does the paper present novel concepts, ideas, tools, or data?

This manuscript tackles an overdue update of assessing how robustly δD-CH_4 can be described by δD-H_2O. Refining this relationships is valuable as isotope enabled Earth System Models could allow projection of δD-CH_4 source characterisation from locations where field measurements are not possible, and, when atmospheric δD-CH_4 dynamics are understood, then this may allow source apportionment to constrain better Earth Surface Fluxes. In addition, the authors explore if the variation in δD-CH_4 not described by δD-H_2O can be understood, exploring the hypothesis that differences in methanogenic pathway exhibit a control. The authors also apportion the database they have constructed into different habitats to explore if there is a habitat specific signature for both δD-CH_4 and $\delta^{13}CH_4$ and using these signatures and published flux estimates, upscale to calculate an atmospheric CH $\delta^{13}CH_4$ and δD-CH_4 paper. It is an ambitious paper of two halves (the controls on isotopic signatures vs. the habitat upscaling) and could be two papers, but their linkage is sensible

3. Are substantial conclusions reached?

Substantial conclusions are reached, but the interrogative approach has weaknesses that propagate through substantial analytical reasoning and so the integrity of the conclusions is questionable. I detail this further below, but until the analytical approaches are reconsidered the conclusions are not securely reached

4. Are the scientific methods and assumptions valid and clearly outlined?

My expertise is in understand the control on methane isotopic composition in the field and lab., and not on flux upscaling, so I can less securely comment on that.

With respect to understanding isotopic compositions: the methods are not all valid, particularly the reconstruction of missing δD-H_2O for a field measurement of δD-CH_4. The interrogation of this relationship (Fig. 2) lacks statistical rigour, and its propagation - a relationship that has bias and significant variability - is unconsidered in all analysis thereafter (as represented by Figs. 3-9 and possibly 10) and so this reasoning is flawed and the interpretations may be wrong.

The authors are not consistent in identifying when processes they are interpreting are based on hypothesised relationships and the impression is given such processes are certain (detailed below).

5. Are the results sufficient to support the interpretations and conclusions?

No due to the flaw above, and its propagation in subsequent analysis. But this can be revisited.

6. Is the description of experiments and calculations sufficiently complete and precise to allow their reproduction by fellow scientists (traceability of results)?

I found it difficult to follow the calculations behind αC – an important part of the manuscript – when I was trying to compare other data sets with their approach.

7. Do the authors give proper credit to related work and clearly indicate their own new/original contribution?

Largely bit not always, for example there is a large section in 4.31. that is repeating suggestions made in section 1.1. of Waldron et al 1999, but this work is unreferenced and so as written implies the review m/s is the first to have suggested this; the abstract does not make clear refining an existing phenomena observed and described similarly previously.

8. Does the title clearly reflect the contents of the paper?

Broadly but not sure how “geographic variability in freshwater methane hydrogen isotope ratios has implications for microbial biogeochemistry” - the microbes are active with no knowledge δD…so this can be refined.

9. Does the abstract provide a concise and complete summary?

Yes, but incorrect interpretation for the reasons above

10. Is the overall presentation well structured and clear?

Yes, it flows quite well.

11. Is the language fluent and precise?

Mostly – some language could be better constrained.

12. Are mathematical formulae, symbols, abbreviations, and units correctly defined and used?

Yes

13. Should any parts of the paper (text, formulae, figures, tables) be clarified, reduced, combined, or eliminated?

It is a paper with a lot of detail and so to follow it all the reader has to concentrate deeply for the results section. As such, and maybe in addition, the discussion from section 4 onwards seems in places repetitive.

14. Are the number and quality of references appropriate?

Broadly yes – I suggest a group whose work may be missing in the intro.

15. Is the amount and quality of supplementary material appropriate?

Yes, very helpful, but sheet 2 could make it clearer if the data offered is used in α_C or these are summarised data from other sources.

Additional feedback

Context: I have not anonymised this review as I believe we should be prepared to stand by our comments, and open reviews promote a fairer review. Thus, for the sake of clarity clear I am lead author in Waldron et al 1999, which is a key paper this BG paper builds on.

As outlined above the aims of this paper are admirable and the ambition to join field research with isotope-enabled ESM an important aspiration. Thus, I very much welcome this paper.

