Response to Anonymous Referee #3[*]

The stable isotope of oxygen ($\delta^{18}O$) is routinely used to characterize ecosystem processes from the cell to the globe (Werner et al., 2011, Biogeosciences). The enzymatic activity of carbonic anhydrase (CA) enhances the exchange of the source water $\delta^{18}O$ signal with CO_2 emitted from points of respiration. We can better constrain estimates of ecosystem metabolism by increasing our knowledge of CA activity under different environmental conditions. Sauze et al. set out to fill these gaps related to soil in our understanding through a series of innovative experiments and modelling. They quantified the isotopic exchange rate (k_{iso}) between H_2O and CO_2 in soils with a range of pH. The authors devised small incubation units to which they could add water of known isotopic composition, known CO_2 gas, and varying amounts of α-CA (28 and 80 mg L^{-1}). By controlling the environment, mainly through maintaining a near constant relative humidity, the authors were able to isolate the impact of soil pH on CA as determined by k_{iso}. They found that the experimental results from three of the soils were congruent with their modelling. For the other three soils that did not conform to the modelling prediction, the authors infer that heterogeneity from the soil column, non-steady state conditions, or phosphate ions may have led to the aberrations.

We are pleased that referee #3 appreciated the originality and significance of our study.

The experiment was carried out well and the analyses were quite thorough, although a few statistical analyses with regards to differences in the isotopic composition of the source water and extracted water are needed.

Referee #3 refers here to results shown in Fig. 5 on the isotopic composition of soil water estimated either from soil water vacuum extraction or from CO_2 gas exchange. We agree that it would be good to perform statistical analyses to test the significance of the difference between the two estimates. However, the two estimates are also depth-dependent which complicates the exercise, notably when CO_2 gas exchange values fall below the deepest point sampled for water extraction (e.g. LeBray1 and LeBray2). It is also not possible to perform such a test on soils where we only had one microcosm per treatment. For the soils that were measured in triplicates for each CA treatment, we tested for significance between the mean ($n = 3$) δ_{sw-eq} values and the mean ($n = 3$) δ_{sw} values measured over the entire soil column and weighted by soil moisture content. It turns out that all the soils tested present a significant difference between δ_{sw} and δ_{sw-eq}. These statistical test results are now gathered into a new table (Table S2).

Soil name	CA treatment	$\langle\delta_{sw}\rangle$	$\langle\delta_{sw\text{-}eq}\rangle$	n	t-test
Le Bray 1	0	-11.97	-7.31	1	-
Le Bray 1	24	-11.24	-6.88	1	-
Le Bray 1	80	-9.57	-7.59	1	-
Le Bray 2	0	-13.56 [a]	-8.79 [b]	3	$P < 0.05$
Le Bray 2	24	-13.31	-8.95	3	$P < 0.05$
Le Bray 2	80	-13.35	-9.41	3	$P < 0.05$
Planguenoual	0	-7.95	-7.31	1	-
Planguenoual	24	-5.50	-7.83	1	-
Planguenoual	80	-5.97	-6.40	1	-
Pierrelaye	0	-11.88	-9.48	3	$P < 0.05$
Pierrelaye	24	-11.40	-9.73	3	$P < 0.05$
Pierrelaye	80	-10.97	-9.61	3	$P < 0.05$
Folleville	0	-8.31	-7.87	1	-
Folleville	24	-6.58	-7.59	1	-
Folleville	80	-6.95	-8.11	1	-
Toulouse	0	-11.03	-9.68	3	$P < 0.05$
Toulouse	24	-10.90	-9.57	3	$P < 0.05$
Toulouse	80	-10.71	-9.68	3	$P < 0.05$

Table S2: Mean δ_{sw} measured over the entire soil column and weighted by soil moisture content and corresponding mean $\delta_{sw\text{-}eq}$ for each soil and CA treatment. For LeBray1, Planguenoual and Folleville, one single microcosm was measured over three consecutive gas-exchange sequence, which did not allow us to test for significance differences between the two means. For the other soils, three different microcosms were measured for each treatment, and care was taken to maintain a relatively homogeneous soil water isotopic composition (Fig. 5) so that statistical tests for significant differences could be performed using the open-source software R v.3.3.1 (R Core Team, 2015).

This table is also referred to in the text at the beginning of section 4.2.

