

Response to Anonymous Referee #2*

*Extracts from reviewer's original comments are indicated in *blue italic*

*Extracts from our original manuscript are indicated in *black italic*

*Proposed modification on our original manuscript are indicated in ***black bold italic***

The paper “The role of soil pH on soil carbonic anhydrase activity” by Sauze, Jones, Wingate, Wohl, and Ogée explores the role of pH on soil carbonic anhydrase (CA) activity by combining a novel experimental setup with a rigorous model framework. The authors are thereby able to compare measured rates of oxygen isotope exchange and CO₂ hydration, and their response to pH, versus theoretical expectations. The results of this study confirm in many cases the mechanistic understanding of the role of pH on soil CA activity. In the process, the authors reveal the potential role of soil complexity on the bulk behaviour of soils including heterogeneous distributions of water content, temperature, porosity, enzymes concentrations, and respiration rates. Using their model framework, Sauze et al. are able to evaluate some of these sources of variability and inform critical and current discussions in soil science, such as whether distinct isotopic pools of water exist in soils. This manuscript thus makes important contributions to the study of the role of soil CA activity and its pH dependence and to a broader body of research in soil science.

We are pleased that referee #2 appreciated the originality and significance of our study.

P2L3: This sentence describing the role of the terrestrial biosphere in compensating for anthropogenic CO₂ emissions is difficult to understand, and should be clarified.

We agree that the sentence was a bit long and we simplified and shortened it:

*The terrestrial biosphere currently **mitigates** about 25% of anthropogenic CO₂ emissions as a result of a small disequilibrium **between two large gross CO₂ fluxes, photosynthetic CO₂ uptake and respiratory CO₂ release** (Le Quéré et al., 2015).*

P2L14: Is this the correct reference for direct CO₂ measurements?

We meant “estimate” gross CO₂ fluxes, as they currently cannot be “measured” at scales above the organ or plot level. We changed the sentence and also added two extra references:

*(...) as it is difficult to **estimate** gross CO₂ fluxes directly (Beer et al., 2010; Wingate et al., 2009, 2010).*

P2L33: Variations in soil properties affecting diffusion rates would also be important, and could be mentioned.

We added this idea:

*Thus variations in soil CA activity **and** CO₂ diffusion rates dictate the shallowest depth*

where full isotopic equilibration between CO_2 and water can occur.

P3L14: More fitting would be to suggest that a direct link between the activity of at least some CA in soils and soil pH should exist because the case was just made that the intracellular CA may not experience environmental pH fluctuations.

We modified the sentence accordingly:

Thus a direct link between (**at least a fraction of**) soil CA activity and soil pH should exist.

P3L22: This is an important point regarding the mode that CA enhancement has been reported in the past. The point would be more effective by clarifying the sentence more. For example, the 'enhancement factor' is not defined before its first mention in line 21 making it difficult for the reader to know how it is different from the uncatalyzed rate mentioned.

We tried to clarify the sentence by explaining a bit more how the enhancement factor was defined previously and how we propose it should be defined from now on:

This is because soil CA activities are often reported as **an enhancement factor** relative to an un-catalysed CO_2 - H_2O isotopic exchange rate, assumed equal to ca. 0.012 s^{-1} at 25°C (Miller et al., 1999). However, because soil pH governs the speciation of CO_2 between the different carbonate forms, with dissolved CO_2 being predominant only in acidic environments ($pH < 6$), the **true un-catalysed rate** ($k_{iso,uncat}$) is not the same for all soils and is strongly reduced in alkaline conditions (Mills and Urey, 1940; Uchikawa and Zeebe, 2012). Thus for the same soil CA activity – or more precisely for the same soil CO_2 -water isotopic exchange rate (k_{iso}) – the enhancement factor **should rather be defined** relative to the true un-catalysed rate ($k_{iso}/k_{iso,uncat}$) and would **then** be much greater in alkaline soils than in acidic ones.

P7L32: What is the meaning of spatially-averaged here? Does this just mean that the kinetic parameters are average values for the volume or mass of soil, or should spatially averaged refer to something more specific? If so, would be good to clarify.

We replaced the term “spatially-averaged” by “community-averaged” to be more specific about the type of averaging.

P10L34: 16S and 18S rRNA or rDNA gene copies. No detectable difference in these gene copies does show no significant change in community structure in response to CA addition, but it does not necessarily mean that native CO_2 hydration rates were un-changed because microbial communities may have modulated their CA gene expression and enzyme production rates, and thus native CO_2 hydration rates, in response to the availability and activity of exogenous alpha CA.