I also recognise that although my research has been predicated towards the interpretation that δD-CH_4 (in shallow freshwater environments) is primarily controlled by δD-H_2O - either directly by incorporation of hydrogen when CO_2 and acetate are used as substrate, or indirectly as organic matter which is turn source hydrogen from environmental water – the community have continued to draw heavily on the hypothesis that methanogenic pathway also imparts a control on δD-CH_4 (heavily influenced by the work of Whiticar), and so it is not surprising the authors explore this approach, although it is not consistent with my research and so I remain sceptical.

I approached this BG manuscript from this position but open to new evidence of the control of methanogenic pathway in explaining the additional variability unexplained by δD-H_2O. For this reason, I welcomed the use of paired α_C as I concur the evidence is strong that changes in methanogenic pathway are reflected by changes in $\delta^{13}C$-CH_4.

I consider I can comment expertly on what controls the isotopic signature of methane and field relationships, but am not expert in estimating global mean compositions using the approaches in the second part of the paper, although I do understand the principles and can assess the scientific communication Thus I comment less on the upscaling.

More detailed review

Expansion on my response to review Q3-5.

The database used:

The substantive conclusions in this manuscript rely on a data set where δD-H_2O does not exist for more than half the data: 53% of the sites do not have field measured δD-H_2O (L88). In these cases,

$\delta D\text{-}H_2O$ is inferred from a reputable global precipitation database and a correlation observed for sites where measured values exist. The authors consider this relationship sufficiently robust to proceed to use the reconstructed $\delta D\text{-}H_2O$ where measured values do not exist. I disagree this is the case.

Figure 2 shows that for a given projected $\delta D\text{-}H_2O_p$, the field (measured) $\delta D\text{-}H_2O$ could be up to 50‰ more depleted or enriched (for example, compare the range in the y-axis for the field data for a predicted $\delta D\text{-}H_2O_p$ for ~ - 125‰ and -150‰,) and can be more enriched than $\delta D\text{-}H_2O$ by up to 20‰ (offset in the x-axis from the 1:1 line). Thus, there is both uncertainty and bias in the relationship that is now used to create $\delta D\text{-}H_2O$ for a field $\delta D\text{-}CH_4$.

The statistical integrity shown elsewhere in the manuscript is lacking in this section on reconstructing $\delta D\text{-}H_2O$, with the authors describing their predictive relationship as showing "generally good agreement" and proceeding to use it. The bias and variability in a predictive $\delta D\text{-}H_2O_p$ and thus how far it may be from the true $\delta D\text{-}H_2O$ appear unconsidered in any further analysis (no errors propagated through for estimated $\delta D\text{-}H_2O$?).

Further, I note that the data in table S3 supplementary information for which there are measured $\delta D\text{-}CH_4\text{-}\delta DH_2O$ fit closely to the in-vitro line from which Waldron et al 1999 project a global relationship - but the data with estimated δDH_2O in table S3 do not. This is important for two reasons:

1. It confirms the predictive relationship in Waldron et al 1999 for $\delta D\text{-}CH_4$ from $\delta D\text{-}H_2O$ still has integrity, more so by adding in another methane-producing environment (innocula), a significant time gap, and another geographic locality.
2. If statement 1 is considered sound, then the poor fit of paired $\delta D\text{-}CH_4\text{-}\delta D\text{-}H_2O$ with predicted $\delta D\text{-}H_2O$ supports the assertion above that the relationship the authors are using here to reconstruct $\delta D\text{-}H_2O$ is questionable.

The revised global database of paired $\delta D\text{-}CH_4\text{-}\delta D\text{-}H_2O$

With the greatest of respect, using the predicted data produces an outcome that is like a 'house of cards' – all subsequent analysis using this data is built on a shaky foundation. I therefore think that incorporating paired $\delta D\text{-}CH_4\text{-}\delta D\text{-}H_2O_p$ in further analysis is flawed and offer two examples why:

1. It creates a new global line for $\delta D\text{-}CH_4\text{-}\delta D\text{-}H_2O$ that may be wrong.

2. It could lead to artefact in interpretation, which indeed may be 'visible' in the dependent analysis. For example, the data in Fig. 3b visually also appears to separate between paired $\delta D\text{-}CH_4\text{-}\delta D\text{-}H_2O$ data that are predicted (inland waters) and measured (wetlands), and if this is the case interpreting a biome difference here, and later in the paper, is also questionable.