*Our results also revealed large differences between the isotopic composition of the water pool "seen" by the CO_2 ($\delta_{sw\text{-}eq}$) and that of cryogenically extracted soil water (δ_{sw}), with significantly ($P < 0.05$) more depleted $\delta_{sw\text{-}eq}$ values compared to δ_{sw} (Fig. 5 **and Table S2**). Interestingly very similar "offsets" between δ_{sw} and $\delta_{sw,eq}$ were also predicted by the numerical model (not shown), except for LeBray1 where even larger offsets were found. For a given soil the offset did not seem to vary with soil CA activity (i.e. the difference between δ_{sw} and $\delta_{sw\text{-}eq}$ was the same for soils with and without CA addition**, see Table S2**) and, at least for the only soil tested, did not seem to be affected by **small changes in** soil water content (similar offsets were observed between LeBray1 and LeBray2). However, in-between the different soils, it seemed that those with the highest CA activity (Planguenoual, Folleville) also had the smallest offset **(Table S2)**. **Also, for LeBray soil, Jones et al. (2017) showed that the offset between δ_{sw} and $\delta_{sw,eq}$ decreased when the soil was approaching saturation.***

The manuscript needs a better description of the experiments performed and the hypotheses/questions asked of each steady-state iteration. I suggest a simple table or figure will suffice. This will let the reader keep better track of 1) some of the terminology used to describe different parameters (e.g., native, non-native), 2) sample size, and the results in general.

The distinction between native and non-native is now clearly defined in the text (see comment below related to page 4 line 30). We also modified the caption of Figure 1 to re-precise sample size:

*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).*

In the introduction the real-world differences between α- and β-CA should be discussed. Furthermore, the use of α-CA can be defended more strongly upfront rather than toward the conclusion of the manuscript.

We added a couple of sentences in the introduction to discuss differences between α- and β-CA:

*Changes in the abundance and diversity of soil microbial communities were proposed as possible drivers of the observed spatial and temporal changes in soil CA activity (Seibt et al., 2006; Wingate et al., 2008, 2009, 2010). In particular, soil pH is known to strongly influence microbial community composition, richness and diversity (Fierer and Jackson, 2006; Griffiths et al., 2011; Hartman et al., 2008; Lauber et al., 2009) and could thus influence soil CA activity indirectly via changes in the microbial populations **that would translate into differences in CA requirements and in the expression of classes of CA with different enzymatic efficiencies**. **Indeed, α- and β-CA classes are not represented equally in all kingdoms. Very schematically, α-CAs tend to be more abundant in algae and micro-algae while β-CAs are more commonly found in fungi (Elleuche & Poggler 2010; Moroney et al., 2001). In addition, α-CAs can be extracellular enzymes unlike β-CAs that are, to our knowledge, only intracellular enzymes.***

The choice of using α-CA for our CA addition treatment is also defended more strongly in the introduction (page 3 line 35):

*"This CA isoform was chosen because it is well characterised in terms of enzymatic activity (Uchikawa and Zeebe, 2012) and pH response (Rowlett et al., 1991****) and it has been demonstrated that its activity was very stable in time even after several hours in solution (Uchikawa and Zeebe 2012)****."*

I also am surprised by the differences in $\delta^{18}O$ between the soil extracted and equilibrated water values. This is important evidence in the on-going debate of the two water world hypotheses (Kirchner, James W. "A double paradox in catchment hydrology and geochemistry." Hydrological Processes 17, no. 4 (2003): 871-874.; McDonnell, J. J. (2014), The two water worlds hypothesis: ecohydrological separation of water between streams and trees?. WIREs Water, 1: 323–329.; Sprenger, M., H. Leistert, K. Gimbel, and M. Weiler (2016), Illuminating hydrological processes at the soil-vegetation-atmosphere interface with water stable isotopes, Rev. Geophys., 54, 674–704.Vargas, A. I., Schaffer, B., Yuhong, L. and Sternberg, L. d. S. L. (2017), Testing plant use of mobile vs immobile soil water sources using stable isotope experiments. New Phytologist, 215: 582–594.).

Initially, we did not want to put too much emphasis on these reported $\delta^{18}O$ differences because our study was mostly focusing on the pH response of the CO_2-H_2O isotopic exchange rate k_{iso}. We agree however that this "side" result on $\delta^{18}O$ is also interesting and we devoted in the end half of the discussion to this finding. In response to a previous comment we have now included a new table (Table S2) reporting results for significant differences between the two $\delta^{18}O$ estimates. We believe it is enough "publicity" for this finding. We decided not to refer to the on-going debate on the "two water world hypothesis" because this would bring too much diversion and also raise some unanswered questions (does gaseous CO_2 "visit" the same soil water pool as the one tapped by plant roots during water uptake?).

