Dear Referee #1

Thank you very much for your valuable comments on our manuscript. We would like to respond to each of your comments one by one.

[1] Section 2.3: While extraction with 2 M KCl is standard for measuring extractable soil nitrate and ammonium, significant loss of soil nitrite can occur during the extraction. This issue is well recognized in the soil nitrogen cycling community (e.g., Homyak et al., 2015). Given that nitrite concentration and $\Delta^{17}O$ measurements are critical to this study, could the authors discuss how potential nitrite loss and associated isotopic fractionation might impact their analysis?

Thank you for your question. While the 2M KCl extraction is widely used for soil nitrite (NO_2^-) analysis (Lewicka-Szczebak et al., 2021; Shen et al., 2003), we also notice the concerns raised by Homyak et al. (2015) regarding possible underestimation of soil nitrite concentrations when using KCl solutions compared to deionized water.

To evaluate this potential issue, we conducted a comparative experiment in April 2022 prior to this study. We collected a soil sample from our study site (secondary warm-temperate forest, Figure 2b), which was thoroughly homogenized and divided into two 50 g subsamples. Each subsample was then extracted with either 50 mL of 2M KCl solution or 50 mL MQ water, following the same analytical procedures described in Sections 2.3 and 2.7 of the manuscript.

Our results showed consistent values between the two extraction methods: the KCl-extracted sample yielded a nitrite concentration of 0.90 μM with $\Delta^{17}O$ of 0.55±0.1‰, while the MQ water-extracted sample showed 0.98 μM nitrite with $\Delta^{17}O$ of 0.62±0.1‰. Because both the concentration and $\Delta^{17}O$ value of soil nitrite in KCl solution and MQ water showed no significant differences, we concluded that for our soil type and experimental conditions, the use of 2M KCl solution introduced negligible bias in terms of nitrite recovery or $\Delta^{17}O$ measurements compared to MQ water extraction.

[2] Section 2.4: The manuscript uses a β (triple oxygen proportionality factor) range of 0.525 to 0.5305 to quantify the potential impact on $\Delta^{17}O$. Although several references are cited, it is unclear why this specific range was chosen. Please clarify. In particular, earlier studies (e.g., Matsuhisa et al., 1978; summarized by Miller, 2002) reported lower β values (e.g., ~0.5164). How would using lower β values affect the results?

Thank you for the comment. Our selection of this range was based on evidence from recent experimental and theoretical studies examining oxygen isotope fractionation across various compounds (CO, O_2, NO, CO_2, NO_2, H_2O, SO_2, SO_3,

CO_3^{2-}, and SiO_2), as documented by (Cao and Liu, 2011; Pack and Herwartz, 2014; Sharp and Wostbrock, 2021). These studies collectively demonstrate that this range encompasses most equilibrium and kinetic fractionation processes. Thus, for the calculation of $\Delta^{17}O$ value of N_2O, we adopted the midpoint value ($\beta = 0.528$) of this range.

Regarding your concern about lower β values reported in earlier work (Matsuhisa et al., 1978), we note that while Matsuhisa et al. (1978) did observe β values as low as 0.5164 in some terrestrial rock and water samples, they ultimately choose 0.52 for the quartz-water system as the most representative value. To address how such lower β values might affect our results, we quantified the possible variations in the $\Delta^{17}O$ values of N_2O during each reaction using $\beta = 0.52$. Our calculations (following the methodology in Section 4.1 and Figure 7 in the manuscript) show that this would introduce variations in $\Delta^{17}O$ values of N_2O (derived from soil NO_2^- and O_2) of less than 0.2 ‰ (Figure R1). This potential variation is significantly smaller than the observed $\Delta^{17}O$ difference between O_2 and NO_2^- in our forested soil samples (average 0.7‰; Figure 4c). We concluded that even using such low β value ($\beta = 0.52$), our key findings or interpretations can't be affected.