With respect to the redefining of a new global $\delta D\text{-}CH_4\text{-}\delta D\text{-}H_2O$ and consideration of how this has changed from the relationship offered in Waldron et al 1999: unless the authors can produce a more robust estimation of $\delta D\text{-}H_2O_p$, the data that uses $\delta D\text{-}CH_4$ paired with predicted $\delta D\text{-}H_2O$ needs to be removed - for as noted earlier, there is insufficient confidence this is an accurate representation of the field situation and may create a false outcome. I suspect this will change the global relationship and increase the slope as paired data with $\delta D\text{-}H_2O_p$ visually appears to dominate the enriched samples.

Then for the comparison with Waldron et al 1999 the following approach would be more robust:

- Please plot both the in-vitro and in-vivo relationship, and for the former its prediction intervals - which are missing from 3b and so give the sense of a poorer fit of Waldron et al 1999 to the bgd expanded field data set here.
- Compare whether the in-vivo line is statistically different to the relationship generated from the data set presented in the bgd manuscript. This will allow confidence in any further discussion on how the relationship has been redefined (than just comparing slopes etc). If the two relationships are indistinguishable statistically, nuanced statements about differences in slope etc are meaningless – all that has happened is that the expanded data set has redefined better the field relationship for $\delta D\text{-}CH_4\text{-}\delta D\text{-}H_2O$ (as indicated likely in Waldron et al, 1999) - noting that this field relationship does not wholly reflect the relationship at production (see next point).
- Assess whether the expanded field data set is predominantly ^{13}C-enriched compared to the in-vivo relationship described in Waldron et al 1999, and therefore consistent with an interpretation that differences in field $\delta D\text{-}CH_4$ may be an artefact of fractionating processes post-production than pathway per se This is advocated as I am still unaware of experimental

evidence methanogenic pathway in shallow freshwaters changes δD-CH_4, but there is evidence of processes, oxidation and mixing, causing enrichment, and so this approach is consistent with scientific principle of parsimony and interpreting data using the simplest approach.

The inference of methanogenic pathway from α_C

To explore why the paired δD-CH_4-δD-H_2O measurements are not fully described by the best fit line, the authors explore whether a difference in (dominant) methanogenic pathway is evident in the data. With no evidence from paired δD-CH_4-$\delta^{13}CH_4$ the authors draw on α_c as a proxy for methanogenic pathway to assess this. Step-wise regression is used to explore this. I think this is interesting and something to revisit when the paired data relying in predicted δD-H_2O has been removed, but currently it is the next floor in the 'house-of-cards', reliant on data that we do not know to be accurate, and therefore the significant relationships that the authors infer changes in methanogenic pathway from, we do not know to be true.

The authors in their revision should be careful in the value of thinking about α_c for the following reasons: some of the literature generating α_c relies on assumption of differences in methanogenic pathway interpreted from differences in δD-CH_4, but there is competng evidence δD-CH_4 cannot be interpreted in this way (so α_c using α_c to infer methanogenic pathway in δD-CH_4 when δD-CH_4 has been used to infer methanogenic pathway becomes a circular, self-supporting and flawed approach).

To help here I would advise the authors to consider Waldron et al 1998 (Geomicrobiology, 15, 157-169), which contributes to the in-vitro line in Waldron et al 1999, but the authors do not cite so I am unsure if they are aware of the detail in this.

Here dominance of methanogenic pathway was changed in mixed culture (as would be found in the field) incubations, and δD-CH_4 monitored with time – so not just one measurement as may be misinterpreted from Waldron et al 1999. Except for one measurement broadly within analytical uncertainty, δD-CH_4 remained constant. However, $\delta^{13}CH_4$ did change and consistently with fractionation ranges for the methanogenic pathways thought to be dominant (as assessed from independent measurements of substrate turnover). I advise the authors to consult Waldron 1998 for two reasons:

1. The authors approach in the bgd paper to draw on δD-CH_4 to represent differences in methanogenic pathway would be stronger if they can provide an explanation for the constancy in δD-CH_4 while $\delta^{13}CH_4$ changes.

2. Waldron et al can also be used to calculate α_c (both from CO_2 and from estimated substrate composition). α_c CO_2-CH_4 generates values of 1.057 for the period when CO_2 reduction is considered dominant (i) and 1.055 when acetoclastic methanogenesis is considered dominant (ii). These are very similar and it would be valuable to understand how the authors interpret this when they infer much wider ranges in α_c. For clarity $\delta^{13}CO_2$ and $\delta^{13}CH_4$ respectively for (i) were -8.3 ‰ and -62‰ , and for (ii) were 1.55‰ and -47.5‰

This concludes my main comment on the parts of the manuscript that I consider are not yet robust enough to concur with the interpretation.
In working through the manuscript, I made the following other comments that the authors should also consider.