We analysed rDNA gene copies, not rRNA, and this is now clarified in the text. We agree that an unchanged community structure does not necessarily translate into no change in CA

activity in response to exogenous CA addition. We thus introduced this possible caveat into our discussion and proposed it as a possible explanation of the reported discrepancies between observed and predicted Δk_h :

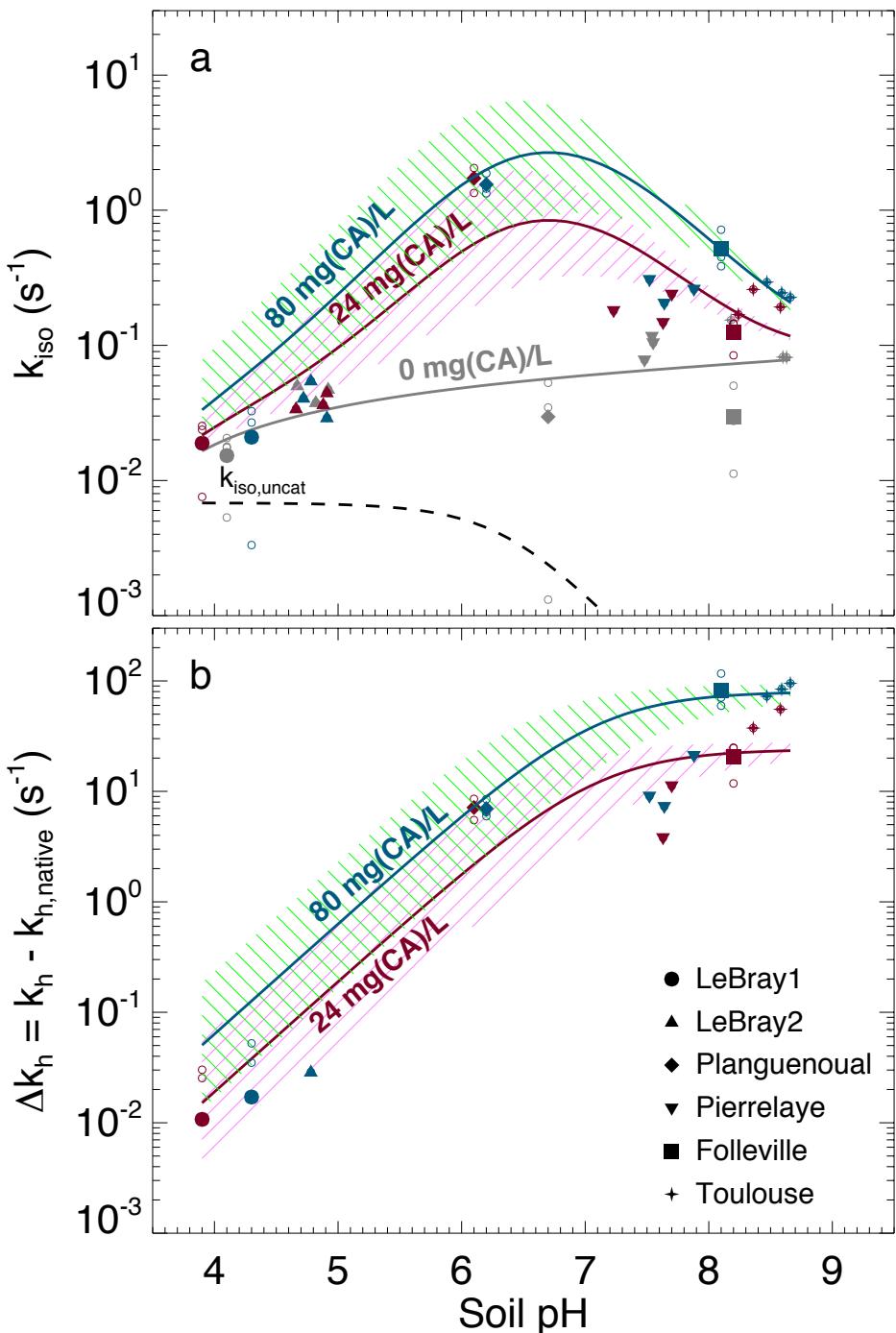
*This approach could have introduced a possible bias in our calculations of Δk_h if the native hydration rates were markedly different between soils with and without CA addition, i.e., if the addition of water with exogenous CA over the 12h-24h prior to our gas exchange measurements was enough to induce changes in microbial growth and diversity **and/or their CA gene expression** compared to soils where only water was added. We estimated the bacterial and fungal abundance using qPCR for some of our microcosms and could not find any clear trend in the number of 16S and 18S DNA gene copies with the amount of exogenous CA added to the soil (not shown). These results suggest that, within the timeframe of our experiment, exogenous CA addition did not affect the community structure. However, conservation of the community structure does not necessarily translate into conservation of the native CO₂ hydration rate as microbial communities may have modulated their CA gene expression in response to the availability and activity of exogenous CA. Actually, the observed values of Δk_h were not always consistent with those predicted for three of the soils (LeBray2, Pierrelaye and Planguenoual), which may indicate changes in native CO₂ hydration rates with exogenous CA addition, that would have biased our Δk_h estimations. Another possible reason for these discrepancies between observed and predicted Δk_h ...*

P11L36: Results for these model results should be given, even if just summarized briefly as a % change from steady-state conditions. Also for P12L16. Could a figure for just one site be added to illustrate the difference between steady and non steady state?

