1) 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.

We would sincerely like to thank the editor for taking the time to handle the review process of this manuscript and for providing their own useful comments that have improved the manuscript. We have addressed the points raised (reproduced in blue) below.

2) 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.

We agree that as the interpretation of the data is dependent on the statistical tests used it is important to be explicit about the steps taken.

3) 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.

Thanks, following this good advice we have worked to streamline the revised manuscript.

4) The description of preparation of soil mesocosms could also be condensed.

Please see the previous comment.

5) 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+.

Thanks for this suggestion, we have modified the text accordingly to use exchangeable in place of available.

6) 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.

Thanks, following this advice we have worked to improve the writing style used in the revised manuscript.

7) 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 carbonic anhydrase.

Please see responses to comments 1) and 4) of Reviewer 1. In brief we have rephrased the hypotheses as suggested and provided caveats about the power of the treatment experiment.

8) 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 kisomay 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 mayalso point at unfavourable conditions for other microbial processes: perhaps also pro-duction of anhydrase activity? In Table 2 the artificially elevated NH4+ 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.

Following this good advice we have removed the unfounded reference to the role of ammonia speciation in explaining the apparent co-correlation between k_{iso}, pH and exchangeable ammonium identified in the Spearman’s rank analysis presented in Table 1. We maintain that the relationship between k_{iso} and exchangeable NH_4^+ is an artefact of it’s relationship with soil pH for two reasons. Firstly, the Spearman's rank correlation between soil pH and exchangeable ammonium is strongly influenced by the co-occurrance of low exchangeable ammonium in the high pH soils that exhibit greater k_{iso} (Figure 2 a) & e); groups Csa, Bsh and Cfa). Secondly, we do not find support for a role of exchangeable ammonium in explaining variability in k_{iso} in the subsequent analyses (Tables S1 & S3). Please also see the response to Reviewer 1, comment 1).

9) It is unfortunate that the authors opted for an enourmous dose of NH4NO3, viz 0.7 mg NH4NO3 g-1 (L168). No doubt such a large addition of NH4+ 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 NH4+ and NO3- levels intreated soil would not reflect environmentally realistic exchangeable N levels. That would severely limit the relevance of Fig. 5. Or is the 0.7 mg NH4NO3 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 NH4+ and NO3- levels alongside changes in pH brought forth by the NH4NO3 administration as based on the fractorial changes in NO3- now presented it is impossible to judge whether or not excessive amounts of N had been added. This firstly needs to be clarified.

The fertilisation rate of 0.7 mg NH4NO3 per gram of dry soil (0.25 mg of N per gram of dry soil) was adopted from Ramirez, Craine and Fierer (2012). The justification for this value (also used in Kaisermann et al., 2018) was that it approximates typically applied fertilizer loads in field studies. We have added a figure to the supplementary material (Figure S3) to provide information about the absolute values of measured parameters in both the untreated controls and treated soils and allow comparison with the wider dataset in Figure 2. The median increase in exchangeable nitrate and ammonium in the treated over untreated soils was 22 and 10 times, respectively.