Abstract generally: Is clear and summarises the paper but projects a future methane emissions scenario (L25-26) before the modelling and assessment of how well this approach can reconstruct current estimates (L27-30) and this seems in the wrong order to me, given the former has a reliance on the latter. Further, the abstract does not acknowledge this research is augmenting the research that historically first documented the global relationship between δD-CH_4 and δD-H_2O easily addressed for example by changing L12 to 'We have refined the existing global relationship between δD-CH_4 - δD-H_2O by the compilation of a more extensive global dataset…."

L28: The authors postulate the mismatch is dependent only on the work of others (emission inventories, etc) and not possibly an error in their approach. Scientifically this is not correct – both 'sides' could have errors.

L19: results do not imply; one interprets data to generate a ‘result’.

L22: high (more ^{13}C-enriched) in rivers and bogs - this is the dataset that has more δD-H_2O projected, so is this an artefact of the modelling than a real biome-specific difference?

L27: integrated (by mass balance) not combined (which is used when sources are added) – which I know the authors have done (L204) but the descriptor is incorrect here.

Intro:

L36: I think the following references is missing: Variability in Atmospheric Methane From Fossil Fuel and Microbial Sources Over the Last Three Decades. / Thompson et al: Geophysical Research Letters, Vol. 45, No. 20, 28.10.2018, p. 11499-11508 (and I invite the authors to wonder if also some of the work from the Royal Holloway group should augment L47-51)

L59 & L83 Citations are given in chronological order of 1999b and 1999a which seems not typical convention to me (uncertain of the referencing convention for BG but for example the two references for Walter K are not in chronological order in the reference list so the in-paper citations would not be b then a due to this convention in the reference list?)

L68: Logic only follows that impact on $\delta^{13}CH_4$ can affect geographic provenancing if reader knows it can also affect δD-CH_4, so does this need to be made explicit?

L70: this implies that different ecosystems have different methanogenic pathways. More accurate text would be “differentiated geographically based on ecosystem differences in the relative strengths of different methanogenic pathways and δ^{13}C of source organic matter” (as per the introduction of the Ganesam paper). Noting relative strengths is important, as a common mistake propagated in the literature and again here (L???) is to assume methanogenesis proceeds by one methanogenic pathway only – this would be rare, with field-based methane production contemporaneous from CO_2 and acetate, and varying temporally in strength as input of fresh OM changes seasonally (or not).

L84-85: sounds a bit defensive? How about “We have advanced existing compilations of freshwater δD-CH_4 by 1,2,3 …? I would remove significantly (statistical connotations) and just say larger as the number speak for themselves.

L91: The aims are clear (good) but ‘then’ and ‘potential’ not needed – the latter as embedded in implications that there is a potential for impact

Methods:

L106 & L117, 9L206 and possibly elsewhere): small w for where, as this follows from an unfinished sentence in both cases with the equation used in between

L136: the five ecosystem categories are not clear from this sentence: ‘lakes’ and ‘rivers’ and then there are five wetlands listed. Further, it is debatable that floodplains are aligned with rivers as CH_4 production would only occur when sediments are deoxygenated from standing water. So I would say more with ponds as the recession of water can be slow and could be like a pond drying in some situations. Noteworthy here is that gas loss from rivers is velocity dependent (see Long et al (2015) Hydraulics are a first order control on CO_2 efflux from fluvial systems Journal of Geophysical Research – Biogeosciences, 120, (doi:10.1002/2015JG002955), and similar references. This will also be the case with methane – possibly more so as insoluble, and may cause an isotope fractionation independent of degassing, and may also be a reason the Amazon rivers in Fig. 5 plot differently.

L139: Similarly, I question the scientific integrity in lumping lakes with rivers here – gas loss from river systems is controlled by hydrological processes primarily and there could be fractionations during emission from lotic systems that are different to lentic systems where diffusion and wind of lake thermal orographic processes control turnover. This starts to become important where these mean sources are used to simulate a resultant atmospheric composition e.g. L227. Thus, the authors should think about how to provide added confidence of the robustness of their catergorisation.