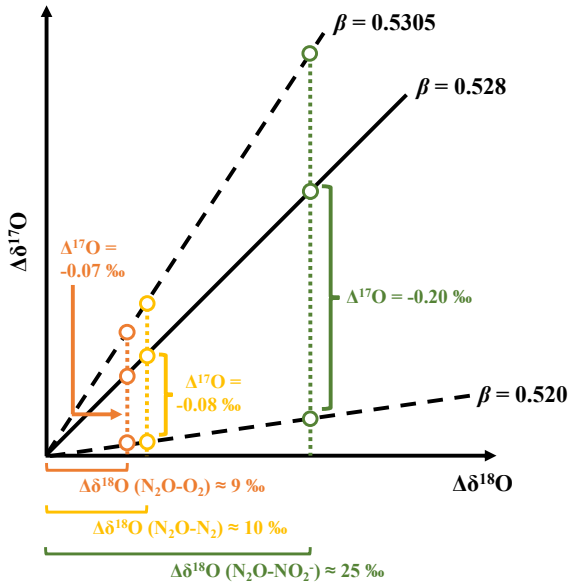

Figure R1. Schematic showing the possible variations in the $\Delta^{17}O$ value of N_2O from that of the source of O atoms (O_2 and NO_2^-) during transformations, including nitrification (orange circles), denitrification (green circles), and reduction (yellow circles), due to variations in isotope fractionation and β from 0.520 to 0.5305

[3] Lines 362–363 and throughout the analysis: A constant $\Delta^{17}O$ value was assumed for O_2 (-0.44‰) and soil H_2O (0.03‰). Please clarify whether these values could vary due to hydrological and biogeochemical cycling. For instance, could O_2 diffusion and heterotrophic consumption affect O_2 $\Delta^{17}O$, or could evaporation significantly alter soil H_2O $\Delta^{17}O$?

Thank you for the question. The $\Delta^{17}O$ values of O_2 (−0.44 ‰) and soil H_2O (+0.03 ‰) used in this study was referred from that of atmospheric O_2 (Sharp et al., 2016) and rainwater (Uechi and Uemura, 2019), respectively.

For soil O_2, Aggarwal and Dillon (1998) measured $\delta^{18}O$ values in soil gas at a depth of 3-4 m at a site near Lincoln, Nebraska, USA ranged from +23.3 ‰ to +27.2 ‰ (Table R1), showing the values were comparable with that of atmospheric O_2 (+23.5 ‰ after adjustment in Aggarwal and Dillon. 1998). This confirms that the isotopic fractionation of soil O_2 induced from soil respiration and diffusion processes wasn't significant. Because the maximum variation in $\delta^{18}O$ from atmospheric O_2 to soil O_2 was less than 1.9 ‰ (27.2 ‰ − 23.5 ‰), using the method presented in Section 4.1 and Figure 7, we quantified the possible variations in the $\Delta^{17}O$ value of soil O_2 from that of atmospheric O_2 to be less than 0.006 ‰. Thus, we ignored the negligible variations in the manuscript.

Similarly, for soil H_2O, Lyu (2021) observed that $\delta^{18}O$ values in soil H_2O at the depths of 0-5 cm, 15-20 cm, and 40-45 cm in a subtropical forest plantation ranged from −4 ‰ to −10 ‰ (Figure R2), which fully overlapped with local rainwater (−1 ‰ to −16 ‰), indicating insignificant isotopic fractionations of soil H_2O during hydrological process such as infiltration and evaporation compared to rainwater. Besides, Aron et al. (2021) compiled $\Delta^{17}O$ values of terrestrial H_2O including rainwater, surface and subsurface water in earth, ranged from +0.06 to −0.06 ‰ and didn't show significant difference with each other, which also indicating that the possible variations of $\Delta^{17}O$ values of soil H_2O compared to that of rainwater should be negligible. Finally, we would like to add the variations of $\Delta^{17}O$ values (+0.06 / −0.06 ‰) of terrestrial H_2O reported in Aron et al. (2021) to Figures 4 and 6 as the uncertainties of $\Delta^{17}O$ values of soil H_2O in the revised manuscript.

