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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.

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SOIL-2020-44 presents on an interesting research into how soil factors would affect O18 exchange between CO2 and H2O. As the 18O levels of emitted CO2 are used for estimating land-atmosphere carbon exchange it presents a well-delineated and timely topic. The experimental assessment of exchange of 18O between soil water and CO2 is by no means trivial, yet as many as 44 soils collected from a wide geographical area (Eurasia and Australia) were assessed for their potential to oxygen isotope exchange. The description was mostly excellent but I concur with the referee that on instances the

text is lengthy. For instance the description of the statistical approach and reporting of fitted general linear models are longer than usual. However, here such an elaborate formulation appeared warranted given the clear collinearity of some of the predictor variables. Still, in particular the introduction can surely be further condensed. Also the lengthy description of the setup to administer air with CO2 with contrasting δ18O requires shortening (e.g. by just referring to or some part of it should be moved to supplementary material. The description of preparation of soil mesocosms could also be condensed. Please also use a different term for available NO3- and available NH4+ (or 'availability of'). Both were measured in typical 1M KCl extracts and are best referred to exchangeable NO3- and NH4+. The entire text is filled with long complex sentences, which on their own are well crafted but do not always allow fluid reading. I would recommend the authors to read their manuscript once more and if they see fit do try to subdivide some of the longer sentences. I concur with the referee that some of the hypotheses require rephrasing and attention needs to be given to his/her possibly justified concern on the NH4NO3 administration's ineptitude to robustly prove that NO3- led to inhibition of of carbonic anhydrase. There are also two other issues that need to be addressed before publication can be considered:

1° The interpretation of the relationship between NH4+ and kiso: On L408 following statement is made 'Notably the weak relationship between changes in kiso and NH4+ availability identified in this experiment (Table 2) suggests the relationship between these variables across the untreated soils (Table 1) does indeed reflect the pH sensitivity of ammonia speciation rather than a direct causal link.'. I would agree that the link is indeed direct, but strongly doubt that NH4+/NH3 speciation is important here. Below pH 7 there is virtually no (toxic) NH3. Most soils had acidic pH so this explanation seems wrong. Instead: high NH4+ levels in upland soils rather suggest nitrification is impeded in some way. There is an obvious link between pH and NH4+ levels: at low pH, nitrification is well known to be slowed: indeed there was a strong negative correlation between both (Table 1) and so the negative correlation between NH4+ and kiso may just be indirect through their mutual relation with pH. Alternatively, higher NH4+

levels point at impeded autotrophic nitrification – a rather energetically unfavourable process and therefore sensitive to environmental constraints. Inhibited nitrification may also point at unfavourable conditions for other microbial processes: perhaps also production of anhydrase activity? In Table 2 the artificially elevated NH_4^+ levels are no longer the resultant from slow or fast pH-dependent nitrification and therefore do not display any relation with kiso. The referee also commented on this matter– please do take that comment into account.

2° It is unfortunate that the authors opted for an enourmous dose of NH_4NO_3, viz 0.7 mg NH_4NO_3 g-1 (L168). No doubt such a large addition of NH_4^+ would have led to serious soil acidification following its partial nitrification during the 1 week pre-incubation at 23°C. And on the other hand the obtained NH_4^+ and NO_3^- levels in treated soil would not reflect environmentally realistic exchangeable N levels. That would severely limit the relevance of Fig. 5. Or is the 0.7 mg NH_4NO_3 g-1 a typing error? In any case in their rebuttal the authors will need to present (not necessarily in the revised manuscript) the absolute increase in NH_4^+ and NO_3^- levels alongside changes in pH brought forth by the NH_4NO_3 administration as based on the fractorial changes in NO_3^- now presented it is impossible to judge whether or not excessive amounts of N had been added. This firstly needs to be clarified.

Minor comment: Remove commas when the citation is part of the sentence throughout the entire text: Jones et al. (2017), not Jones et al., (2017).

L21 suggest to omit 'future'

L31 'the δ18O of leaf-atmosphere CO_2 exchange' reads strange, sounds clearer without 'exchange'

L 35 CO_2 that interacts with a leaf – interacts seems too vague, could this be reworded?

L36 'undergo considerable enrichment' of what?

L40 'alters the δ18O of atmospheric CO_2' was not really clear to me. Perhaps write

'alters the δ18O of CO_2 in soil vs. atmospheric CO_2'?

L51 is 'of soil atmospheric CO_2' not better?

L56 'abiotically invades' sounds awkward, perhaps write 'diffuses into the soil pore network'

L65 delete 'true', is confusing here

L99 better 'agricultural soils'?

L166 'an additional three replica incubations' correct English?

2.1 Not clear if soils were kept cool during transport

L273 remove 'presumably reflecting the pH dependency of NH_4^+ and ammonia speciation' this does not belong in the M&M section

L293-294 do not seem entirely accurate: there seems to be a higher kiso for the Csa group.

L370-375 is quite unclear: are the 0.04 to 13 s-1 field values derived from your lab measured 0. To 0.4 s-1 kiso estimates? + which older studies. The whole part starts quite sudden. In fact for the sake of clarity the apparent issue of comparing lab and field estimates is perhaps best omitted from the paper entirely – also further on.

L431 'between untreated and treated' is not a very clear phrasing – spell out better what you are referring at.

L434 'A significant challenge to using this relationship to predict kiso is likely the availability of suitable pedotransfer functions,particularly for NO_3^- availability and microbial biomass, to estimate patterns in the proposed drivers (Van Looy et al., 2017).' Is an understatement. It will be impossible to predict the ephemeral soil NO_3^- levels with a simple pedotransfer function. The authors best refer to use soil N models to predict NO_3 levels and use these as inputs into Eq 6.

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