During the past two decades, there has been limited progress in advancing understanding of controls on $\delta^2H(CH_4)$ values in freshwater environments and improving estimates of δ^2H values of CH_4 emissions. This study: (i) updates and attempts to refine the relationship between $\delta^2H(H_2O)$ and $\delta^2H(CH_4)$ first reported by Waldron et al. (1999b), (ii) evaluates the extent to which factors other than $\delta^2H(H_2O)$ may influence $\delta^2H(CH_4)$ values in freshwater environments, (iii) uses the refined relationships to estimate new δ^2H values for CH_4 emissions from freshwater sources, and (iv) weights CH_4 fluxes reported by Saunois et al. (2020) with a mixture of old and new δ^2H and $\delta^{13}C$ values to estimate global δ^2H and $\delta^{13}C$ values for atmospheric CH_4. In my opinion, the study offers new insights that are worthy of publication pending revision.

General comments

Site level mean values - The study has produced a thorough compilation of stable isotope data related to CH_4 from freshwater environments. The availability of $\delta^2H(CH_4)$ values presumably was the key criterion for inclusion in the data base. The supplemental file contains a summary of the data, showing the number of samples from each site and site-level mean isotopic values as described in section 2.3.1.

While I appreciate the motivation to avoid introducing bias towards sites that have larger datasets, this approach does limit the extent to which the study can comment meaningfully on differences between environments. $\delta^2H(CH_4)$, $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ values all exhibit significant ranges and trends with depth in the subsurface of wetlands. That information is lost when profiles of δ-values are averaged. In peatlands where CH_4 production pathways change with depth or CH_4 oxidation occurs, δ-values determined from an average of shallow and deep layers has little meaning in the context of production pathways or evidence for CH_4 alteration. The pooled δ-values also do not take into account differences in the amount of CH_4 or CO_2 at different depths. Moreover, δ-values from deep peat typically will have little bearing on the stable isotope composition of CH_4 emitted from a wetland. Venting of accumulated gas bubbles from deep peat can occur (e.g., Glaser et al, 2004) but there is little evidence that such events are common. The bulk of CH_4 production occurs at shallow depths (from water table level to ~50 cm depth) where the supply of labile substrates from plant roots is greatest and temperature is highest during summer. The residence time of CH_4 at those depths is shortest (e.g., Lombardi et al., 1997; Bowes and Hornibrook, 2006) and most of the CH_4 produced seasonally is either consumed or evaded to the atmosphere.

If subsurface data must be averaged to avoid bias, then I suggest using a consistent depth range (e.g., 0 to 50 cm) to (i) generate mean δ-values that are more likely to represent δ-values of CH_4 emissions, and (ii) enable analysis of α_C and α_H values that are more likely to be related to one methanogenic pathway or exhibit the influence of methane oxidation rather than a blend of pathways and processes across a range of depths. An important advance in this study was the attempt to discern the relative impact of factors other than $\delta^2H(H_2O)$ on $\delta^2H(CH_4)$ values. Use of site level means for δ-values raises concern about the validity of the α_C and α_H values calculated to assess breakpoints in CH_4 production pathways and oxidation.

'***Bottom-up' mixing model*** - I appreciate that considerable effort was invested in attempting to upscale $\delta^2H(CH_4)$ and $\delta^{13}C(CH_4)$ values; however, it is questionable whether that portion of the manuscript has potential to advance discourse on global isotope-weighted CH_4 budgets. A more valuable outcome of this work would have been the one identified by that authors in lines 441-

443: "A logical next step in predicting global freshwater δ^2H-CH_4 source signatures would be to combine high-resolution mapping of wetlands and inland waters, maps of the global distribution of δ^2H_p, and regression relationships between δ^2H-CH_4 vs. δ^2H_p." In my view, production of a global gridded map of $\delta^2H(CH_4)$ values for freshwater environments would have a more suitable application of the outcomes from the data analysis. It would provide a useful counterpart to the $\delta^{13}C(CH_4)$ global map for wetlands published by Ganesan et al. (2018). I realize at this stage in the process that would take the second half of the manuscript in a very different direction. As things stand, the weighted atmospheric $\delta^2H(CH_4)$ and $\delta^{13}C(CH_4)$ values that were calculated are difficult to reconcile with atmospheric data and KIEs associated with sinks for atmospheric CH_4. It's possible that the values may be offering new insights but it seems more likely that there are issues with attribution of δ^2H and $\delta^{13}C$ values to CH_4 sources.