Table R1. Concentration and isotopic compositions of soil gas oxygen and carbon dioxide from the midwestern USA site (Aggarwal and Dillon. 1998).

Location	Depth (m)	Date	CO_2 (%)	O_2 (%)	$\delta^{18}O$-O_2 [a] ‰, SMOW	$\delta^{13}C$-CO_2 [a] ‰, PDB
1b	2.9	June 94	1.8	13.8	27.2	−21.8
	2.9	Dec. 94	2.3	15.1	24.2	−21.7
2b	3.4	June 94	0.7	16.3	25.1	−22.4
2b	3.4	Dec. 94	0.9	15.2	23.3	−22.8
3b	4.1	June 94	0.7	15.7	25.3	−22.1
3b	4.1	Dec. 94	1.4	15.2	25.1	−24.0
5b	3.2	June 94	0.8	17.6	24.7	−21.7
5b	3.2	Dec. 94	1.0	16.3	24.7	−21.0
4b	3.2	June 94	2.3	17.1	24.5	−20.0
4b	3.2	Dec. 94	3.0	16.0	24.0	−19.9

[a] Isotopic values reported are averages of duplicate analyses with a standard deviation of 0.3‰ for $\delta^{18}O$ and 0.2‰ for $\delta^{13}C$. The oxygen isotope ratios have been adjusted to an atmospheric oxygen $\delta^{18}O$ of 23.5 ‰.

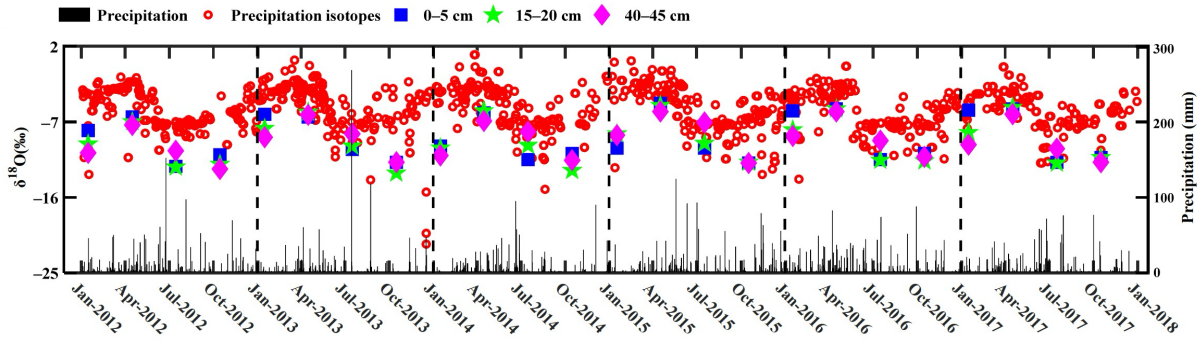

Figure R2. Temporal variations of the amount of precipitation, $\delta^{18}O$ in precipitation, and weighted average $\delta^{18}O$ in soil water source during winter, spring, summer, and autumn, at 0–5 (0–5 cm), 15–20 (15–20 cm), and 40–45 (40–45 cm) cm depths (Lyu. 2021).

[4] Lines 378–397: The characterization of $\delta^{18}O$ offsets between O_2 and N_2O, and between NO_2^- and N_2O, does not necessarily represent true isotope effects between N_2O and its oxygen precursors because field-measured N_2O is a mixture of multiple sources. For example, in Fig. 6a, the actual $\delta^{18}O$ difference between NO_2^- and N_2O may be larger than calculated if O_2-derived N_2O has $\delta^{18}O$ values similar to that of O_2. Similarly, the O_2-N_2O difference may be smaller than estimated. This mixing effect could confound the use of $\delta^{18}O$ differences to estimate $\Delta^{17}O$ variations and warrants further clarification.

