Response to Anonymous Referee #1

We would like to thank the referee for taking the time to review this manuscript. Their comments helped us to greatly improve the manuscript. You will find a point-by-point response to these comments (reproduced in blue) below.

1) My main concern is with the interpretation of the results of ammonium nitrate (NH4NO3) treatment. The authors attributed the decrease of kiso following NH4NO3 addition to the inhibition of carbonic anhydrase caused by NO3-. However, other possible mechanisms, namely, inhibition through increased ammonium content or decreased pH cannot be ruled out by the experimental design, nor by the statistical analysis that follows.

In essence, NH4NO3 addition may affect kiso through these causal pathways:
- NH4NO3 addition → [NH4+] increase → kiso decrease
- NH4NO3 addition → [NH4+] increase → pH decrease → kiso decrease
- NH4NO3 addition → [NO3-] increase → kiso decrease

To accept Hypothesis 3, the authors must show evidence that after controlling for all confounding variables, including pH and [NH4+], there is still a robust decrease of kiso with the increase of [NO3-]. Given the absence of a randomized design and the small sample size (n=14) for NH4NO3 addition treatment, it is difficult to identify [NO3-] as the unique cause for carbonic anhydrase inhibition. One possible solution could be to treat pH and [NH4+] as instrumental variables, but this would require them to show strong correlation with [NO3-]. The best way would be to separate different causes through experimental design.

We agree that the experimental design of the ammonium nitrate treatment is not sufficient to fully tease apart the combined systematic effects (i.e. increased nitrate and ammonium availability and decreased soil pH) of the treatment on k_{iso}. As the reviewer states, a more extensive controlled factorial experiment would be required to achieve this. However, the results of this experiment (Section 3.2; Figure 5; Table S3), that show changes in k_{iso} are most strongly linked to changes in nitrate availability (pathway 3 above) and to a lesser degree soil pH (pathway 2 above) but do not appear related to changes in ammonium availability (pathway 1 above), are still informative to the interpretation of the wider study. Across the untreated soils we clearly identify soil pH, nitrate availability and microbial biomass as explaining variations in k_{iso} (Section 3.1; Figure 3; Table S1). Agreement between the results of both these analyses helps reinforce the importance of pH and nitrate (pathways 2 and 3) but not, directly at least, a role for ammonium (pathway 1). We have adjusted the text in the abstract and Section 4 to acknowledge that limitations of the experimental treatment prevent the definite conclusion that only nitrate, and not some combination of effects, influences the decrease in k_{iso} observed following the fertilisation treatment.

Abstract: "This effect appears to be supported by a supplementary ammonium nitrate fertilisation experiment conducted on a subset of the soils"

Section 4: "It is important to note that whilst the relationship between the changes in k_{iso} and exchangeable NO_3^- are supported by observations from the untreated dataset, the experimental design used in this addition experiment is not sufficient to fully test the influence of the combined changes in soil pH, exchangeable NO_3^-, exchangeable NH_4^+ and microbial biomass on k_{iso}. Further controlled, factorial experiments are needed for this purpose."

2) A minor concern I have is that this study was partially motivated by the use of δ18O of CO2 to estimate terrestrial photosynthesis. While the validity of this method has been demonstrated at the global scale by Welp et al. (2011), I would caution that it is un-clear whether the current in-situ observational network would provide sufficient data to resolve regional-scale photosynthesis. Nevertheless, in my opinion, soil–atmosphere CO2 isotope exchange is an interesting topic for its own sake, regardless of whether δ18O-CO2 can provide constraints on terrestrial photosynthesis with accuracy and spatio-temporal resolution as high as those of other photosynthetic tracers in vogue (e.g., solar-induced chlorophyll fluorescence).

