

Response to Matthias Sprenger (Referee #1)

We would like to thank Matthias Sprenger for taking the time to review the manuscript. We have reproduced his comments, in blue, along with our responses in below.

To my understanding, the non-destructive soil water $\delta^{18}\text{O}$ estimations would be either limited to an integrated signal (no depth information) or would need to be conducted with in-situ devices sampling the soil vapor. Thus, the title is a bit misleading as one is often interested in the depth information of the soil water isotope composition. I think that this lack of depth information should be also discussed in the manuscript.

We agree with this comment, the value of soil water $\delta^{18}\text{O}$ estimated does indeed reflect an integrated signal. In addition the depth to which this signal is integrated is hard to define because it is a function of the effective rate of diffusion which controls the residence time of CO_2 in the soil profile and the rate of hydration which acts to impart the soil water signature on the CO_2 . In the case of this study, conducted on shallow soil microcosms with minimal heterogeneity in the soil water content and isotopic composition, the signal likely reflects the influence of the total soil column. We have altered the title to clarify that we refer to the soil water composition associated with hydration of CO_2 and the atmospheric signal rather than propose a sensible approach to non-destructively obtain depth-resolved soil water profile data. We have also expanded these points in the text, as detailed below.

Title “*Non-destructive estimates of soil carbonic anhydrase activity and associated soil water oxygen isotope composition*”

P4 L4 “*The appropriate value for δ_{eq} is then conceptually related to the shallowest depth at which respired or atmospheric CO_2 has sufficient time to fully equilibrate with soil water (Miller et al., 1999; Wingate et al., 2009). For example, Wingate et al. (2009) estimate this depth as the soil depth below which CO^{18}O molecules would take more than 4 times longer to diffuse out of the soil than it would take them to re-equilibrate with soil water. However, whilst use of this setting-point is a convenient approximation in field settings (Wingate et al., 2009, 2010), some degree of exchange still occurs above this depth (Kapiluto et al., 2007).*”

P14 L20 “*Given the relatively constant profile of $\delta_{sw,ce}$ with depth (Fig 3) and the fact that total soil depth (z_{max}) was shallower than that required for full convergence between the semi-infinite and finite soil depth model solutions (Table 3, Fig S2), the estimates of $\delta_{sw,eq}$ reported likely reflect the influence of interaction between CO_2 and soil water across the total soil depth (Kapiluto et al., 2007).*”

I do not agree with the interpretation of Figure 5 that $\delta_{sm,eq}$ is in equilibrium with waters in hygroscopic water (see P14 L31). Given that the difference between $\delta_{sm,eq}$ and $\delta_{sm,ce}$ is smallest for wettest soils reveals the opposite: The wetter the soil, the smaller is the ratio between volumes of soil water in soil pores and volume of waters in soil pores plus hygroscopic waters. If equilibration would preferably take place with the hygroscopic water, the differences should be highest for wetter soil, as the hygroscopic water would become small relative to the bulk pore water volume (Figure 1).

We understand and agree with the point made that essentially highlights some deficiency in our explanation. Whilst CO_2 appears to be heavily influenced by hygroscopic water in the main experimental tests (conducted at about 20 % WFPS), it is also clear that the proportion of non-hygroscopic to hygroscopic water that CO_2 has to interact with increases with water content (Figure 1 in the reviewer comment; future readers please note the y-axis in the lower panel, as drawn, should be V_h/V_{nh} rather than V_{nh}/V_h). This occurs because as water content increases, non-hygroscopic water occupies more pore space that CO_2 must diffuse through, thus undergoing further hydration and equilibration with this more mobile pool of water. This results in a better agreement between the signal imparted on the CO_2 and that of the bulk soil water. We have now clarified this point in the discussion.

P15 L19-L27: “*However this requires us to consider that CO_2 is being heavily influenced by exchange with hygroscopic water under our experimental conditions. Such interaction between CO_2 and hygroscopic water may be plausible as this is where microbial communities expressing CA are likely to be present and active. If interaction with hygroscopic water were the cause of this observation, we should expect to see a smaller offset between $\delta_{sw,eq}$ and $\delta_{sw,ce}$ at higher water content because, as water content increases, so does the proportion of non-hygroscopic to hygroscopic water that CO_2 interacts with during the slow process of liquid phase diffusion (4 orders of magnitude lower than gas phase diffusion). We estimated that, even at the uncatalysed rate of hydration, CO_2 molecules would be fully equilibrated if they had to diffuse through about 0.5 mm of water. Whilst this is not realistic for water films adsorbed onto pore-surfaces, such path-lengths are plausible for filled capillaries as the soil-pore network approaches saturation (Lebeau and Konrad, 2010; Tokunaga, 2011; Tuller and Or, 2001).*”

Lebeau, M. and Konrad, J.-M.: A new capillary and thin film flow model for predicting the hydraulic conductivity of

unsaturated porous media, *Water Resources Research*, 46(12), W12554, doi:10.1029/2010WR009092, 2010.

