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SOILD

Interactive comment

Interactive comment on "Oxygen isotope exchange between water and carbon dioxide in soils is controlled by pH, nitrate availability and microbial biomass through links to carbonic anhydrase activity" *by* Sam P. Jones et al.

Anonymous Referee #1

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General comments

This study reports the rates of oxygen isotope exchange between soil pore-space water and the ambient CO2, k_{iso}, from a large set of soil samples ($n = 44$) collected from seven ecoclimatic zones in Eurasia and Australia. k_{iso} is an essential parameter for quantifying soil influences on the δ^{18}O signature of atmospheric CO2. The variability of k_{iso} with soil and ecoclimatic features is poorly understood due to the paucity of data and the lack of standardization in measurement and reporting. This study marks a

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Discussion paper

valuable contribution as it shows that soil pH and NO_3^- content are the most important factors controlling k_{iso} variability. The study may be considered for publication after concerns are addressed.

My main concern is with the interpretation of the results of ammonium nitrate (NH_4NO_3) treatment. The authors attributed the decrease of k_{iso} following NH_4NO_3 addition to the inhibition of carbonic anhydrase caused by NO_3^-. 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, NH_4NO_3 addition may affect k_{iso} through these causal pathways:

- NH_4NO_3 addition $\rightarrow$ $[NH_4^+]$ increase $\rightarrow$ k_{iso} decrease
- NH_4NO_3 addition $\rightarrow$ $[NH_4^+]$ increase $\rightarrow$ pH decrease $\rightarrow$ k_{iso} decrease
- NH_4NO_3 addition $\rightarrow$ $[NO_3^-]$ increase $\rightarrow$ k_{iso} 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 k_{iso} with the increase of $[NO_3^-]$. Given the absence of a randomized design and the small sample size ($n = 14$) for NH_4NO_3 addition treatment, it is difficult to identify $[NO_3^-]$ as the unique cause for carbonic anhydrase inhibition. One possible solution could be to treat pH and $[NH_4^+]$ as instrumental variables, but this would require them to show strong correlation with $[NO_3^-]$. The best way would be to separate different causes through experimental design.

A minor concern I have is that this study was partially motivated by the use of δ^{18}O 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 unclear 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 $\delta^{18}O_{CO_2}$ can provide constraints on terrestrial photosynthesis with accuracy and spatiotemporal resolution as high as those of other photosynthetic tracers in vogue (e.g., solar-induced chlorophyll fluorescence).

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.

The hypotheses need to be accurately framed. Hypothesis 2 is a complicated statement, and the only part *testable* based on your experiments is that k_{iso} 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 k_{iso} increases with $[\mathrm{NO_3^-}]$, but not whether $[\mathrm{NO_3^-}]$ 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?

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.

Specific comments

- 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 k_{iso}.

- P1L19–20: "[. . .] potentially reflecting the direct or indirect inhibition of carbonic anhydrases" - Is there a way to tell which mechanism is more likely?
- P2L31: "because the δ^{18}O 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).
- 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).
- P3L81–82: "Whilst the sensitivity of soil k_{iso} 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?
- P4L99: Be specific about "the inorganic nitrogen chemistry of soil solutions."
- P5L133–134: Does sieving affect carbonic anhydrase activity in soils?
- P7L195–198: What was the precision of the IRIS for CO2 and $\delta^{18}O_{CO_2}$ measurements when averaged in 40 intervals?
- 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?
- 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

the reader to reference across multiple publications. For the Australian sites, I do not see any such data.

- P10L281: What does the "two-term model" mean? What are the predictors?
- P10L282: Have soil texture, carbon content, and nitrogen content been considered in the aforementioned model selection procedures?
- 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 NH_4NO_3 treatment results. If NO_3^- concentration does not control k_{iso} in natural soils, why would adding NH_4NO_3 cause k_{iso} to decrease through carbonic anhydrase inhibition? One possible scenario could be that the variation in k_{iso} that is attributable to soil pH is so large that any influence from NO_3^- 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 $[NO_3^-]$.
- 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 $\ln\left[NO_3^-\right]$?
- 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.
- 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.
- P15L435: What are the "pedotransfer functions?"

Technical comments

- P1L10: “gross primary *production*” vs. P1L25 “gross primary *productivity*” (emphases mine), pick one.
- P1L11: “ecosystem-scale” → “ecosystem scale”
- P1L15: Add a comma before “indicating [. . .].”
- P1L33: “the leaves of plants” → “leaves”. Pleonasm.
- 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.
- 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.
- P3L83: “it’s” → “its”
- 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.
- P4L95: “non-carbon” → “non-carbonate”
- P5L123: “principle” → “principal”
- P5L124: “indicted” → “indicated”
- P6L171: This should be section 2.2, not 2.1.

- P11L312–316 and P12L330–337: It is inconvenient to track which model is which. Please consider listing model diagnostics in supplementary tables.
- Figure 3: It is difficult to distingush high values from low values indicated by the color bars. Try to increase the contrast.
- 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.

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