Thank you for your comment. You mentioned that the true field-measured N_2O is a mixture of multiple sources is correct. However, the mixture ratios of N_2O produced through nitrification and denitrification were unknown. Thus, in our theoretical calculations for the possible variations in the $\Delta^{17}O$ values of N_2O in Section 4.2, we separated the nitrification and denitrification to discuss the possible variations.

After we supposed that if all O atoms in N_2O were derived from O_2 (Lines 377-378), the average in $\delta^{18}O$ from O_2 to N_2O due to nitrification ($\Delta\delta^{18}O$ (N_2O-O_2)) was estimated to be 9 ‰ on average. Similarly, after we supposed that if all O atoms in N_2O were derived from NO_2^- (Lines 391-392), the average variation in $\delta^{18}O$ from NO_2^- to N_2O due to fractionation ($\Delta\delta^{18}O(N_2O-NO_2^-)$) was estimated to be 25 ‰ on average.

[5] Fig. 7 and related discussion: I commend the authors for conducting a sensitivity analysis to assess how much $\Delta^{17}O$ variation may stem from biogeochemical processes versus purely geochemical processes (i.e., β variability). However, applying the β range to the net $\delta^{18}O$ difference between N_2O and oxygen sources treats the N_2O-producing processes as a single step. In reality, processes like nitrite reduction involve multiple sub-steps (e.g., NO_2^- to NO, NO to N_2O, isotope exchange with H_2O), each potentially associated with different β

values. This could lead to larger $\Delta^{17}O$ variations than those estimated from a single-step approach. This limitation should be discussed.

Thank you for your comment. Because the β values for processes of NO_2^- to NO and NO to N_2O should be included in the range of 0.525 to 0.5305 (Cao and Liu, 2011; Matsuhisa et al., 1978; Pack and Herwartz, 2014; Sharp and Wostbrock, 2021), the processes of NO_2^- to NO and NO to N_2O were merged into the process of denitrification for the theoretical calculations for the possible variations in the $\Delta^{17}O$ values of N_2O. As a result, the calculated possible variations in $\Delta^{17}O$ during denitrification (less than 0.075 ‰) incorporated the β variability across these sub-steps.

Besides, because the estimated $\Delta^{17}O$ values of soil NO_2^- included the effect of oxygen isotope exchange between soil NO_2^- and H_2O, the oxygen isotope exchange between soil NO_2^- and H_2O can't affect the $\Delta^{17}O$ values of N_2O.

Additionally, because we concluded the contributions of O atoms derived from soil H_2O were minor during the reduction of NO_2^- and oxidation of NH_4^+ to produce N_2O, the discussion for possible variations in $\Delta^{17}O$ values of N_2O due to the process of the contribution of O atoms derived from soil H_2O was ignored.

[6] Lines 434–438: It is unclear how the 24% contribution of soil H_2O was derived.

This calculation was based on isotopic mass balance. In the plot fertilized with CS, the average $\Delta^{17}O$ value of N_2O emitted from the soil 2 and 6 days after the fertilization was +7.79 ‰ (Lines 429-431). The $\Delta^{17}O$ value of the possible source of O atoms in N_2O was +10.30 ‰ for soil NO_2^-, +0.03 ‰ for soil H_2O, and −0.44 ‰ for O_2, respectively. If all O atom in N_2O were derived from soil H_2O (+0.03 ‰), the contribution of O atoms derived from soil H_2O was calculated to be 24 % ((10.30 ‰ – 7.79 ‰) / (10.3 ‰ – 0.03 ‰)). If the O_2 also contributes to the N_2O production, the contribution of O atoms derived from soil H_2O should be smaller (less than 24 %). As a result, we concluded that the maximum possible contribution of O atoms derived from soil H_2O during the reduction of NO_2^- to N_2O was 24 % (Lines 428-439). We would like to clarify that in the revised manuscript.

Additionally, Fig. 6b shows that the $\Delta^{17}O$ of N_2O in the CS plot was significantly lower than that of NO_2^- six days after tracer addition. This suggests that soil H_2O may have played a significant role during nitrite reduction to N_2O.