Tokunaga, T. K.: Physicochemical controls on adsorbed water film thickness in unsaturated geological media, *Water Resources Research*, 47(8), W08514, doi:10.1029/2011WR010676, 2011.

Tuller, M. and Or, D.: Hydraulic conductivity of variably saturated porous media: Film and corner flow in angular pore space, *Water Resources Research*, 37(5), 1257–1276, doi:10.1029/2000WR900328, 2001.

P1 L30: As outlined above, I do not understand that conclusion as I would interpret the Figure 5 differently. Thanks, we have altered this in line with the previous comment.

P1 L30: “These offsets suggest that, at least at lower water contents, CO₂-H₂O isotope equilibration primarily occurs with water pools that are bound to particle surfaces, which are expected to be depleted in ¹⁸O compared to bulk soil water.”

P4 L8: I am missing a clear research question here. You present the research gap, but do not state any hypothesis or research question before getting to the objectives in L9ff.

The main research question is whether soil CA activity can reasonably estimated from gas flux measurements in the absence of independent information about soil water isotopic composition. Secondly, we aim to better understand CO₂-H₂O isotope equilibration in soil. This has been clarified in the text.

P4 L9-L16: “Given the need to make an assumption about the soil water pool with which CO₂ is interacting, the potential for spatial and temporal variability of δ_{sw} , and limited a priori information with respect to appropriate sampling resolution and depth (Miller et al., 1999; Riley, 2005), approaches allowing CA activity to be estimated in the absence of this information are desirable.

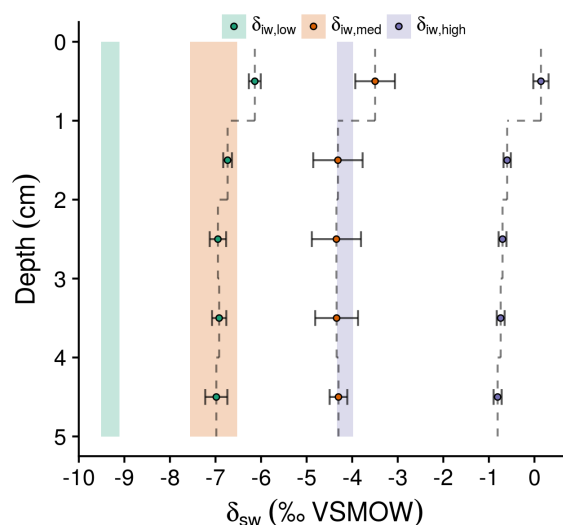
Here we test whether soil CA activity can be reasonably estimated in the absence of independent information about δ_{sw} and investigate assumptions about soil CO₂-H₂O isotope equilibration. To do so we develop a novel approach to obtain solutions for v_{inv} and δ_{eq} , as a function of the response of δ_R to variations in δ_a , from gas flux measurements. ”

P12 L16: I suggest providing statistical tests rather than using “broadly”. Also for the L19 “distinct”.

We have not conducted statistical tests on the slopes and intercepts as these could potentially vary as function of soil properties between incubations e.g. soil depth, bulk density etc. After taking these variances into account we test differences for our terms of interest (Table 3). The words 'broadly' and 'distinct' were chosen to clearly indicate that these visual descriptions of Figure 4 rather than statistical statements.

P12 L31: You do not present $\delta_{sm,eq}$ in the Figure 3. Please add.

We have not plotted the estimates of $\delta_{sw,eq}$ in Figure 3, as these reflect an integrated signal with depth and also make the plot harder to read (see Figure 3 MS below). Estimates of $\delta_{sw,eq}$ are provided in Table 3.



“Figure 3 MS: Depth profiles of the $\delta^{18}O$ of soil water (δ_{sw}). Points and error bars indicate mean and standard

deviation δ_{sw} determined following cryogenic extraction of water ($\delta_{sw,ce}$) from incubated soils, at intervals of 0-1, 1-2, 2-3, and 4-5 cm below the surface. Shaded areas indicate mean and standard deviation δ_{sw} determined to be in equilibrium with CO_2 ($\delta_{sw,eq}$) from gas flux measurements. Colours indicate the three different irrigation water $\delta^{18}O$ (δ_{iw}) treatments.”