We agree with the reviewer that the current in-situ observational network of $\delta^{18}O$ in atmospheric CO_2 is rather coarse, at least compared to the network for total CO_2 mixing ratio. However, there are still more than 50 atmospheric stations measuring $\delta^{18}O$ in CO_2, spread across all latitudes and continents, with some of them covering several decades of measurements. The extremely large north-south gradient of $\delta^{18}O$ in CO_2 and its seasonal and interannual dynamics brings unique information on the seasonality and inter-annual variability of the northern hemisphere CO_2 sink, which is the strongest land carbon sink at the global scale and with the largest long-term trend (Ciais et al. 2019). Currently, this information is obscured by the lack of understanding of how soil dwelling organisms (and their carbonic anhydrase activity) affect this signal (Wingate et al. 2009). This study presents the largest soil dataset ever gathered on soil $\delta^{18}O$-CO_2 exchange. The in-depth analysis of the drivers of soil carbonic anhydrase activity that this study brings also serves as an important stepping-stone to study other emerging tracers of the carbon cycle including the $\Delta^{17}O$ anomaly in CO_2 (Koren et al. 2019) and COS (Campbell et al., 2017). For all these reasons, it would seem awkward not to mention the implication this study will have in the future development of independent tracers to study the global carbon cycle. We also agree that solar-induced chlorophyll fluorescence (SIF), another independent proxy of photosynthesis, has the advantage of being detected from space, conferring a global coverage. However the relationship between SIF detected from space and land photosynthesis is still not well understood, notably in disentangling structural and physiological factors. For this reason, SIF is most interesting at very high spatial resolution, which is only possible since the late 2010s, with satellite instruments like TROPOMI launched in 2017 or FLEX that will be launched in 2022. Here we do not pretend that $\delta^{18}O-CO_2$ is a more powerful tracer compared to other tracers of global photosynthesis (i.e. SIF or COS), but we are convinced that $\delta^{18}O-CO_2$ contains unique independent and historical information, strongly linked to the global water and carbon cycle, that cannot be discarded. This study, with its extensive survey of soil types and biomes, addresses one of the key knowledge gaps that currently prevent the routine use of $\delta^{18}O-CO_2$ as a global carbon tracer, and should motivate the community to reconsider this independent tracer in global climate models, thus constraining our understanding of variability in the northern hemisphere land carbon sink. Hopefully, this may also stimulate the development of a denser observational network of $\delta^{18}O$ in CO_2, which is now possible with the next generation of laser-based CO_2 isotope analysers.

Campbell, J. E., Berry, J. A., Seibt, U., Smith, S. J., Montzka, S. A., Launois, T., Belviso, S., Bopp, L. and Laine, M.: Large historical growth in global terrestrial gross primary production, Nature, 544(7648), 84, https://doi.org/10.1038/nature22030, 2017.

Ciais P, Tan J, Wang X et al. (2019) Five decades of northern land carbon uptake revealed by the inter-hemispheric CO2 gradient. Nature, 568, 221–225.

Koren G, Schneider L, van der Velde IR et al. (2019) Global 3-D Simulations of the Triple Oxygen Isotope Signature Δ17O in Atmospheric CO2. Journal of Geophysical Research-Atmospheres, 127, 73.

Wingate L, Ogee J, Cuntz M et al. (2009) The impact of soil microorganisms on the global budget of δ18O in atmospheric CO2. Proceedings of the National Academy of Sciences of the United States of America, 106, 22411–22415.

3) The writing needs more clarity and conciseness. As a rule of thumb, try not to make sentences more complicated than the ideas they convey. In a paragraph, stick to one point and avoid switching topics or walking back and forth. For example, much of the discussion had the main points hidden in the middle of a paragraph and could use some restructuring. Break long paragraphs if necessary.

Thanks, we have worked to improve the clarity and conciseness of the revised manuscript following this good advice.

4) The hypotheses need to be accurately framed. Hypothesis 2 is a complicated statement, and the only part testable based on your experiments is that kiso increases with soil pH. The rest of Hypothesis 2 describes possible mechanisms and they cannot be answered by your experiments. In Hypothesis 3, you can only test whether kiso increases with [NO3-], but not whether [NO3-] binds carbonic anhydrases or how it inhibits carbonic anhydrase. These two hypotheses should be precisely worded as testable hypotheses. The hypotheses you actually tested were stated in P14L382–383, so why not simplify them just like that?

We thank the reviewer for this suggestion and we have simplified our hypotheses to reflect the reviewer's comments. This section now reads: "Based on the potential controls on k_{iso} presented above we tested three specific, non-exclusive, hypotheses; 1) k_{iso} increases as microbial biomass increases (H1), 2) k_{iso} increases as soil pH increases (H2), and 3) k_{iso} decreases as NO_3^- availability increases (H3)."

5) Finally, I encourage the authors to make the data sets publicly available in a data repository. This would make the study more easily discoverable and facilitate data reuse in future studies, for example, comparison across sites and parameterization of related soil processes in a land biosphere model.

We agree with the reviewers comment and have submitted the dataset (Nov 2020) from this paper to PANGAEA (https://pangaea.de/) for archiving (see also comment 15).

Specific comments

6) P1L13: "The expression and activity of carbonic anhydrase [. . .]" - You may need to tell the reader that carbonic anhydrase regulates the hydration of CO2 in soil pore-space water before you mention that it drives kiso.

Thanks. We have rephrased this sentence as suggested: “As the enzyme carbonic anhydrase enhances the rate of CO_2 hydration within the water-filled pore spaces of soils it is important to develop understanding of how environmental drivers can impact carbonic anhydrase expression and activity and alter k_{iso}.”

7) P1L19–20: “[. . .] potentially reflecting the direct or indirect inhibition of carbonic anhydrases” - Is there a way to tell which mechanism is more likely?