These are my two main concerns with the manuscript in its present form. I am supportive of publication in a revised form. The work has potential to be a useful contribution and stimulate further efforts to characterise δ^2H values of CH_4 produced and emitted from freshwater environments.

Specific comments

Citations within the text do not appear to be listed consistently either alphabetically or chronologically.

Line 38: 'clearly' = 'unequivocally' ?

Lines 51-52: 'recent technological developments'. An additional sentence or two about laser-based methods would be helpful for a broader readership.

Lines 53-57: Rigby *et al.* (2012) also demonstrated the utility of a multi-isotope approach for global methane cycle characterization.

Lines 87-88 (and elsewhere): 'data is' should be 'data are'

Line 105: A citation for Coplen (2011) could be added for the definition of delta that (correctly) does not include a 'x 1000' factor.

L129: The citation for John Lansdown's thesis should be:

Lansdown J. M. (1992) The carbon and hydrogen stable isotope composition of methane released from natural wetlands and ruminants. Ph.D. dissertation, Univ. of Washington.

(The citation can be confirmed at: https://dggs.alaska.gov/pubs/id/28259)

L156 – Is the annual estimate of δ^2H_p weighted by the relative amounts of precipitation during different seasons?

L200: δ^2H (superscript missing)

L258-L259 “55 sites are classified as wetlands, including 16 bogs, 14 swamps and marshes, 12 fens, and 8 rice paddies.”
>> Are the classifications for bogs and fens based upon pore water chemistry and vegetation surveys? The word ‘bog’ sometimes is used in site names that are other wetland types, in particular, fens.

Table 1: Origins of some data are unclear. When indicated as ‘no specific measurement in database’, what does it mean to say ‘we used the isotopic values and uncertainties for X’? Which literature source? Also, only C3 $\delta^{13}C$ values appear to be used for biomass burning. Grassland and savanna wildfires presumably generate CH_4 that has more positive $\delta^{13}C$ values from burning of C4 grasses.

L266-L271 The comparison of modelled δ^2H_p values and measured $\delta^2H(H_2O)$ values for 62 sites is important for validating the approach on which estimating $\delta^2H(CH_4)$ relies. The text is not clear though with respect to causes in deviation from a 1:1 relationship. Presumably “δ^2H-H_2O is generally higher” means ^2H-enrichment is evident in the measured data. Is the statement about ‘overall smaller water volumes’ meant to infer evaporative enrichment of ^2H?

L282-L283 “Both relationships result in a large amount of unexplained residual variability, implying the importance of other variables in controlling δ^2H-CH_4.”

I’ll expand here on the point raised in my general comments. The extent to which residual variability exists is likely underestimated because of the use of site-level means. There are relatively few data sets globally that contain subsurface profiles of both $\delta^2H(H_2O)$ and $\delta^2H(CH_4)$ values. Four of those data sets are shown in the enclosed figure which was published in Hornibrook and Aravena (2010): Turnagain Bog (open triangles; Chanton et al. 2006), Sifton Bog (open diamonds; Hornibrook et al. 1997), Point Pelee Marsh (open circles; Hornibrook et al. 1997) and Ellergower Moss (open squares; Waldron et al. 1999a). The arrows indicate the direction of increasing depth in peat for Turnagain Bog, Sifton Bog, Point Pelee Marsh and Ellergower Marsh. The figure also includes δ^2H values of coexisting CH_4 and H_2O values from Alaskan peatlands along a N-S transect (filled triangles; Chanton et al. 2006) and regression equations (Table 6.2 from Hornibrook and Aravena, 2010 also enclosed) from a number of studies including Waldron et al. (1999b; line 5) and Whiticar et al. (1986; lines 1 and 2).