L145-148: Such categorisation is good, and the open access data set is very welcome. This categorisation relies on the integrity of the interpretation, but this integrity is important as the data analysis relies on this. With 131 sites it is impossible for the reviewer to know each site and so as a check I can only look at my own data: L61 in the excel files. These methane samples were collected in-situ from porewater diffusing into samplers embedded in the peat (the GBC abstract notes in-situ and the methods clarifies at depth sampling) so I would classify as more aligned with dissolved porewater than diffusive flux (which is normally associated with the potential for oxidation and change in δ values). Further I comment in the GBC paper there is a dynamic zone and interpret that is the section from which gas can be emitted. Mean δD-CH_4 here is -332 ± 17‰, more depleted the -294 ± 39‰ used in the table and subsequent data analysis. Thus, some feedback from the authors in the revised manuscript that their interpretations are not sensitive to the variation their interpretation of environment and which data to use would be valuable.

L152 – typically small – as this manuscript relies on several source of data estimation (here, δ^2H_2O, it would be good to provide estimates as to what the maximum is this would manifest in δD (recognising that it changes with resolution and scale of figure and so this is challenging, but saying small is insufficient).

L177: the authors need to unpick for the reader the statement more as they have with L179 onwards. I am thus left to interpret the reasoning. I assume it is based on considerations that methanogenic pathway influences δD-CH_4? If so please see earlier substantive comments on this and decide whether to proceed in the revised manuscript.

L200: Clarify where the flux estimate comes from at this point – I presume from Saunois et al as in L209, but this should be clarified when first introduced. I am not expert enough to judge if the methodology for the bottom up flux section is sound, but it seems reasonable to me.

L 267: given the statistical approaches such as Monte Carlo bootstrapping used with the flux estimate section previously I would have expected more rigorous comparison should be undertaken here to show if there is a statistical offset between measured and predicted δD-H_2O than relying on descriptors of "generally good agreement" and using RMSE. The RMSE is a red herring if the lines generating 19 and 23 ‰ do not overlap - ?

Fig 2: Should the predicted (postulated and therefore dependent) not be regressed onto the measured (the true field value, so measured and independent and as a control of δD-CH_4 the one to get as close to the true value as possible)?

Fig 3B: this needs revisited once the δD-CH_4 -δD-H_2O predicted data has been removed as described above. There may still be an inland water specific difference here, but again that this may not be controlled by anything more complex than lentic and lotic freshwater systems having generalised differences in gas transport mechanism (ebullition or diffusion). These would be influenced by atmospheric and sediment interface boundary layer dynamics, transit time, depth of oxidative zone, lake stratification, and surface roughness, with the latter in turn influenced by wind speed, depth of water, and river flow velocity, slope. In other words, considerable methane isotope fractionation (enrichment) is possible, or not.

Fig 4. It is good to see this plotted but not surprising given δD-H_2O varies with latitude and δD-CH_4 varies with δD-H_2O. The same difficulties in estimating field δD-H_2O from modelled δD-H_2O are evident when considering δD-CH_4 as a function of predicted δD-H_2O. The authors need to note here that there may be an imbalance of where methane is sampled from globally and so if more measurements existed from the higher latitudes then there may be as much scatter as with the lower latitudes.

Section 3.4 jumps to something completely different with L313 "shifts to being controlled by changes in methanogenic pathway to being controlled by ….". There has not been clear discussion from the authors to date they are considering changes in methanogenic pathway of δD-CH_4 so this seems out of context. And yet L317 goes on to consider this in more detail. The key message in the Waldron et al 1999 paper is that considering methanogenic pathway a control on δD-CH_4 is misplaced and that "that 50% of the variation in natural δD-CH_4 samples can be explained by δD-H_2O, with isotopic fractionation post-production, or mixing with gas already fractionated likely responsible for most of the noise in the natural system". The analysis prior to section 3.4 may be more likely to support this interpretation than refute it, particularly when the data in Fig. 3.2. is appropriately compared (as described earlier), and so now considering data as a function of methanogenic pathway seems to be ignoring this. Indeed the authors observe they find no relationship between $\delta^{13}C$-CH_4 and δ^2H-

$CH_{4,w0}$ which would be expected if δ^2H-CH_4 was influenced by methanogenic pathway as $\delta^{13}C$-CH_4 is (Fig. 5a). Thus, the authors should not make clearer statements such as L312 of "shifts from being controlled by variation in methanogenesis pathway" are inferred controls.