Thank you for your comment. Compared to the value observed 2 days after fertilization, the $\Delta^{17}O$ value of N_2O emitted from the soil in the CS plot 6 days after fertilization became lower than that of soil NO_2^- (Figure 6b), implying that (1) the soil H_2O have played a significant role 6 days after fertilization as suggested, or (2) the

relative contribution of nitrification to N_2O production increased 6 days after fertilization. Because the main pathway to produce N_2O was nitrification in the NF plot (no fertilizer addition) (Figure 6b), the diminishing fertilization effect over time resulted in reduced N_2O production through denitrification was responsible for the relative contribution of nitrification to N_2O production increased 6 days after fertilization. The significant decrease in N_2O flux from 112.3 to 39.4 μg N m^{-2} h^{-1} between 2 and 6 days after fertilization further confirm the diminishing fertilization effect over time.

Importantly, similar to 2 days after fertilization, the $\Delta^{17}O$ value of N_2O emitted from the soil in the CS plot 6 days after fertilization (+7.36 ‰) remained closer to that of soil NO_2^- (+12.32 ‰) than that of atmospheric O_2 (−0.44 ‰) and H_2O (+0.03 ‰), consistent with our conclusion (Lines 428-432) that the denitrification became the main pathway of N_2O production in the CS plot.

[7] Lines 444–449 and Fig. 6b: Apparent differences in $\Delta^{17}O$ between soil H_2O and N_2O cannot be used to conclusively rule out H_2O contributions during N_2O production. In the NF and U plots, the $\Delta^{17}O$ of soil H_2O lies between that of NO_2^- and N_2O, and both soil H_2O and NO_2^- have higher $\Delta^{17}O$ than O_2. Could significant H_2O exchange during N_2O production explain these observations, leading to a mixed $\Delta^{17}O$ signal from both H_2O- and O_2-derived N_2O?

Thank you for your comment.

In NF plot, the average $\Delta^{17}O$ value of N_2O (−0.35 ‰) measured 2 and 6 days after fertilization was close to that of O_2 (−0.44 ‰) compared to that of soil H_2O (+0.03 ‰) and soil NO_2^- (+0.38 ‰) (Figure 6b), implying that the O atoms in N_2O mainly derived from O_2 rather than soil H_2O. Thus, the H_2O contribution during N_2O production can't be significant in this case.

In U plot, while the significant H_2O contribution during N_2O production could explain the $\Delta^{17}O$ value of N_2O becoming higher than that in NF plot after fertilization, the observed increases in the emission flux of N_2O from the soil in NF plot (from 4.7 to 63.7 μg N m^{-2} h^{-1}; Table S1 in supplement) can't be explained by the significant H_2O contribution during N_2O production. Thus, we maintain our conclusion that the increase in N_2O production through NO_2^- reduction was responsible for the $\Delta^{17}O$ values of N_2O produced in the U plot in response to fertilization of urea/NH_4^+ for the reasons described in the manuscript (Lines 490-514).

[8] Section 4.5: Early in the manuscript, the authors argue that bulk isotopic and SP-based techniques for N_2O source apportionment are limited due to isotopic fractionations during cycling (lines 56-61), whereas $\Delta^{17}O$ measurements may be more robust. After presenting the results, I would encourage the authors to revisit this point with more specificity. Given potential complications such as H_2O exchange and multiple contributing sources (H_2O, O_2, NO_2^-), can $\Delta^{17}O$

measurements realistically achieve quantitative source apportionment? If so, what would the total uncertainty be, considering analytical precision, β variability, and uncertainties from the Keeling approach? Under what conditions would $\Delta^{17}O$ approaches be preferable to conventional methods, and when might they be less effective?

Thank you for your suggestion. We would like to add the uncertainty information for using $\Delta^{17}O$ as a natural signature for identifying N_2O production pathways including H_2O contributions, analytical precision, and β variability in Section 4.5 in the revised manuscript.