Page 1 Line 12: delete "nonetheless"

It is important to tell the reader this lack of understanding is currently a weakness of the ^{18}O methodology to partition gross CO_2 fluxes.

Page 2 Line 3: Biosphere "absorbs" - unconventional way of describing flux and pools

We replaced "absorbs" by "mitigates":

*The terrestrial biosphere currently **mitigates** about 25% of anthropogenic CO_2 emissions as a result of a small disequilibrium **between two large gross CO_2 fluxes, photosynthetic CO_2 uptake and respiratory CO_2 release** (Le Quéré et al., 2015).*

Page 2 Line 17: delete "advanced over recent years" given the literature list dating back to 1997 (and earlier?), the method is established.

Done.

Page 2 Line 37: perhaps simple instead of crude.

Done.

Page 2 Line 40: since you introduce these values can you report the accepted global photosynthesis rates?

This is now done:

*Subsequently, using the lower range of soil CA activity estimates made by Wingate et al. (2009), an atmospheric $CO^{18}O$ inversion was performed and led to a rate of global photosynthesis of ca. 175 GtC yr^{-1} over the period 1980-2010 (Welp et al., 2011), a **surprisingly high value compared to the accepted global estimate of 115-130 GtC yr^{-1} (Beer et al., 2010; IPCC 2013)**.*

Page 3 Line 4: You introduce microbial communities as a possible explanation, but not how the microbes might alter CA activity. I assume it might have something to do with total biomass and functional characteristics of the communities.

We have clarified this point now by adding two extra sentences referring to the different classes of CA, their abundance in different kingdoms and their differences in intrinsic activities (i.e. enzymatic parameters). See general comment above about the introduction.

Page 3 Line 18: please provide a citation for this value.

We added a reference to the text:

*This is because soil CA activities are often reported relative to the un-catalysed CO_2-H_2O isotopic exchange rate ($k_{iso,uncat}$), usually assumed equal to ca. 0.012 s^{-1} at 25°C **(Miller et al., 1999)**.*

Page 3 Line 23: I think you mean soil solution here. Perhaps specify in your hypotheses that you are primarily interested in the direct effects of pH and not the indirect effects anticipated from shifts in microbial diversity and function.

Indeed we meant to refer to the chemical composition of the soil "solution". We also modified the hypothesis to specify that we primarily looked at soil pH because of its (direct) role on CO_2 speciation:

*The chemical composition of the soil **solution** is another potentially important factor that should be considered when reporting soil CA activity. [...]*
***Because of the direct role of pH on CO_2 speciation and CO_2 hydration rate**, we hypothesised that exogenous CA activity should be inhibited in acidic soils, but that the native soil phosphate concentration might also influence the activity of CA for soils differing in pH.*

Page 4 Line 30: I suggest to introduce the "native" or control term here and please explain the situation to which you arrived at estimates of "un-catalysed rates" (page 9 line 28).

We now clarified in the text what the terms "native" and "non-native" mean:

CA activities from soil microcosms without CA powder addition are qualified hereafter as "native" and CA activities related to the CA addition are called "non-native" and estimated, for a given soil, as the difference between the activities on the CA-added microcosms and their native rates.
We theoretically estimated the uncatalysed rate depending on temperature and pH and simply

compared our measured isotopic exchange rates compared to the theory. A reference to the theoretical calculation (Uchikawa and Zeebe 2012) is now given.

We chose these CA concentrations because they should correspond to the upper range of CA concentrations in natural soils (Ogée et al. 2016). This is now explained in the text:

*For a set of gas exchange measurements, lyophilised α-CA powder from bovine erythrocytes (C3934-100MG, Sigma-Aldrich, France) was diluted into the irrigation water. For each set of experiments, CA concentrations of ca. 24 and 80 mg L^{-1} were used. **We chose these concentrations because they would correspond to the upper range of CA concentrations expected in natural soils, assuming a cytoplasmic CA concentration of 0.1mM (Ogée et al. 2016)***.