We added the results of the non-steady state simulations on all the soils in the form of a Supplementary figure:

*Surprisingly, the results from this numerical model differed only marginally from those shown in Fig. 5 and Fig. 6 (see **Supplementary Material Fig. S1**).*

Figure S1: same as Fig. 6 but with k_{iso} values retrieved from the non-steady state model as described in the main text.



P12L1: Reader should be pointed to Table 1 to look for phosphate concentrations.

We added a reference to the table:

Another factor that could explain the deviation of Δk_h from theory is the presence of phosphate ions in the soil solution (Table 1) that could either activate or inhibit CA compared to its activity in the absence of such anions (Rowlett et al., 1991; Rusconi et al., 2004).

P13L1-16: Interesting results and informative discussion

Thanks!

Table 1: citations for literature data should be provided

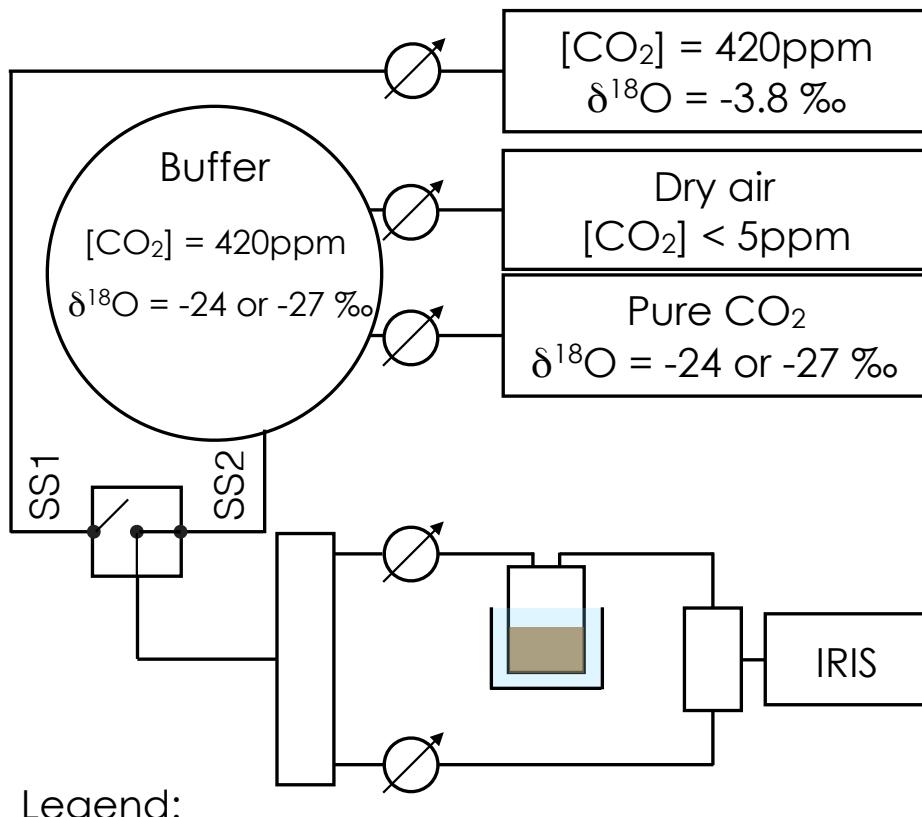
References have been added in the Table 1 caption.

Table 1: main characteristics of the soils investigated in this study. Numbers in italics indicate literature data (Achat et al. 2014).

Figure 1: the ‘automatic trigger’ terminology seems a bit odd if the text just calls the component a 3-way valve

Figure legend changed, with “3-way valve” instead of “automatic trigger”.

Figure 1: Schematic of the experimental setup used to estimate simultaneously the CO_2 - H_2O isotope exchange rate (k_{iso}) in a soil microcosm and the oxygen isotopic composition of the soil water pool with which the CO_2 equilibrates (δ_{sw-eq}). The soil microcosm consists of 280–300 g of dry soil previously re-humidified to 25% of the water holding capacity using mineral water containing different amounts of exogenous CA powder. The soil column is thermally regulated using a 6.5L water bath and the air entering the chamber is a mixture of CO_2 in dry air whose oxygen isotopic composition is alternatively enriched (steady state 1, -3.8‰ VPDBg) and depleted (steady state 2, between -24‰ and -27‰ VPDBg, depending on the experiment).