We would like to thank you for the helpful comments. We hope that our responses to your comments are satisfactory.

Sincerely,
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Reference

Aggarwal, P. K. and Dillon, M. A.: Stable Isotope Composition of Molecular Oxygen in Soil Gas and Groundwater: A Potentially Robust Tracer for Diffusion and Oxygen Consumption Processes, Geochimica et Cosmochimica Acta, 62, 577–584, https://doi.org/10.1016/S0016-7037(97)00377-3, 1998.

Aron, P. G., Levin, N. E., Beverly, E. J., Huth, T. E., Passey, B. H., Pelletier, E. M., Poulsen, C. J., Winkelstern, I. Z., and Yarian, D. A.: Triple oxygen isotopes in the water cycle, Chemical Geology, 565, 120026, https://doi.org/10.1016/j.chemgeo.2020.120026, 2021.

Cao, X. and Liu, Y.: Equilibrium mass-dependent fractionation relationships for triple oxygen isotopes, Geochimica et Cosmochimica Acta, 75, 7435–7445, https://doi.org/10.1016/j.gca.2011.09.048, 2011.

Homyak, P. M., Vasquez, K. T., Sickman, J. O., Parker, D. R., and Schimel, J. P.: Improving Nitrite Analysis in Soils: Drawbacks of the Conventional 2 M KCl Extraction, Soil Science Society of America Journal, 79, 1237–1242, https://doi.org/10.2136/sssaj2015.02.0061n, 2015.

Lewicka-Szczebak, D., Jansen-Willems, A., Müller, C., Dyckmans, J., and Well, R.: Nitrite isotope characteristics and associated soil N transformations, Sci Rep, 11, 5008, https://doi.org/10.1038/s41598-021-83786-w, 2021.

Lyu, S.: Variability of δ^2H and $\delta^{18}O$ in Soil Water and Its Linkage to Precipitation in an East Asian Monsoon Subtropical Forest Plantation, Water, 13, 2930, https://doi.org/10.3390/w13202930, 2021.

Matsuhisa, Y., Goldsmith, J. R., and Clayton, R. N.: Mechanisms of hydrothermal crystallization of quartz at 250°C and 15 kbar, Geochimica et Cosmochimica Acta, 42, 173–182, https://doi.org/10.1016/0016-7037(78)90130-8, 1978.

Pack, A. and Herwartz, D.: The triple oxygen isotope composition of the Earth mantle and understanding $\Delta^{17}O$ variations in terrestrial rocks and minerals, Earth and Planetary Science Letters, 390, 138–145, https://doi.org/10.1016/j.epsl.2014.01.017, 2014.

Sharp, Z. D. and Wostbrock, J. A. G.: Standardization for the Triple Oxygen Isotope System: Waters, Silicates, Carbonates, Air, and Sulfates, Reviews in Mineralogy and Geochemistry, 86, 179–196, https://doi.org/10.2138/rmg.2021.86.05, 2021.

Sharp, Z. D., Gibbons, J. A., Maltsev, O., Atudorei, V., Pack, A., Sengupta, S., Shock, E. L., and Knauth, L. P.: A calibration of the triple oxygen isotope fractionation in the SiO_2–H_2O system and applications to natural samples, Geochimica et Cosmochimica Acta, 186, 105–119, https://doi.org/10.1016/j.gca.2016.04.047, 2016.

Shen, Q. R., Ran, W., and Cao, Z. H.: Mechanisms of nitrite accumulation occurring in soil nitrification, Chemosphere, 50, 747–753, https://doi.org/10.1016/S0045-6535(02)00215-1, 2003.

Uechi, Y. and Uemura, R.: Dominant influence of the humidity in the moisture source region on the ^{17}O-excess in precipitation on a subtropical island, Earth and Planetary Science Letters, 513, 20–28, https://doi.org/10.1016/j.epsl.2019.02.012, 2019.