To distinguish whether the impact of nitrate is direct or indirect an integrated study looking into changes in the concentration of carbonic anhydrase protein and the abundance of carbonic anhydrase transcripts alongside measurements of k_{iso} would be required. Additionally it would also be important to do some detailed protein studies that show the physical interaction of nitrate with the carbonic anhydrase protein and develop a method that could quantify the binding efficiency of nitrate to carbonic anhydrase for a few of the dominant soil carbonic anhydrases e.g. the beta-CA class. Collectively these different experiments would help us tease apart the direct and indirect effects of nitrate on carbonic anhydrase in soils.

8) P2L31: “because the δ18O of leaf–atmosphere CO2 exchange tends to be enriched [. . .]” - More precisely, this is because leaf preferentially uses lighter isotopologues of CO2, which diffuse faster than heavier ones. See Farquhar et al. (1993) Nature (https://doi.org/10.1038/363439a0).

Actually, diffusion is not the only reason. We agree that the oxygen isotope composition of leaf-atmosphere CO_2 exchange is partly explained by fractionation during diffusion, but not only by this. The isotopic exchange between CO_2 and water is also very important (Farquhar et al. 1993). In contrast the influence of oxygen isotope fractionation during other steps of fixation (e.g. carboxylation) is limited because carbonic anhydrase concentrations are sufficiently high enough for the isotopic equilibration between CO_2 and water to be extremely rapid (Ogée et al. 2018). By analogy to ^{13}C fractionation during photosynthesis, Farquhar et al. (1993) described the leaf as consuming isotopically lighter CO_2 in terms of ^{18}O, thereby leaving behind CO_2 enriched in ^{18}O in the intercellular air space to diffuse back to the atmosphere. However, the analogy works because the CO_2 inside the leaf equilibrates its oxygen isotopes with evaporatively enriched leaf water. Thus, the mechanism is very different than for ^{13}C, and primarily driven by leaf water isotopic composition and secondarily by diffusion.

Farquhar, G. D., Lloyd, J., Taylor, J. A., Flanagan, L. B., Syvertsen, J. P., Hubick, K. T., Wong, S. C. and Ehleringer, J. R. (1993) Vegetation effects on the isotope composition of oxygen in atmospheric CO2, Nature, 363(6428), 439–443, doi:10.1038/363439a0.

Ogée J, Wingate L, Genty B (2018) Estimating mesophyll conductance from measurements of C18OO photosynthetic discrimination and carbonic anhydrase activity. Plant Physiol., 178, 728–752.

9) P2L44: “Comprising at least six distinct families, [. . .]” - There are seven now, with the newly discovered ι-CA in phytoplanktons. See Jensen et al. (2019) ISME J (https://doi.org/10.1038/s41396-019-0426-8).

Thanks! We have corrected this and updated the references and manuscript text accordingly. “Comprising at least seven distinct families, carbonic anhydrases have independently evolved in all domains of life in order to catalyse the reversible hydration of carbon dioxide (CO_2) to bicarbonate (Jensen et al., 2019)”

10) P3L81–82: “Whilst the sensitivity of soil kiso to the presence of specific functional groups, like phototrophs which employ carbonic anhydrases in their carbon concentration mechanisms [. . .]” - Are phototrophs abundant in soil microbial communities?

In a review of the literature Wingate et al., 2009 estimated that soil algal populations of between 10^3 - 10^6 per gram of soil are typically present in most soils. If cyanobacteria are further included, phototrophs can indeed form an important part of the soil microbial community under many conditions (Muriel Bristol Roach, 1927; Seppey et al., 2017). This may be either as superficial crusts or within the near surface. Whilst they are likely to be less ubiquitous than fungi and bacteria, the possibility of specialised, carbonic anhydrase dependent, carbon concentration mechanisms might suggest their presence could have a disproportionately strong influence on k_{iso}. In a previous study looking at the role of phototrophs on carbonic anhydrase activity (Sauze et al., 2017) we developed a qPCR approach that helped us show that the putative natural abundance of soil phototrophs derived from the number of 23S reads were relatively small under darkened conditions compared to the bacterial (16S) and fungal (18S) abundances but their relative abundances increased significantly when incubated in the light. This probably and unsurprisingly suggests that such an influence might be somewhat dependent on the canopy cover and light conditions of the system in question.

Muriel Bristol Roach, B. (1927). On the algae of some normal English soils. *The Journal of Agricultural Science, 17*(4), 563-588. doi:10.1017/S0021859600018839

Seppey, C. V. W., Singer, D., Dumack, K., Fournier, B., Belbahri, L., Mitchell, E. A. D. and Lara, E.: Distribution patterns of soil microbial eukaryotes suggests widespread algivory by phagotrophic protists as an alternative pathway for nutrient cycling, Soil Biology and Biochemistry, 112, 68–76, doi:10.1016/j.soilbio.2017.05.002, 2017.