The approach of using site-level means reduces each of those depth trends to a single point in $\delta^2H(H_2O)$ vs. $\delta^2H(CH_4)$ space. The δ^2H values of CH_4 emitted to the atmosphere are likely to be similar to the most ^2H-depleted values in each trend which corresponds to CH_4 in shallow peat near the water-air interface and within the root zone where CH_4 may be transported to the atmosphere via plant aerenchyma. Averaging $\delta^2H(CH_4)$ values from all depths (2 m for Sifton Bog and Pelee Marsh; 6 m for Ellergower moss) yields a mean that is substantially more ^2H-rich.

Again, I appreciate the goal of not biasing the analysis to these larger data sets but a single mean for each site does not reflect the considerable residual variability that exists with depth as $\delta^2H(CH_4)$ values shift away from the global $\delta^2H(H_2O)$ vs. $\delta^2H(CH_4)$ regression line. Moreover, the $\delta^{13}C(CH_4)$ and $\delta^{13}C(CO_2)$ depth trends from these sites yield systematic shifts in α_C values that are lost when the $\delta^{13}C$ values similarly are reduced to unitary site-level means.

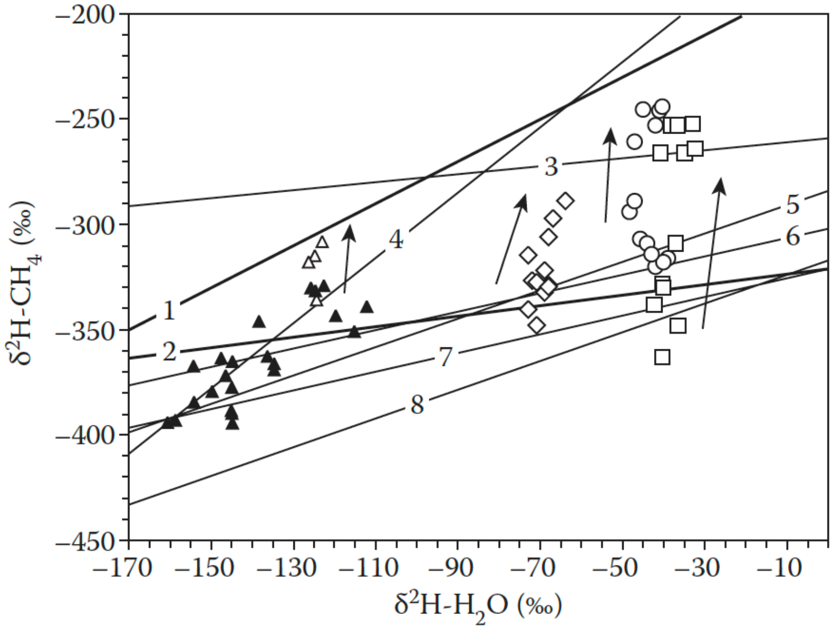

TABLE 6.2

Equations Relating δ^2H Values of Coexisting CH_4 and H_2O Shown in Figure 6.1

No.	Equation	Origin	Source
1	$\delta D\text{-}CH_4 = 1.000\ \delta D\text{-}H_2O - 180(\pm 10)$‰	CO_2 reduction in situ	Whiticar, Faber, & Schoell (1986)
2	$\delta D\text{-}CH_4 = 0.250\ \delta D\text{-}H_2O - 321$‰	acetate fermentation in situ	Whiticar et al. (1986)
3	$\delta D\text{-}CH_4 = 0.19\ \delta D\text{-}H_2O - 259$‰	*Methanosaeta thermophila*	Valentine et al. (2004)
4	$\delta D\text{-}CH_4 = 1.55(\pm 0.46)\ \delta D\text{-}H_2O - 145(\pm 30)$‰	Alaskan peatland transect	Chanton, Fields, & Hines (2006)
5	$\delta D\text{-}CH_4 = 0.675(\pm 0.10)\ \delta D\text{-}H_2O - 284(\pm 6)$‰	global freshwater in situ	Waldron et al. (1999)
6	$\delta D\text{-}CH_4 = 0.437(\pm 0.05)\ \delta D\text{-}H_2O - 302(\pm 15)$‰	acetate fermentation in vivo	Sugimoto & Wada (1995)
7	$\delta D\text{-}CH_4 = 0.444(\pm 0.03)\ \delta D\text{-}H_2O - 321(\pm 4)$‰	mesophilic incubations in vivo	Waldron et al. (1998)
8	$\delta D\text{-}CH_4 = 0.683(\pm 0.02)\ \delta D\text{-}H_2O - 317(\pm 20)$‰	CO_2 reduction in vivo	Sugimoto & Wada (1995)

L308-L309 “We do not find evidence for a piece-wise linear relationship between δ^{13}C-CH$_4$ and δ^2H-CH$_{4,\mathrm{w0}}$ (Fig. 5a), nor did we find a significant simple linear correlation between these variables.”

>> It may be worth exploring whether any relationships exist in the full data sets rather than site-level means.