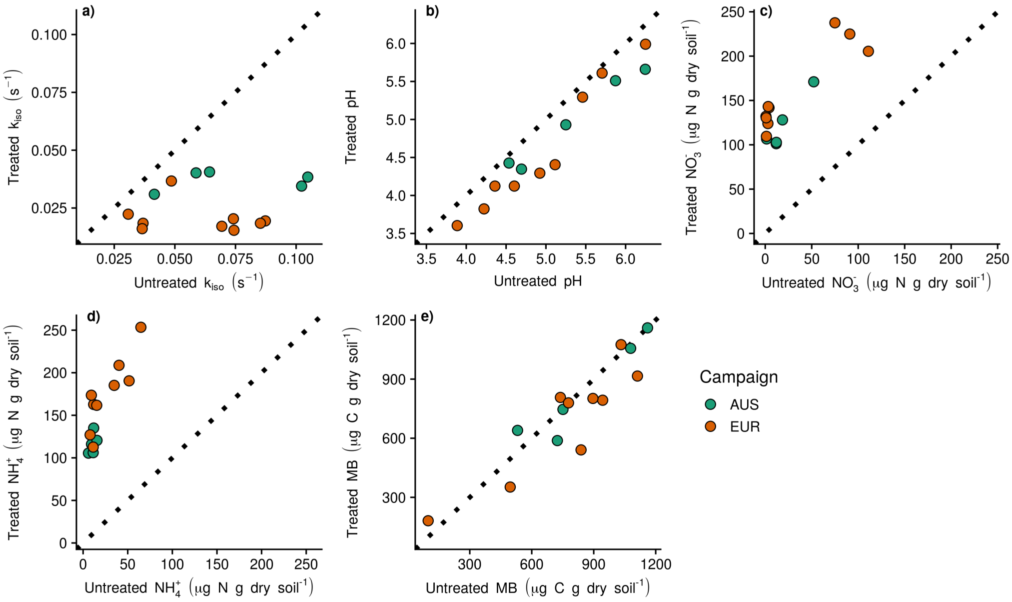

Figure S3: Mean a) k_{iso}, b) pH, c) exchangeable nitrate (NO_3^-), d) exchangeable ammonium (NH_4^+) and e) microbial biomass (MB) for the untreated control and the corresponding treated soils. Dashed lines indicating the 1:1 line with points below the line representing a decrease in treated relative to untreated soils and points above the line representing an increase. Points falling along the line indicate no change.

Ramirez, K. S., Craine, J. M. and Fierer, N.: Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes, Global Change Biology, 18(6), 1918–1927, https://doi.org/10.1111/j.1365-2486.2012.02639.x, 2012.

Kaisermann, A., Jones, S. P., Wohl, S., Ogée, J. and Wingate, L.: Nitrogen Fertilization Reduces the Capacity of Soils to Take up Atmospheric Carbonyl Sulphide, Soil Systems, 2(4), 62, https://doi.org/10.3390/soilsystems2040062, 2018.

10) 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).

Thanks, we have corrected this.

11) L21 suggest to omit 'future'

Thanks, done.

12) L31 ‘the δ18O of leaf-atmosphere CO2 exchange’ reads strange, sounds clearer with-out ‘exchange’

We have modified this sentence to make it clearer in general but maintain the use of the word ‘exchange’ as it is important to do so.

13) L 35 CO2 that interacts with a leaf – interacts seems too vague, could this be reworded?

This has been reworded in response to Reviewer 1, comment 27). It now reads: “This is 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).”

14) L36 ‘undergo considerable enrichment’ of what?
Thanks, we have rephrased this section : “As leaf water pools are small and undergo considerable evaporation, the $\delta^{18}O$ of leaf water is generally enriched relative to that of the soil water (Wingate et al., 2010). Consequently, the $\delta^{18}O$ of CO_2 molecules that have interacted with leaf and soil water pools are also isotopically distinct from one another and can be used to constrain the contribution of soils and vegetation to the atmospheric $CO^{18}O$ mass balance (Francey & Tans, 1987).”

15) L40 ‘alters the δ18O of atmospheric CO2’ was not really clear to me. Perhaps write ‘alters the δ18O of CO2 in soil vs. atmospheric CO2’?
We have simplified this sentence “Currently, the abundance and activity of carbonic anhydrases in soil is poorly understood complicating our abilities to predict the influence of soil communities on the oxygen isotope composition of atmospheric CO_2.”

16) L51 is ‘of soil atmospheric CO2’ not better?

This section has been restructured in response to Reviewer 1, comment 28).

17) L56 ‘abiotically invades’ sounds awkward, perhaps write ‘diffuses into the soil porenetwork’

We specifically used the term “invade” as we were referring to the invasion flux of CO_2 (also known as the piston velocity of the overlying air-column). However, to simplify we have changed the text to “that diffuses within the soil profile”

18) L65 delete ‘true’, is confusing here

Thanks, deleted.

19) L99 better ‘agricultural soils’?

Thanks, corrected ‘agriculture’ to ‘agricultural’

20) L166 ‘an additional three replica incubations’ correct English?

We have re-written this paragraph to improve the clarity of our methodology.