Sauze, J., Ogée, J., Maron, P.-A., Crouzet, O., Nowak, V., Wohl, S., Kaisermann, A., Jones, S. P. and Wingate, L.: The interaction of soil phototrophs and fungi with pH and their impact on soil CO2, CO18O and OCS exchange, Soil Biology and Biochemistry, 115(Supplement C), 371–382, doi:10.1016/j.soilbio.2017.09.009, 2017.

Wingate L., Ogée J., Cuntz M., B. Genty, I. Reiter, U. Seibt, D. Yakir, K. Maseyk , E.G. Pendall, M.M. Barbour, B. Mortazavi, R. Burlett, P. Peylin, J. Miller, M. Mencuccini, J.H. Shim, J. Hunt, J. Grace (2009) The impact of soil microorganisms on the global budget of $\delta^{18}O$ in atmospheric CO_2. *Proceedings of the National Academy of Sciences of America*, 106, 22411–22415.

11) P4L99: Be specific about "the inorganic nitrogen chemistry of soil solutions."

We have changed this sentence to the following:

"In this respect, the fact that nitrate (NO_3^-) has also been shown to inhibit carbonic anhydrases (Peltier et al., 1995) suggests that the application of common fertilisers such as ammonium nitrate may exert a considerable control on carbonic anhydrase activity. Indeed, this hypothesis is supported by recent ammonium nitrate fertilising experiments that demonstrated decreases in carbonyl sulphide exchange (Kaisermann et al., 2018b), also catalyzed by carbonic anhydrases , but the influence on k_{iso} has yet to be considered."

12) P5L133–134: Does sieving affect carbonic anhydrase activity in soils?

Our experiments did not test for the impact of sieving on soil carbonic anhydrase activity and as far as we are aware this has not been reported in the literature, thus the nature of these effects is not well understood and is discussed in L360 to L380.

13) P7L195–198: What was the precision of the IRIS for CO2 and δ18O-CO2 measurements when averaged in 40 intervals?

We have added this information from Jones et al., 2017: "The associated precision for the total concentration and $\delta^{18}O$ of CO_2 was 0.02 ppm and 0.06 ‰ VPDBg respectively."

Jones, S. P., Ogée, J., Sauze, J., Wohl, S., Saavedra, N., Fernández-Prado, N., Maire, J., Launois, T., Bosc, A. and Wingate, L. (2017) Non-destructive estimates of soil carbonic anhydrase activity and associated soil water oxygen isotope composition, Hydrology and Earth System Sciences, 21(12), 6363–6377, doi:https://doi.org/10.5194/hess-21-6363-2017.

14) P7L210: Eq. (1) requires a steady-state condition. What is the turnover time for gas exchange in the cuvette? Could you show that the measurement period (12, P1L191) is much longer than this turnover time?

As in Jones et al. (2017) the turnover time was less than 10 minutes. We have added this information to the text: "The turnover time of air in the jar was less than 10 minutes". Each jar was flushed for 20 or 22 minutes before the measurement period (L189-193) and 22 or 24 minutes before the first used chamber measurement was made. These timings reflect the need to balance the trade-off between approximate steady-state conditions and changes in the isotopic composition of the soil water pool (Jones et al. 2017).

Jones, S. P., Ogée, J., Sauze, J., Wohl, S., Saavedra, N., Fernández-Prado, N., Maire, J., Launois, T., Bosc, A. and Wingate, L. (2017) Non-destructive estimates of soil carbonic anhydrase activity and associated soil water oxygen isotope composition, Hydrology and Earth System Sciences, 21(12), 6363–6377, doi:https://doi.org/10.5194/hess-21-6363-2017.

15) P8L238–239: Please considering providing a table of site information and soil characteristics, either as a supplementary table or a metadata file in the online data set associated with this study. Although such information is available for European sites in Kaisermann et al. (2018) ACP, it would not be convenient for C4 the reader to reference across multiple publications. For the Australian sites, I do not see any such data.

We have submitted the dataset used for archiving in PANGAEA (https://pangaea.de/) and will include the relevant information in the finalised manuscript or as an amendment once the archiving process is complete (see also comment 5).

16) P10L281: What does the “two-term model” mean? What are the predictors?

Two-term models are those limited to 2 or less predictive terms. We have rephrased this to make it clearer:
“The same approach was also applied to the 27 soils from the EUR sampling campaign and extended to consider the relationships with soil texture and carbon and nitrogen contents to investigate their utility in upscaling efforts. To prevent over-fitting, these models were limited to a maximum of two of predictive terms. The predictive terms considered were soil sand, silt, clay, carbon and nitrogen content, the ratio of carbon to nitrogen content and soil pH.”

