June 15, 2025

Dr. David McLagan
Editor of Biogeosciences

Title: Triple oxygen isotope evidence for the pathway of nitrous oxide production in a forested soil with increased emission on rainy days
Authors: Weitian Ding et al.
MS No.: egusphere-2025-996

Dear Dr. David McLagan:

Thank you very much for handling our manuscript. We would like to thank the referees as well for the constructive comments on our manuscript.

We have carefully studied the comments and revised the manuscript accordingly. We include below point-by-point responses to the comments, and detailed descriptions of the modifications we made to the manuscript. Besides, we also uploaded the revised manuscript in MS Word, in which all the revisions from BGD version were recorded.

Specifically, we have incorporated clarifications in response to Reviewer 1's comments [1], [3], [6], and [8] into the revised manuscript. Additionally, we have implemented some of Reviewer 2's comments throughout the revised manuscript.

We hope that with these changes you will find our revised manuscript appropriate for publication in your journal.

Sincerely yours,
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Cc: Drs. Urumu Tsunogai and Fumiko Nakagawa

Response to the referee #1:

[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?

While the 2M KCl extraction is widely used for soil nitrite (NO_2^-) analysis (e.g., Lewicka-Szczebak et al., 2021; Shen et al., 2003), Homyak et al. (2015) raised the concerns that the recovery of soil NO_2^- could be low 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, 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 used in this study.

Our results showed consistent values between the two extraction methods: the KCl-extracted sample yielded a NO_2^- concentration of 0.90 μM with $\Delta^{17}O$ of 0.55±0.1‰, while the MQ water-extracted sample showed a NO_2^- concentration of 0.98 μM with $\Delta^{17}O$ of 0.62±0.1‰. Because both the concentration and $\Delta^{17}O$ value of soil NO_2^- 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 NO_2^- recovery or $\Delta^{17}O$ measurements compared to MQ water extraction.

We emphasized this in the revised manuscript (P13/L276-283) and supplement (P2/L36-52).

[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?

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 (β = 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 β = 0.52. Our calculations

(following the methodology in Section 4.1 and Figure 7) 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 (β = 0.52), our key findings or interpretations can't be affected.
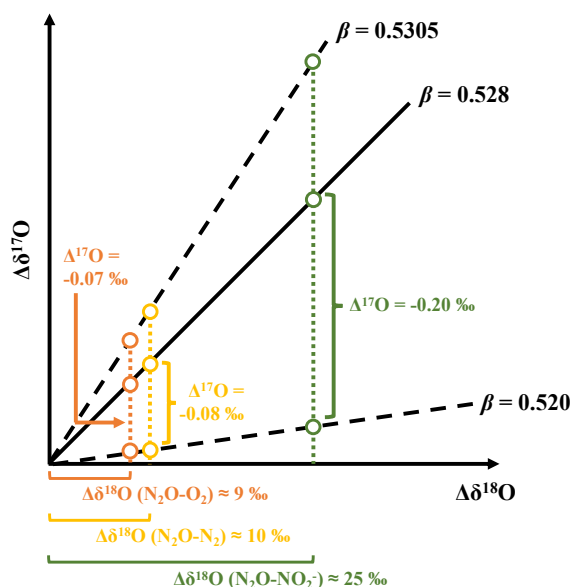
Figure R1. Schematic showing the possible variations in the $\Delta^{17}O$ value of N_2O from that of the source of O atoms (O_2 and NO_2^-) during transformations, including nitrification (orange circles), denitrification (green circles), and reduction (yellow circles), due to variations in isotope 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 $\Delta^{17}O$?

While the $\Delta^{17}O$ values of soil O_2 and H_2O used in this study were referred from atmospheric O_2 and rainwater, respectively, the processes in soil, including diffusion and respiration of O_2 and evaporation and infiltration of rainwater, may cause significant isotopic fractionations of $\delta^{18}O$, which could consequently alter the $\Delta^{17}O$ values of atmospheric O_2 and rainwater. Thus, we evaluated the possible variations in the $\Delta^{17}O$ values of O_2 and H_2O in soil compared to those of atmospheric O_2 and rainwater. The details are presented below.

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 3.7 ‰ (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 0.01 ‰. 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 added 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.

We added this information into the revised manuscript (P20-21/L424-451).

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 (%)	$\delta^{18}O\text{-}O_2$[a] ‰, SMOW	$\delta^{13}C\text{-}CO_2$[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 ‰.
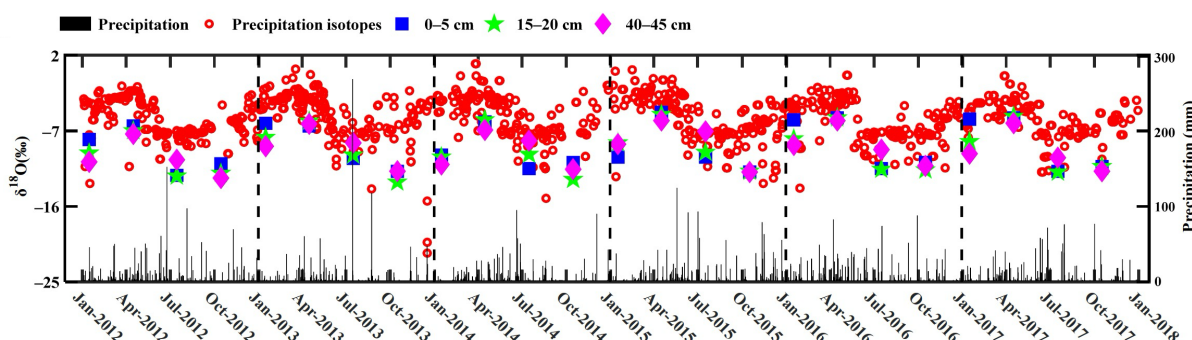
Figure R2. Temporal variations of the amount of precipitation, $\delta^{18}O$ in precipitation, and weighted average $\delta^{18}O$ in soil water source during winter, spring, summer, and autumn, at 0–5 (0–5 cm), 15–