We modified the sentence as follows:

*Apart from this addition of CA into the irrigation water, all other **preparation** steps **of the soil microcosms** were kept identical **to the ones** described above for **the microcosms** without CA addition.*

This is now done:

*Prior to gas exchange measurements, each soil pot was **closed using a screw-tight lid connected to inlet and outlet tubes (Fig. 1) and immerged into a 6.5L water bath, thermally-regulated at 20°C**. An acclimation time of at least 20 minutes was used to allow the soil column to re-equilibrate to the new air supply CO_2 composition **and the new temperature**. The soil CO_2 efflux and its oxygen isotopic composition were then measured using the experimental setup illustrated in Fig. 1.*

We now explain in more details why we used different $\delta^{18}O$ composition for the two air supplies corresponding to the two steady states (SS1 and SS2) and how we did it:

*To simultaneously retrieve soil CA activity, reported here as the CO_2-H_2O isotopic exchange rate k_{iso}, and the $\delta^{18}O$ of the soil water pools with which CO_2 equilibrates (δ_{sw-eq}), we designed a system that allowed us to measure CO_2 isotope fluxes under two, quasi-simultaneous isotopic steady states that only differ in the isotopic composition of the CO_2 entering the soil chamber (Fig. 1). The air supplied to the chamber came directly from a **tank containing dry** air during the*

first steady state (SS1) and from a mix of dry, CO₂-free air and a tank of pure CO₂ during the second steady state (SS2). **In practice, the air was supplied to the microcosms during SS2 using a compressor (FM2 Atlas Copto, Nacka, Sweden), coupled to a chemical scrub column (Ecodry K-MT6, Parker Hannifin, Cleveland, OH, US) that removed water vapour and CO_2 from the air before being mixed with pure CO_2, with a $\delta^{18}O$ isotopic compositions significantly different from the CO_2-in-air mixture used in SS1.** *During SS2 mixing valves adjusted the CO_2 concentration of the inlet air to maintain it close to the value of the inlet air used in SS1 within acceptable error (423 ± 5 ppm), whilst their oxygen isotope compositions differed markedly (Fig. 2). The transition between SS1 and SS2 was operated by means of a three-way valve (Fig. 1) and a transition period of 20 minutes was necessary to attain the new steady state (Fig. 2).*

Page 5 Line 24: each is singular in this case: "Each line was measured", "only the last 40s of measurement was averaged"

Corrected:

To minimise carry-over effects caused by this residence time, each line (inlet or chamber air or calibration tanks) **was** *measured for 2 minutes and only the last 40 s of measurements* **were** *averaged to provide a single mean and standard deviation.*

Page 5 Line 29: do you mean over the measurement time? i.e., the measurement period?

The measurement and mean and standard deviation calculations on the calibration tank are the same as for the inlet or chamber air *i.e.* we measured them 2 minutes and used only the last 40 s for our calculations. This is now clarified in the text:

Calibration tank mixing ratios for the different isotopologues ($^{12}CO_2$, $^{13}CO_2$ and $CO^{18}O$) were averaged as described above **(2 minutes of measurement and only the last 40 s were averaged)** *and interpolated in time using a spline function.*

Page 6 Line 1: what do you mean precisely by "were propagated"?

We meant "mathematically propagated". We changed the wording, with the hope that the way we reported measurement errors is now clearer:

Standard deviations on CO_2 mixing ratios of the different isotopologues were **used to compute measurement error on total CO_2 concentration**.

Page 6 Line 17: The equation in parentheses is difficult to decipher, perhaps separate it from the text.

We could include this equation into Eq. 4 but we are afraid that it would make the new equation too long and difficult to read. Because the equation in the text is just a definition while the equation we used to compute δ_F is what is written in Eq. 4 we felt more important to emphasize Eq. 4. We thus preferred to keep things as they are.

We added a few word to describe V_{inv}, using the exact wording used by Tans (1998):

*F is the soil CO_2 efflux (µmol m^{-2} s^{-1}) and V_{inv} (m s^{-1}) is the piston velocity **(i.e. the rate at which a column of air gets pushed into the soil; Tans, 1998)**.*

Done.

We now define D_{iso} sooner, i.e. just after Eq. 7:

*...where z_{max} is soil depth and $z_1 = D_{iso}/V_{inv}$ with **$D_{iso} = D_{eff}/(1 - \varepsilon_D)$ and D_{eff} (m^2 s^{-1}) is the effective diffusivity of gaseous CO_2 through the soil matrix (Tans, 1998; Wingate et al., 2010). The latter was computed using the formulation of Moldrup et al. (2003) for repacked soils: $D_{eff} = (\varphi - \theta)^{2.5}/\varphi$ D_0, where φ (m^3 m^{-3}) is total soil porosity and D_0 (m^2 s^{-1}) is the molecular diffusivity of CO_2 in soil air at temperature T_s (K): $D_0 = 1.381$ 10^{-5} $(T_s/273.15)^{1.81}$ (Massman, 1998).** The right-hand side of Eq. 6b was then used to estimate (...).*
The soil CO_2–H_2O isotopic exchange rate (k_{iso}, in s^{-1}) was then derived from the piston velocity according to:

$$k_{iso} = \frac{V_{inv}^2}{D_{iso} B \theta} \qquad\qquad (8)$$

*where B (m^3 m^{-3}) is the solubility coefficient for CO_2 in water (Weiss, 1974) **and** θ (m^3 m^{-3}) is the volumetric soil water content.*

Done.