17) P10L282: Have soil texture, carbon content, and nitrogen content been considered in the aforementioned model selection procedures?

Yes, the same model selection procedures were used. Please see previous comment where this is now explicitly stated in the text.

18) P11L305: “Correlations between all other variable pairings were weaker and non-significant ($p > 0.05$).” - I find this observation in apparent conflict with the interpretation of NH4NO3 treatment results. If NO3- concentration does not control kiso in natural soils, why would adding NH4NO3 cause kiso to decrease through carbonic anhydrase inhibition? One possible scenario could be that the variation in kiso that is attributable to soil pH is so large that any influence from NO3- concentration is obscured. To test whether this would be the case, Spearman’s rank correlation would be insufficient. You would need to control for the variation due to pH before testing the effect of [NO3-].

Spearman’s rank correlation is used to identify the strongest patterns between pairs of variables without making a priori assumptions about the data. This is particularly useful as it helps us identify potential co-correlations such as that between pH and ammonium availability that may confound the subsequent analyses discussed in the paragraph following that referred to in this comment.

Subsequent use of multiple generalised linear models lets us test these relationships in a more satisfactory fashion. This analysis bears out the main result of the Spearman’s rank correlation i.e. that most of the variability in k_{iso} is explained by soil pH. However, after controlling for the effect of pH the inclusion of nitrate availability and biomass both significantly increase the degree of variability explained (see also Table S1). This indicates that nitrate concentration does indeed control k_{iso} in natural soils. Figure 3 b shows the nature of this relationship with nitrate concentration, particularly under acidic conditions, causing k_{iso} to decrease.

19) P13L357: While the fraction of explained deviance is high, this is a small sample with n=14 and uncertainty associated with # the model could be large. What is the confidence interval of the coefficient of ln NO3-?

We agree that the sample size is small and report this model simply as the best fit to the data out of the variables considered in order to understand the influence of the treatment on the rate of exchange. Indeed, the uncertainty is large particularly at higher values of change. Please see the confidence interval provided in Figure 5.

20) P13L376–380: “Whether the potential [. . .] remains an unresolved but key question.” - Not sure what you are trying to mean with this sentence. Please clarify it.

Thanks, we have re-phrased this to make it clearer.

“Understanding why k_{iso} has the potential to be orders of magnitude greater in the field compared to values observed in incubation studies is a key question for the future. The abundance and activity of carbonic anhydrases may be reduced during the process of sieving soils and incubating them for prolonged periods in the dark. For example, the exclusion of intact roots and mycorrhizal fungi interacting within the rhizosphere might reduce kiso (Li et al., 2005). Equally the suppression of phototrophic community members by incubating mesocosms in the dark (Sauze et al., 2017) may also contribute to differences in k_{iso} between the field and incubated mesocosm experiments. Furthermore, we cannot rule out the possibility that determining k_{iso} accurately under field conditions is less reliable. For example the calculation of k_{iso} relies on determining the isotopic composition of the soil water pool in equilibrium with CO_2. Given the potential for increased heterogeneity in the isotopic composition of the soil water pool in natural conditions this may make it more challenging to determine kiso robustly in the field (Jones et al., 2017).”

21) P15L425: “The absence of strong patterns with climate or land-cover in this study may well reflect the fact that the temperature and moisture conditions used are unrepresentative of field conditions especially for colder and drier sites.” - Or, it could also be that soil texture and composition are the main controls.

It is true that the conditions experienced by the microbes in their natural environments can be very different from those experienced in our experiment. This would definitely be interesting to look at in the future with a different experimental and mechanistic modelling approach. However, the aim of the present study was to standardise moisture and temperature conditions to the best of our abilities and investigate how the gas exchange rates and enzyme activity of these different communities compared. Opting for this experimental design meant we were not able to attribute statistically whether differences in activity were underpinned by land-use or climate class in a way that would facilitate a simple scaling up approach, Our study indicates other soil traits such as pH have the potential to provide more reliable spatial predictions of k_{iso}. With larger databases perhaps land-use or climate patterns will begin to emerge as important large-scale drivers of soil function and predictors of soil-atmosphere gas exchange but for the moment it remains unclear as these datasets are rare in the community.

22) P15L435: What are the “pedotransfer functions?”
Pedotransfer functions are predictive functions used to estimate certain soil properties from more readily available data. We have altered this sentence to provide more clarity on the message we are trying to communicate:

“A significant challenge to using this statistical relationship to predict k_{iso} is underpinned by our capacity to describe the spatial and temporal variations in the important drivers of k_{iso}, namely soil pH, microbial biomass and exchangeable NO_3^-. Fortunately, a number of promising spatial databases are evolving for soil characteristics such as pH and microbial biomass likewise a number of land surface models can now estimate the spatial and temporal dynamics of the biosphere N cycle convincingly (Zaehle, 2013).”