Figs. 5b=c. The uncertainty around what α_c should be for different methanogenic pathways has been described earlier in this review. But additionally, although breakpoint analysis was used, there is a high dependence in this on data set that has enriched δ^2H-CH_4 to generate opposing trends. The eye is drawn by the projected pathways, but if these was not included as we cannot be sure it is oxidation[1] and all the remaining data was considered in a weighted regression would there be trends?

[1] If the high δ^2H-CH_4 is from the Amazonian rivers, there are shales in this basin that fuel C cycling (Vihermaa et al) and this could be thermogenic: δ^2H-CH_4 is also consistent with this.

Vihermaa L.E., Waldron S. , Garnett M.H., and Newton J. (2014) Old carbon contributes to aquatic emissions of carbon dioxide in the Amazon. Biogeosciences, 11, 3635-3645. (doi: 10.5194/bgd-11-1773-2014).

It is remarkable Fig 7 is so consistent – this is very interesting. Is it what we would expect?

L370 discussion is over-interpretations given the differences between sites are not statistically significant. It would be ok to say the prevalence of more depleted CH_4 is greater in the ecosystems sampled but for example this could represent accessibility of field sites, or differential investment into research measurements in these areas, than group compositional differences per se. Ecosystem types are not evenly distributed by latitude (L370) – nor is resource for investment in field research with tropical regions of the Earth lacking measurement due to access or financial constraints – we need to start recognising what we have not measured is as important as what we measure.

Fig. 10 is tiny and needs to be bigger

L426 "roughly as strong a predictor". Too big a leap: explain how – from ice core gases "roughly is a colloquialism"

L487 – as noted earlier, the paired measured values plot on Waldron et al 1999 In-vitro line, consolidating further the significant of this line. Please acknowledge this.

L508 – in the revised manuscript please detail the % variation explained by δD-H_2O and then additionally by α_c should this prove to still be important

L510 – this is the crux of what is new to explore in isotope biogeochemistry of methane and also the role of methanol substrates.

L519 – same comments as before about is there really a relationship, but why more points classified as oxidised with this pairing than with α_c?

L551- Much of 4.31. is repeating statements first described in Waldron et al 1999 section 1.1., paragraph starting "In addition…" but this is not referenced and as written implies the authors are the primary source of this thinking. This is not the case and should be referenced appropriately to indicate this was first noted 20+ years ago.

L564 – please note pure cultures are not representative of the field processes of methane production and thus the batch cultures and other experimental data collated in Waldron et al 1998, 1999 are. This is not clear from the statement.

L569, please reverse the order of the references or remove Whiticar 1999. The Waldron 1999 paper is the one that is particularly focussed on the global relationship between δD-CH_4 - δD-H_2O, and constructs the first global relationship, which this paper finds with new data is similar. This gives appropriate credit to the conceptual understanding. The Whiticar paper coplots δD-CH_4 - δD-H_2O but does not assert that " δ^2H-H_2O is a primary determinant of δ^2H-CH_4 on a global scale", rather the focus is on the interpretation of how δ^2H-CH_4 reflects methanogenic pathway or marine vs. freshwater.

To conclude: this has been an uncomfortable review for me to undertake as my position of not anonymising the review puts me up for public scrutiny, and a misinterpreted that I am trying to defend my work and am unwilling to accept an addition to this. This does not represent my professional scientific principles, I would urge the authors to accept this is not the case - indeed in the 1999 GCA paper I welcome refinement of my work. However, the authors have still not presented here compelling evidence that δD-CH_4 can represent well different methanogenic pathways and so the reliance of this in the manuscript I find troubling. I consider the α_c approach may be valuable in helping constrain the signal in δD-CH_4 that is not defined by δD-H_2O, but the current manuscript is not constraining uncertainty sufficiently and the approach is therefore flawed. I would urge the authors to find a way to better constrain projected δD-H_2O and revisit this, or work with only measured data and revisit this. Their refined analysis should undertake rigorous statistical comparison with the existing field δD-CH_4 - δD-H_2O relationship from Waldron et al 1999 to say whether it is different (although the new larger dataset will likely be a more representative relationship that the community can go forward with), and adopt a parsimonious interpretation of variation within the data set, as that is least likely to induce an erroneous interpretation. The biome specific considerations and upscaling should also be revisited if the removal of biased and inaccurate data pairings changes the source bulk compositions, and further thought should be given to the basis for source differentiation based on scenarios of methane production and loss in this upscaling.