Changed.

We edited the sentence as follows:

A small water volume (0.2-1.0 µL) from each vial was sampled using a 5-µL syringe (SGE Analytical Science, Ringwood, Australia) and injected through a septum in a vaporiser unit

*maintained at 80°C to **ensure complete vaporisation of the liquid water straight after injection***.

Effectively it is a data filter, we edited the sentence:

*Each vial was then measured eight times in total and only the last five measurements, subject to **data** filtering, were retained and averaged.*

We edited the sentence:

***Based on measurements on the internal standard used for quality check,** the accuracy (**i.e.** the mean absolute difference between calibrated and true $\delta^{18}O$ values) and reproducibility (**i.e.** the standard deviation of these means) **of** our $\delta^{18}O$ measurements were always below 0.15 ‰ and 0.1 ‰ respectively.*

We changed this sentence (also to address reviewer 2's comment) and replaced near-common to very similar:

*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}-$\delta_{sw\text{-}eq}$ space.*

We believe that this comment refers to the following sentence where we talk about estimating k_{iso} and $\delta_{sw\text{-}eq}$ "separately" (as opposed to "graphically"). We realised that the wording was misleading and replaced it by "numerically":

*Combining the two steady states from the same sequence and using the iterative procedure described above, it is also possible to estimate k_{iso} and $\delta_{sw\text{-}eq}$ **numerically**, as **indicated** by the symbols in Fig. 4.*

The word "sequence" refers here to a full sequence of measurement as described in Fig.2. This is now specified in the text (the notion of "sequence" is also better explained earlier in the text, in response to some comments from reviewer 2):

*Combining the two steady states from the same sequence **of measurement (Fig.2)** and using the iterative procedure described above, it is also possible to estimate k_{iso} and $\delta_{sw\text{-}eq}$ separately, as demonstrated by the symbols in Fig. 4.*

Our estimations of $\delta_{sw\text{-}eq}$ were always significantly different from the $\delta^{18}O$ value of the irrigation water and the cryogenically-extracted waters (t-test, $P < 0.05$). The results from these statistical tests have been incorporated into the revised manuscript (a table has also been added in the Supplementary material, see above):

*These estimated values of $\delta_{sw\text{-}eq}$ were **significantly different (P < 0.05) from** the $\delta^{18}O$ of irrigation water (-10.1 ‰VSMOW) **and from the mean, cryogenically-extracted soil water averaged over the entire soil column and weighted by volumetric soil water content** (Fig. 5).*

The term "native" is associated to the soil microcosms where no CA was added (it could also have been called control). This was clarified in section 2.2 and is re-explained again at the very beginning of section 3.2 (see above).

The un-catalysed rate and the referring symbol $k_{iso,uncat}$ is introduced in the introduction (page 3) but we felt the need to recall the symbol and its meaning here to help the reader understand Fig. 6.

This is now done:

*This influence of soil pH on the enhancement of k_h by exogenous CA was anticipated as the k_{cat}/K_M **(appearing in Eq. 10)** is known to be strongly reduced in acidic pH with a pH response of the form (Rowlett et al., 1991):*

We are convinced that Figs. 6a and 6b should stay together as we derived the latter from the former. The optimum in k_{iso} with pH is clearly explained in the text referring to the theoretical curves drawn in Fig. 3 (end of section 2.5 and beginning of section 3.2).

We agree that the title of the subsection was confusing. We changed it to:

*Can we predict the enhancement in soil CA activity **associated with** exogenous CA **addition**?*

Page 10 Line 20: Since this is the first sentence of your discussion, perhaps you can set the reader up for what the topic of discussion is for this paragraph. The term native doesn't appear until page 9 line 28 and I don't think the term non-native is ever defined in the prior text. This exemplifies why a table or figure explaining the experiment will help the reader.

We now define more clearly what we mean by native and non-native rates in the Material and Methods, and the definition of the non-native rate is also re-established in this first sentence of the discussion.