Technical comments

23) P1L10: “gross primary production” vs. P1L25 “gross primary productivity ” (emphases mine), pick one.

Thanks. L25 changed to “gross primary production”.

24) P1L11: “ecosystem-scale” → “ecosystem scale”

Thanks. Corrected.

25) P1L15: Add a comma before “indicating [. . .].”

Thanks. Corrected.

26) P1L33: “the leaves of plants” → “leaves”. Pleonasm.

Thanks. Changed to “This is the case because leaves contain...”

27) P2L35: “causing CO2 that interacts with a leaf but is not fixed to inherit the isotopic composition of the leaf water pool” - A difficult sentence. Please clarify.
Thanks we have simplified this: “This is the case because leaves contain considerable concentrations of carbonic anhydrase that catalyses the hydration of aqueous CO_2 and the exchange of oxygen isotopes between CO_2 and water molecules. The rate of this exchange is rapid and causes the majority of CO_2 within a leaf to inherit the isotopic composition of the leaf water pool (Gillon & Yakir, 2001).”

28) P2L44–P3L73: This paragraph has a lot to unpack. In my opinion, to bring clarity to this paragraph, you may consider splitting it into two. Describe the abiotic reaction of oxygen isotope exchange first, and then introduce the role of carbonic anhydrases in accelerating the reaction towards equilibrium. I would consider splitting the paragraph at line 62 and rearraging sentences for a clean separation.

We have rearranged and edited this section as suggested:

“The oxygen isotope composition of atmospheric CO_2 is influenced by leaves and soils because oxygen isotopes are exchanged between water and CO_2 through the reverse dehydration step of the reversible hydration reaction between aqueous CO_2 and bicarbonate (Mills & Urey, 1940). In a closed system at chemical equilibrium, CO_2 will reach isotopic equilibrium with water after some time depending on the rate of oxygen isotope exchange, k_{iso} (s^{-1}), (Uchikawa & Zeebe, 2012). In soils the greater abundance of water molecules causes endogeneous CO_2 or atmospheric CO_2 that diffuses within the soil profile to inherit the $\delta^{18}O$ of the soil water (Tans, 1998). The degree to which the $\delta^{18}O$ of CO_2 reflects a given soil water pool is determined by the residence time of dissolved CO_2 and the apparent k_{iso} (Miller et al., 1999). Longer residence times or greater k_{iso} move the system closer to isotopic equilibrium. Resulting from the interconversion of aqueous CO_2 and bicarbonate, k_{iso} is expected to vary as a function of the combined rates of CO_2 hydration, k_h, and hydroxylation reactions and the pH dependent speciation of dissolved inorganic carbon (Uchikawa & Zeebe, 2012). Under acidic and neutral conditions interconversion is dominated by hydration, whilst the hydroxylation becomes important under alkaline conditions as the concentration of hydroxyl anions increases (Figure 1 a). The presence of carbonic anhydrases increases the rate of the hydration reaction, k_h, and the overall rate of interconversion between CO_2 and bicarbonate. However, the influence of carbonic anhydrases, for a given concentration and efficiency, is also limited by the presence of high proton concentrations under acidic conditions that inhibit de-protonation required for enzyme regeneration (Rowlett et al., 2002; Sauze et al., 2018). The k_{iso} resulting from these reactions is dependent on the relative abundance of CO_2, which is the dominant form of dissolved organic carbon under acidic conditions, to carbonic acid, bicarbonate and carbonate in the system (Figure 1 b). In alkaline conditions, the predominance of bicarbonate and carbonate acts to inhibit the rate of k_{iso} associated with the hydration reaction and limit the influence of hydroxylation (Figure 1 c).

Comprised of at least seven distinct families, the carbonic anhydrases have independently evolved in all domains of life in order to catalyse the reversible hydration of carbon dioxide (CO_2) to bicarbonate described above (Jensen et al., 2019). Whilst this reaction occurs abiotically, the need for carbonic anhydrases stems from the fact that enhanced rates of hydration, k_h , are required to control the transport and availability of CO_2, bicarbonate and protons in numerous metabolic processes (Smith & Ferry, 2000). Unsurprisingly given their apparent ubiquity, evidence of carbonic anhydrase activity in soils indicates the expression of these enzymes directly supports the viability of microbial communities and thus plays a role in the wider biogeochemical function of the soil environment (Li et al., 2005). . The fact that k_{iso} inferred from patterns in the $\delta^{18}O$ of CO_2 fluxes observed under field (Seibt et al., 2006; Wingate et al., 2008, 2009, 2010) and laboratory conditions (Jones et al., 2017; Meredith et al., 2019; Sauze et al., 2017, 2018) can exceed uncatalysed rates by up to three orders of magnitude indicates a particular need to better understand variations in the expression of carbonic anhydrases and the controls on their activity in soil environments."