Legend:

- mass flow controller (MFC)
- 3-way valve
- manifold
- soil
- water bath

Figure 3: What is the basis for the expectation that beta CAs are the most abundant in soils? Provide citation or justification.

We changed the figure caption slightly and also added a reference:

These theoretical curves have been obtained using **the un-catalysed rate formula compiled in Uchikawa and Zeebe (2012)** and enzymatic parameters of $k_{cat}/K_M = 70 \text{ s}^{-1} \mu\text{M}^{-1}$ and $pK_a = 7$, which are typical values for CA-catalysed CO_2 hydration (Rowlett et al. 2002; Smith & Ferry 2000).

Figure 4: It is not clear which lines and points in Figure 4 correspond with LeBrayI soil versus an aCA addition of 24 mg L-1, which are both stated in the caption. If the aCA data were plotted for some soils, wouldn't the kiso values be different? If they are not significantly different, as suggested in Fig 6, a justification for plotting results from the no-addition and addition should be given because that reasoning is not clear at the beginning of the results

section. Would it be worthwhile switching the order of 3.1 and 3.2 or referencing 3.2 as justification?

Section 3.1 is required to understand results presented in section 3.2 as it explains, for each soil microcosm and CA treatment, how we were able to retrieve values of k_{iso} and δ_{sw-eq} . We added a sentence in section 3.1 to reinforce the idea that results shown in Fig. 4 are just an example:

This approach, when presented graphically, leads to a plot with up to six curves (2 curves per sequence, see Fig. 4 in the case of LeBray1 with 24mg/L of exogenous CA addition) that intersect at very similar locations within the k_{iso} - δ_{sw-eq} space.

We also modified the figure caption:

Figure 4: The CO_2 - H_2O isotopic exchange rate (k_{iso}) and isotopic composition of soil water equilibrated with CO_2 (δ_{sw}) retrieved using the two-steady-state approach described in the main text (Eqs. 6a and 6b), for one single microcosm (LeBray1 with an α -CA addition of 24 mg L⁻¹). Relationships between k_{iso} and δ_{sw} for steady-state 1 (dotted lines) and steady-state 2 (solid lines) are also shown. In this example the microcosm was measured over 3 consecutive sequences, resulting in 3 curves for each steady state and 3 intersection points that coincide well with the two-steady-state solution for each sequence (black squares).

Figure 5: May be useful to state why plotted without distinction (CA conc shouldn't affect result for water isotopic composition) and restate why CO_2 gas exchange results shift with depth (Eq xx)

Caption of Figure 5 has been amended accordingly:

Figure 5: The isotopic composition of soil water at different depths in the replicated soil microcosms from each site, estimated either by vacuum distillation and water isotope analysis (δ_{sw} , blue squares) or online CO_2 - H_2O isotopic exchange using the two steady-state approach (δ_{sw-eq} , at depth z_{eq} , black circles). Profiles for the different CA treatments are plotted together without distinction (because exogenous CA addition should not affect the isotopic composition of soil water). The blue vertical line also indicates the isotopic composition of the irrigation water used for the re-wetting of the air-dried soils. According to Eq. 11, the addition of exogenous CA shifts the gas exchange results (δ_{sw-eq}) to shallower depths (z_{eq}).

Figure 6: difficult to see diamond points - shift CA concentration labels. Why are some LeBray2 points missing in 6b? What are the open circles representing? State in caption.

Figure has been redrawn with shifted CA concentration labels and the fit to the “native” k_{iso} values has been modified (no extrapolation outside the measured pH range, polynomial fit rather than a spline fit) which led to a smoother “basal” line. The associated caption has also

been changed to:

Figure 6: (a) measured CO_2-H_2O isotopic exchange rates (k_{iso}) in the different soils for different levels of α -CA addition and (b) associated enhancement hydration rates ($k_h - k_{h,native}$) caused by the α -CA addition. In panel a, the un-catalysed isotope exchange rate ($k_{iso,uncat}$, see Uchikawa and Zeebe (2012)) is shown for reference (black dotted curve). The pH dependence of the native isotope exchange rates (grey points in panel a) is interpolated over the entire pH range explored here using a third-order polynomial fit (grey curve in panel a). The range of the theoretical rates above this native rate curve that we would expect from α CA addition of 24mg/L (purple curve and hatched area) and 80mg/L (green curve and hatched area) are also shown and have been obtained using $k_{cat}/K_M = 30 \pm 5 s^{-1} \mu M^{-1}$ and $pK_a = 7.1 \pm 0.5$. For those microcosms that were measured multiple times (several sequences), smaller open symbols are displayed to indicate the results from each individual sequence. In some cases, (e.g. LeBray 2), some points could not be displayed in panel b because the k_{iso} measured after CA addition was smaller than the mean native k_{iso} , resulting in negative Δk_h values (within the measurement uncertainty).