Page 10 Line 28: please explain "native hydration". Is this potential water remaining within the soil from the field or elsewhere that has potentially mixed with the irrigation water?

See comment above.

Page 11 Line 11: this sentence is a little convoluted, it reads at first as if the soil pH is going to have a response when in fact this metric is intrinsic to the soil.

We do not understand this comment. There is no reference to pH in this sentence or any other sentence before and after this passage.

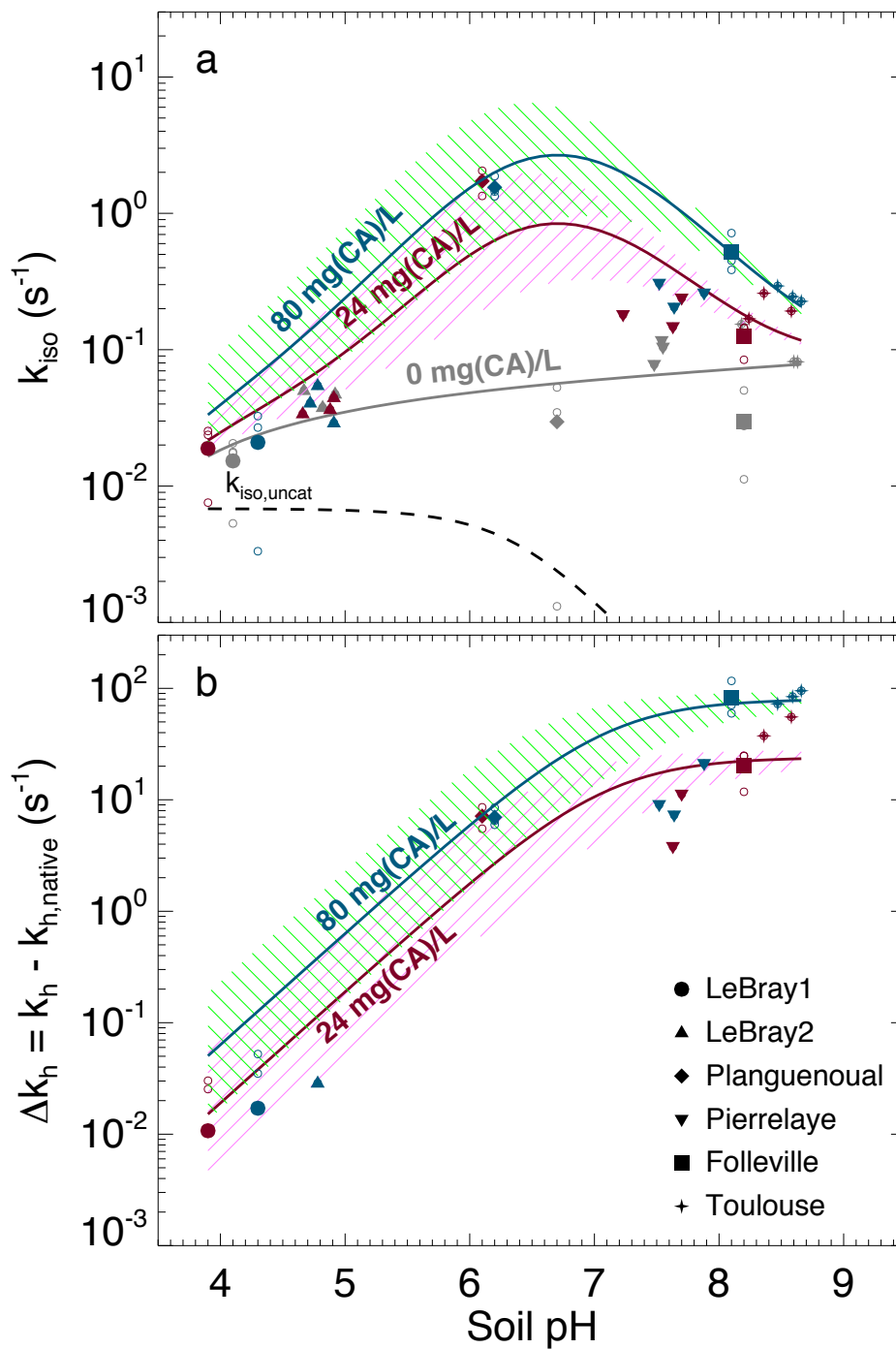*Page 11 Line 22: deviations from non-steady state instead of non-steadiness.*

Changed.