29) P3L83: "it's" → "its"

Thanks. Corrected.

30) P3L87–89: "Such an observation may result from changes in size or composition of the microbial communities involved as discussed (Sauze et al., 2017, 2018)." - This is a reiteration of P3L79–81.

Removed.

31) P4L95: "non-carbon" → "non-carbonate"

Thanks. Changed to "The chemistry of other anions".

32) P5L123: "principle" → "principal"

Thanks. Corrected.

33) P5L124: "indicted" → "indicated"

Thanks. Corrected.

34) P6L171: This should be section 2.2, not 2.1.

Thanks. We have corrected section '2.1 Gas exchange measurements' to '2.2 Gas exchange measurements'.

35) P11L312–316 and P12L330–337: It is inconvenient to track which model is which. Please consider listing model diagnostics in supplementary tables.

We have added three tables to the supplement listing the relevant models discussed in the text.

Table S1: Ranking and included terms for a subset of the generalised linear models tested to predict variations in the rate of oxygen isotope exchange, k_{iso}, for the entire dataset (n = 44). Model selection was limited to a maximum of four predictive terms and the intercept. The terms MB, NO_3^- and NH_4^+ are the natural logarithms of microbial biomass and nitrate and ammonium availability. Selected terms or interactions within each model are indicated by + symbols whilst - symbols indicate their omission. The interactions Campaign:pH and Campaign:MB are omitted from the table for brevity as they were not selected in any of the models shown. Model ranking was based on comparison of sample size corrected Aikake's Information Criterion (AICc) with ΔAICc indicating the difference in AICc from the best model. ΔAICc of 2 or more indicates real differences in model performance.

Rank	Intercept	Campaign	pH	MB	NO_3^-	NH_4^+	Campaign: NO_3^-	pH: MB	pH: NO_3^-	MB: NO_3^-	$NO3^-$: NH_4^+	ΔAICc
1	+	-	+	+	+	-	-	-	+	-	-	0.00
2	+	-	+	+	+	-	-	-	-	-	-	6.10
3	+	+	+	+	+	-	-	-	-	-	-	7.06
4	+	-	+	+	+	-	-	+	-	-	-	7.07
5	+	+	+	-	+	-	+	-	-	-	-	7.09
6	+	-	+	+	+	-	-	-	-	+	-	8.79
7	+	+	+	-	+	-	-	-	-	-	-	12.43
8	+	-	-	+	+	+	-	-	-	-	+	13.27
16	+	-	+	-	-	-	-	-	-	-	-	21.56
19	+	-	-	-	-	+	-	-	-	-	-	26.48
21	+	-	-	+	-	-	-	-	-	-	-	43.64
28	+	-	-	-	-	-	-	-	-	-	-	47.91
33	+	+	-	-	-	-	-	-	-	-	-	50.15
34	+	-	-	-	+	-	-	-	-	-	-	50.21

Table S2: Ranking and included terms for a subset of the generalised linear models tested to predict variations in the rate of oxygen isotope exchange, k_{iso}, for the relatively invariant soil properties of the EUR campaign dataset (n = 27). Model selection was limited to a maximum of two predictive terms and the intercept. The terms C, N and CN are soil carbon and nitrogen content and their ratio. Selected terms or interactions within each model are indicated by + symbols whilst - symbols indicate their omission. Model ranking was based on comparison of sample size corrected Aikake's Information Criterion (AICc) with ΔAICc indicating the difference in AICc from the best model. ΔAICc of 2 or more indicates real differences in model performance.