“An NH_4NO_3 addition experiment was also conducted in both campaigns. This involved the preparation of three additional replicated incubations as described above, for nine of the EUR sites and five of the AUS sites. Prior to the pre-incubation step, 0.7 mg of NH_4NO_3 g dry soil^{-1} was dissolved in water and used to adjust the water content of three of the replicated incubations. These were then incubated with the three other ‘control’ incubations in the same control chamber.”

21) 2.1 Not clear if soils were kept cool during transport

We have clarified that EUR samples were transported at ambient temperatures and that AUS samples were returned to the laboratory on the day of sampling to minimise the time the samples spent without definite temperature control.

22) L273 remove ‘presumably reflecting the pH dependency of NH4+ and ammonia speciation’ this does not belong in the M&M section

Thanks, this has been deleted.

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

It's true there are two sites associated with the Csa group that have high values above the 0.75 quantiles (i.e. the upper hinge of the box). However, these values are lower than the unique case for Bsh and an individual from the Cwa group. Furthermore the median of the Csa group clearly overlaps with the 0.50 to 0.75 quantile of both the Cfa and Cwa groups. On balance we feel that this does not support the conclusion that there is a robust pattern associated with these climate and landcover classifications. We do not attempt to test this statistically simply because despite our efforts the number of replicates for land-cover and climate still remains relatively low and their distribution is unbalanced.

24) L370-375 is quite unclear: are the 0.04 to 13 s-1 field values derived from your labmeasured 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.

We feel it is important to include the comparison of k_{iso} observed in this study with those from the literature. This type of information has not typically been presented in recent publications in part because it is not always easy to compare reported values on an equal basis. However, by attempting to do so we do observe that there are potentially significant discrepancies that should not be ignored. We have adjusted and rephrased this paragraph to hopefully make it clearer:
"The observations of this study, with a median of 0.07 s^{-1}, are in good agreement with a number of previous studies (Jones et al., 2017; Sauze et al., 2018, 2017) that estimated k_{iso} values ranging from 0.03 to 0.15 s^{-1} for sieved soils incubated in the dark. However, these rates are somewhat lower than those reported by Meredith et al. (2019) for dark incubated soils with a median and range of 0.46 s^{-1} and 0.08 to 0.88 s^{-1}, respectively. These greater k_{iso} values reported by Meredith et al. (2019) are more comparable to those, ranging from 0.01 to 0.75 s^{-1}, reported by Sauze et al. (2017) for soils with well developed algal communities. Direct comparison of our estimates k_{iso} with those observed in the field is challenging because these older studies (Seibt et al., 2006; Wingate et al., 2008, 2009, 2010) estimated soil carbonic anhydrase activity as a range of enhancement factors over a temperature sensitive uncatalysed rate of hydration . However, using the mid-point of the enhancement factors and soil temperatures reported by Wingate et al. (2009), we estimate that k_{iso} varied between 0.04 and 13 s^{-1} with a median of 0.31 s^{-1} across the seven ecosystems considered in their analysis . Understanding why k_{iso} has the potential to be orders of magnitude greater in the field compared to values observed in lab 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 k_{iso} (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 k_{iso} robustly in the field (Jones et al., 2017)."

25) L431 'between untreated and treated' is not a very clear phrasing – spell out betterwhat you are referring at.
We have rephrased this to hopefully make the meaning clearer:

"Indeed, the ability of this model to reasonably predict fractional changes in k_{iso} between untreated control soils, that were used to build the model, and their fertiliser treated counterparts, that were not used to 'train' the model selection process, is encouraging (Figure 4 b)."

26) L434 'A significant challenge to using this relationship to predict kiso is likely the avail-ability of suitable pedotransfer functions,particularly for NO3−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 NO3- levels with asimple pedotransfer function. The authors best refer to use soil N models to predictNO3 levels and use these as inputs into Eq 6.

We have rephrased this section in response to Reviewer 1, comment 22) and refer to the need for dynamic models in predicting these parameters: "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)."

Zaehle S. 2013 Terrestrial nitrogen–carbon cycle interactions at the global scale. Phil Trans R Soc B 368: 20130125. http://dx.doi.org/10.1098/rstb.2013.0125