Page 11 Line 26: "was run" or simply "ran"

Corrected.

The check for non-steady state was cursory and not all the assumptions were easily understood. While the exercise is interesting, it is not possible for the reader to determine how robust the results are. I think the authors need to decide how important this issue is to their results and either fully address the issue to the best extent possible or shorten the explanation and report that the non-steady state effect needs to be addressed by further experimentation and modelling.

The results to the non-steady-state simulations are now provided in the Supplementary material.

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

Figure: Plots of (a) k_{iso} (s^{-1}) and (b) $\Delta k_h = k_h - k_{h,native}$ (s^{-1}) versus Soil pH. Curves for 80 mg(CA)/L, 24 mg(CA)/L, 0 mg(CA)/L, and $k_{iso,uncat}$ are shown. Site legend: LeBray1 (●), LeBray2 (▲), Planguenoual (◆), Pierrelaye (▼), Folleville (■), Toulouse (+).

Page 13 - Conclusion: Can you bring out the larger relevance once more? How might the effect of pH influence our estimates of ecosystem carbon balance at different scales?

We added an extra sentence on the relevance of this work in a broader context:

*Our experimental results demonstrate that our two steady-state approach is robust and sensitive enough to detect changes in the CO$_2$-H$_2$O isotope exchange rate when the concentration of CA enzyme in the soil matrix is augmented artificially. We also found that natural variations in soil pH had a strong control over the variability of soil CA activity, with a smaller influence of the phosphate ion concentration, and these variations reassuringly followed similar patterns to those observed in other studies on α-CA activity in buffered solutions. **This is a real advancement in our understanding of the spatial variations of soil CA activity across biomes reported by**

Wingate et al. (2009) and the associated impact on the atmospheric budget of $CO^{18}O$. However, our results should still be taken with caution. Although α-CAs may be present in certain soil microbial communities with a high abundance of phototrophs such as cyanobacteria and micro-algae, the majority of microbial CAs in soils are more likely represented by the β-CA class (Smith and Ferry, 2000).

Figure 2. Please insert the step number within the top or bottom panel. Check the grammar within the caption; for example, I believe you want to say you measured the two calibration bottles in step 1.

Done:

*Figure 2: Typical time-series of the measured CO_2 mixing ratio and isotope composition ($\delta^{18}O$) over the course of a working sequence. The sequence is composed of 7 steps **(indicated in panel b) to successively measure**: (1) two calibration bottles spanning the expected range of CO_2 mixing ratios, (2) inlet and outlet lines of the soil microcosm, measured 4 times consecutively, using a CO_2 with an enriched $\delta^{18}O$ (steady state 1), (3) calibration bottles, (4) the outlet of the chamber during the switch of the air supplying the soil chamber (front), (5) calibration bottles, (6) inlet and outlet lines of the soil chamber, measured 4 times consecutively, using a CO_2 with a depleted $\delta^{18}O$ (steady state 2) and (7) calibration bottles.*
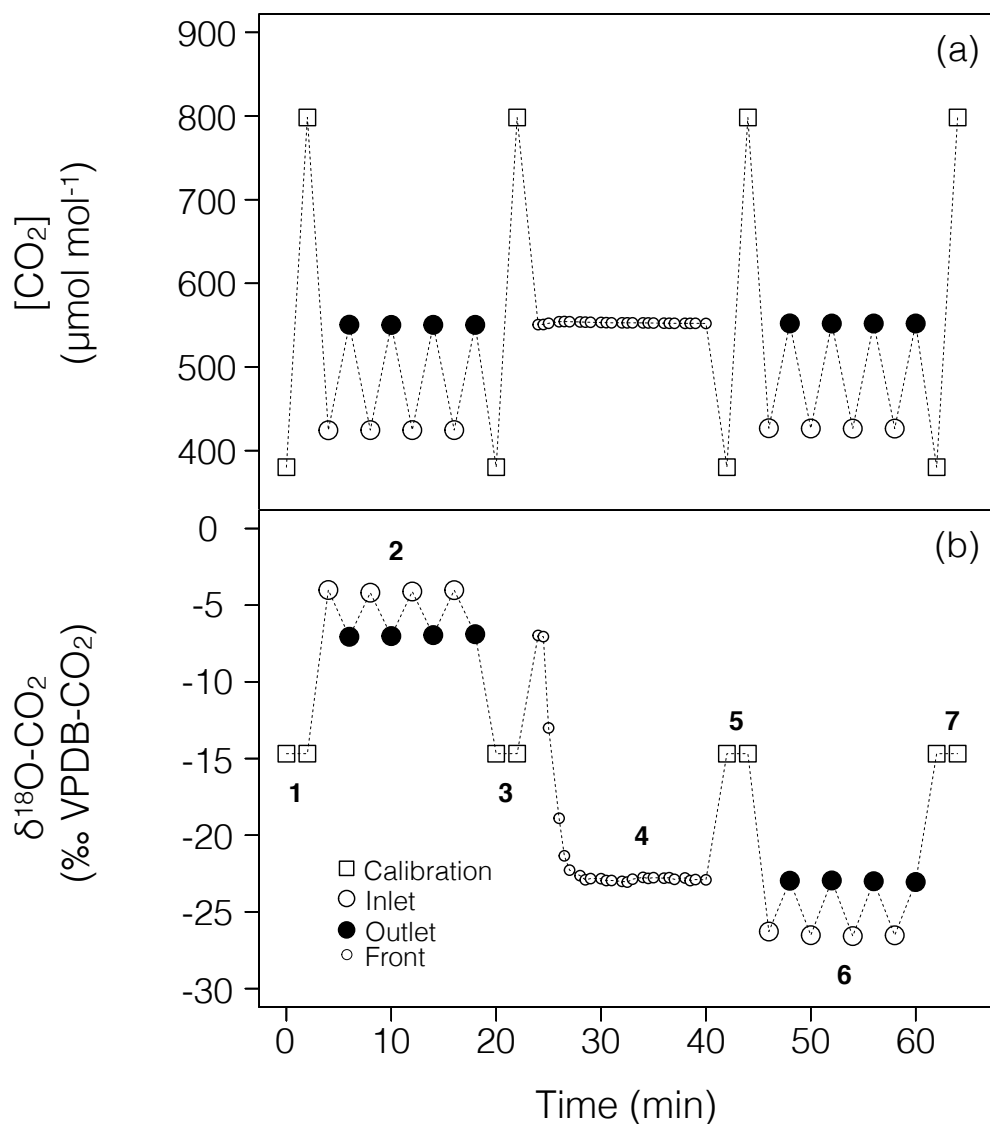
Figure 3. I recommend that the use of a-CA in the experiments is clarified in the figure. Perhaps also how this figure is related to the experimental results.