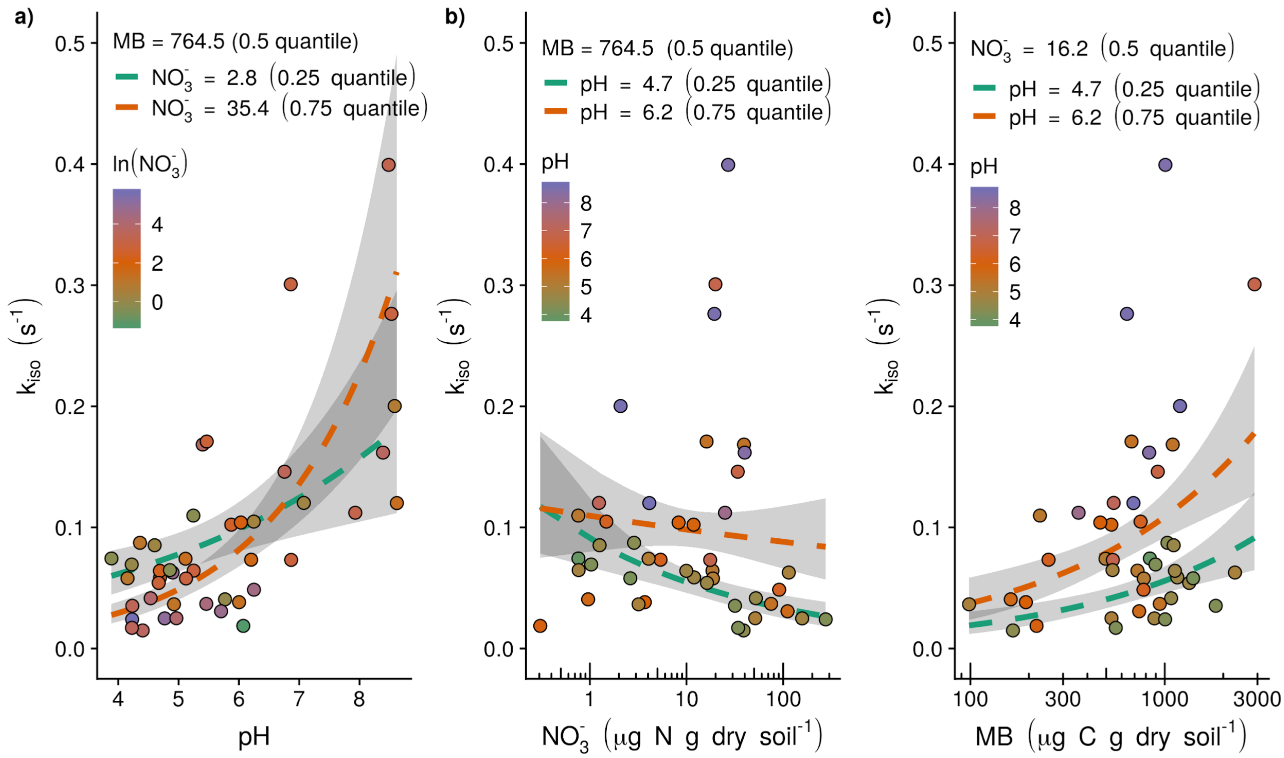
Rank	Intercept	pH	Sand	Silt	Clay	C	N	CN	ΔAICc
1	+	+	-	-	+	-	-	-	0.00
2	+	+	+	-	-	-	-	-	0.57
3	+	+	-	-	-	+	-	-	1.32
4	+	+	-	-	-	-	-	-	1.85
5	+	+	-	-	-	-	-	+	1.92
6	+	+	-	+	-	-	-	-	2.46
7	+	+	-	-	-	-	+	-	4.57
8	+	-	-	-	-	-	+	-	21.26
9	+	-	-	-	-	-	-	-	22.07

Table S3: Ranking and included terms for a subset of the generalised linear models tested to predict variations in the change in rate of oxygen isotope exchange, k_{iso}, following ammonium nitrate addition (n = 15). Model selection was limited to a maximum of one predictive term and the intercept. The terms MB, NO_3^- and NH_4^+ are differences in microbial biomass and nitrate and ammonium availability following ammonium nitrate addition whilst the prefix ln indicates the natural logarithm of these differences. Selected terms or interactions within each model are indicated by + symbols whilst - symbols indicate their omission. Model ranking was based on comparison of sample size corrected Aikake's Information Criterion (AICc) with ΔAICc indicating the difference in AICc from the best model. ΔAICc of 2 or more indicates real differences in model performance.

Rank	Intercept	Campaign	pH	MB	NO_3^-	NH_4^+	lnMB	lnNO_3^-	lnNH_4^+	ΔAICc
1	+	-	-	-	-	-	-	+	-	0.00
2	+	-	-	-	+	-	-	-	-	8.65
3	+	-	+	-	-	-	-	-	-	13.20
4	+	-	-	-	-	-	-	-	-	15.95
5	+	+	-	-	-	-	-	-	-	17.38
6	+	-	-	-	-	-	+	-	-	18.34
7	+	-	-	+	-	-	-	-	-	18.80
8	+	-	-	-	-	-	-	-	+	19.10
9	+	-	-	-	-	+	-	-	-	19.21

36) Figure 3: It is difficult to distingush high values from low values indicated by the color bars. Try to increase the contrast.

Figure 3 has been revised to hopefully increase the contrast of the plot gradients and use a more accessible colour palette.



37) Figure S1: Remove the ocean background and other unnecessary information. Please simplify this figure to make the ecoclimatic classification more evident. Consider putting the legend outside of the figure canvas to avoid interference.

Figure S1 has been revised to mask the ocean, move the legend outside of the map area and reduce the classes to only reflect those covered by the samples obtained.