P14 L14: I do not like “immobile” water pool and encourage to use a different term, as the soil water held at low pressure heads is less mobile, but not stagnant. However, I know that this is widely used and common nomenclature is missing. Maybe “less mobile” or “water at lower pressure heads”? Or instead “mobile and immobile” using “bulk soil water”?

We agree that the terminology “mobile” and “immobile” water is too strong and misleading. We replaced it here with the terms macro-pore and micro-pore to better reflect the differences between relatively free and bound water pools.

P14 L30: “Differences in the water pools characterised by different methodologies for determining the isotopic composition of soil waters are well known, with the cryogenic extraction method being expected to remove macro-pore, micro-pore, hygroscopic and potentially crystalline water, whilst the static equilibration of soils with CO_2 is expected to principally reflect only the macro-pore and micro-pore pools (Hsieh et al., 1998b; Orłowski et al., 2016b; Sprenger et al., 2015).”

P15 L2-L6: This reads more like results and introducing a new figure would also better fit to the results section.

Classically, this is true. However, as these measurements were conducted post-hoc to test the explanation proposed in the discussion, we feel that the current placement better reflects the development of the work.

P15 L7: You do not have a data point at 95% water filled pore space. Therefore, I prefer you refer here to 75%.

We have amended the figure to the range of data shown (see below) and altered the text accordingly.

P15 L30: “The fact that these relationships indicate the offset decreases at higher water contents may indeed support the inference that estimates of $\delta_{sw,eq}$ are being influenced by fractionation between surface and bulk water pools.”

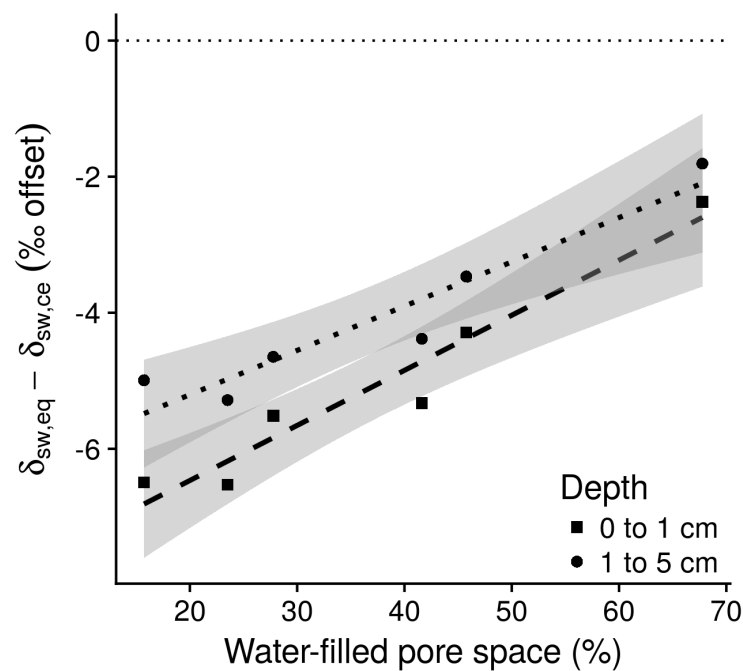


Table 2: Be consistent with the decimal places for the delta-values.

Done, thanks.

Table 3: In the 5th column, it should be “ã” not “Ã”

Done, thanks.

Figure 2: Is the dotted grey line showing the measurements at 1Hz and the dots, diamonds and triangles are showing the average values integrated over time?

The symbols indicate corrected average values. The uncorrected 1 Hz data is not plotted as it is difficult to coherently combine corrected and uncorrected values on the same plot. The dashed line is provided as a visual aid for sequence

order. We have amended the caption to clarify this point.

“Figure 2: An example of the gas exchange measurement sequence, scanning sequentially calibration cylinders, the chamber line during a stabilisation period, calibration cylinders again, and finally the chamber and bypass lines, for the three different $\delta^{18}\text{O}$ of CO_2 delivered to the inlet of the incubation system (δ_b). In this case, the δ_b inlet conditions, whose changes are indicated by the vertical dashed lines, started with $\delta_{b,\text{med}}$ and ended with $\delta_{b,\text{low}}$. Symbols represent the calibrated average values and the dotted line is provided as a visual aid and does not correspond to raw 1-Hz data, (a) total CO_2 concentration and, (b) $\delta^{18}\text{O}$ of CO_2 .”

Figure 3: Consider adding the $\delta_{\text{sm,eq}}$ as you refer to that in the manuscript.

See above (P12 L31 comment)