We added a sentence in the caption to say that using parameter values more typical of α-CA would not change qualitatively the figure. We also gave the reference for the uncatalysed rate calculations:

Figure 3: Theoretical rates of CO_2 hydration (k_h) and CO_2-H_2O oxygen isotope exchange (k_{iso}) as a function of pH, for 3 levels of carbonic anhydrase concentration. These theoretical curves have been obtained using **the un-catalysed rate formula compiled in Uchikawa and Zeebe (2012) and** *enzymatic parameters of $k_{cat}/K_M = 70$ s^{-1} μM^{-1} and $pK_a = 7$, which are typical values* **for CA-catalysed CO_2 hydration (Rowlett et al. 2002; Smith & Ferry 2000). Using enzymatic parameter values more specific to the αCA powder used here for the CA treatment (i.e. $k_{cat}/K_M = 30 \pm 5$ s^{-1} μM^{-1} and $pK_a = 7.1 \pm 0.5$), would not change qualitatively this figure.**

Figure 4. It is probably worthwhile to report the results for all the soils or at least place them in the supplementary. Please reference where the k_{uncat} is presented in the text.

The δ_{sw} values are now provided in a supplementary table and a reference for the k_{uncat} calculations has been added to 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, for LeBray1 soil and an αCA 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 3 sequences were used, resulting in 3 curves for each steady state and 3 intersection points. The pH-dependent, un-catalysed CO_2-H_2O isotopic exchange rate **(Uchikawa and Zeebe 2012)** is also indicated by the grey horizontal line.*

Figure 6. The caption does not reference a or b. In the text, I could not find a reference to a. Please explain what appears to be model uncertainty. How is the fact that three of your soils did not conform to your model reconciled within fig 6?

We clarified this figure caption that clearly lacked a lot of information:

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 $\alpha-CA$ 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} μM^{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).**

A reference to panel 6a is now done in the text (page 9, lines 30-33):

The addition of exogenous CA generally led to higher k_{iso} values compared to the native rates, and also enhanced CO_2 hydration rates k_h, with marked differences depending on the pH range (Fig. 6a).

The discrepancies between some data points shown in panel b and the theory is clearly addressed in the discussion of the paper.