L377 – L378. “Similarly, we did not observe any significant differences in $\delta^{13}C$-CH_4 values between wetland ecosystems in this dataset based on a Kruskal-Wallis test, nor between inland waters and wetlands based on a U-test.”
>> I recommend examining whether this is the case if CH_4 data are used from a common depth interval rather than site-level means.

L441-L443: “A logical next step in predicting global freshwater δ^2H-CH_4 source signatures would be to combine high-resolution mapping of wetlands and inland waters, maps of the global distribution of δ^2H_p, and regression relationships between δ^2H-CH_4 vs. δ^2H_p.”
>> I agree with the authors and suggest this would be a worthwhile output to include in this manuscript instead of the global upscaling estimate.

L445-L464 Section 4.2. This section would benefit from acknowledging and discussing the study by Rigby et al. (2012).

L500-L504 In addition to the caveat noted that CH_4 data exhibiting ^2H-enrichment due to methane oxidation are uncommon, the amount of CH_4 emitted to the atmosphere bearing the effects of methanotrophy is likely to be small. Bacteria oxidation is highly efficient in the subsurface of wetlands and little CH_4 tends to escape to the atmosphere via diffusion through porewater. This comment applies to peatlands. The situation is different in inland water environments.

L510–L518 I was pleased to see incorporation of these alternate explanations for relationships between δ^2H and $\delta^{13}C$ values of CH_4. Methanogenic pathways are not the only potential explanation.

L592-L593 – Bellisario et al. (1999) provides a good example of how $\delta^{13}C(CH_4)$ values vary along a trophic gradient in a wetland complex. Differences in $\delta^{13}C$ values of CH_4 emissions and porewater CH_4 values in minerotrophic vs. ombrotrophic wetland are demonstrated in Hornibrook and Bowes (2007) and Hornibrook (2009). Landscape scale measurements (atmospheric inversions and aircraft measurements; Fisher et al., 2017) also show that northern wetlands contain sources of ^{13}C-poor CH_4 that differ from values of ~-62 to -58 permil typically attributed to northern peatlands in isotope-weight CH_4 budgets. Characterization of sites as ombrotrophic or minerotrophic on the basis of water chemistry and vegetation surveys is essential for making these distinctions.

L617 to L622 It is unclear how a more negative than expected value for estimated $\delta^{13}C(CH_4)$ can be explained by (2) source signatures being biased toward more positive $\delta^{13}C$ values.

Ed Hornibrook
24 December 2020

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