20 (15–20 cm), and 40–45 (40–45 cm) cm depths (Lyu. 2021).

[4] Lines 378–397: The characterization of δ¹⁸O offsets between O₂ and N₂O, and between NO₂⁻ and N₂O, does not necessarily represent true isotope effects between N₂O and its oxygen precursors because field-measured N₂O is a mixture of multiple sources. For example, in Fig. 6a, the actual δ¹⁸O difference between NO₂⁻ and N₂O may be larger than calculated if O₂-derived N₂O has δ¹⁸O values similar to that of O₂. Similarly, the O₂-N₂O difference may be smaller than estimated. This mixing effect could confound the use of δ¹⁸O differences to estimate Δ¹⁷O variations and warrants further clarification.

You mentioned that the true field-measured N₂O is a mixture of multiple sources is correct. However, the mixture ratios of N₂O produced through nitrification and denitrification were unknown. Thus, in our theoretical calculations for the possible variations in the $\Delta^{17}O$ values of N₂O in Section 4.2, we separated the nitrification and denitrification to discuss the possible variations.

After we supposed that if all O atoms in N₂O were derived from O₂, the average in $\delta^{18}O$ from O₂ to N₂O due to nitrification ($\Delta\delta^{18}O$ (N₂O−O₂)) was estimated to be 9 ‰ on average. Similarly, after we supposed that if all O atoms in N₂O were derived from NO₂⁻, the average variation in $\delta^{18}O$ from NO₂⁻ to N₂O due to fractionation ($\Delta\delta^{18}O$(N₂O−NO₂⁻)) 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 Δ¹⁷O variation may stem from biogeochemical processes versus purely geochemical processes (i.e., β variability). However, applying the β range to the net δ¹⁸O difference between N₂O and oxygen sources treats the N₂O-producing processes as a single step. In reality, processes like nitrite reduction involve multiple sub-steps (e.g., NO₂⁻ to NO, NO to N₂O, isotope exchange with H₂O), each potentially associated with different β values. This could lead to larger Δ¹⁷O 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 NO₂⁻ to NO and NO to N₂O 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 NO₂⁻ to NO and NO to N₂O were merged into the process of denitrification for the theoretical calculations for the possible variations in the $\Delta^{17}O$ values of N₂O. 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 NO₂⁻ included the effect of oxygen isotope exchange between soil NO₂⁻ and H₂O, the oxygen isotope exchange between soil NO₂⁻ and H₂O can't affect the $\Delta^{17}O$ values of N₂O.

Additionally, because we concluded the contributions of O atoms derived from soil H₂O were minor during the reduction of NO₂⁻ and oxidation of NH₄⁺ to produce N₂O, the discussion for possible variations in $\Delta^{17}O$ values of N₂O due to the process of the contribution of O atoms derived from soil H₂O was ignored.

[6] Lines 434–438: It is unclear how the 24% contribution of soil H₂O 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 ‰. 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 the O atoms with low $\Delta^{17}O$ values in N_2O were derived from soil H_2O (+0.03 ‰) in the CS plot, the contribution of O atoms derived from soil H_2O was calculated to be 24 % ((10.30 ‰ – 7.79 ‰) / (10.30 ‰ – 0.03 ‰)). If the O_2 also contributed to the N_2O production in the CS plot, the contribution of O atoms derived from soil H_2O should be further reduced. As a result, we determined that the maximum possible contribution of O atoms derived from soil H_2O during the reduction of NO_2^- to N_2O was 24 %.

We clarified that in the revised manuscript (P22/L476-482).

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.

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 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?

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^+.

[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?

We added the uncertainty information for using $\Delta^{17}O$ as a natural signature for identifying N_2O production pathways including H_2O contributions and β variability in Section 4.5 in the revised manuscript (P26-27/L575-579).

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Response to the referee #2:

1. The study site is very local. The study site's soil type, vegetation, and climate should be contextualized relative to other ecosystems to assess broader applicability.

In this study, we focused on monitoring the temporal variations of $\Delta^{17}O$ of soil N_2O to evaluate whether the $\Delta^{17}O$ of N_2O can be a signature for identifying the main pathway of N_2O production.

While the current work emphasizes temporal variations of $\Delta^{17}O$ of soil N_2O at a forested soil, we acknowledge the importance of contextualizing these findings across diverse ecosystems. In futural studies, we plan to investigate the spatial variations of $\Delta^{17}O$ of soil N_2O to access the broader applicability and the connections with the variations of soil type, vegetation, and climate.
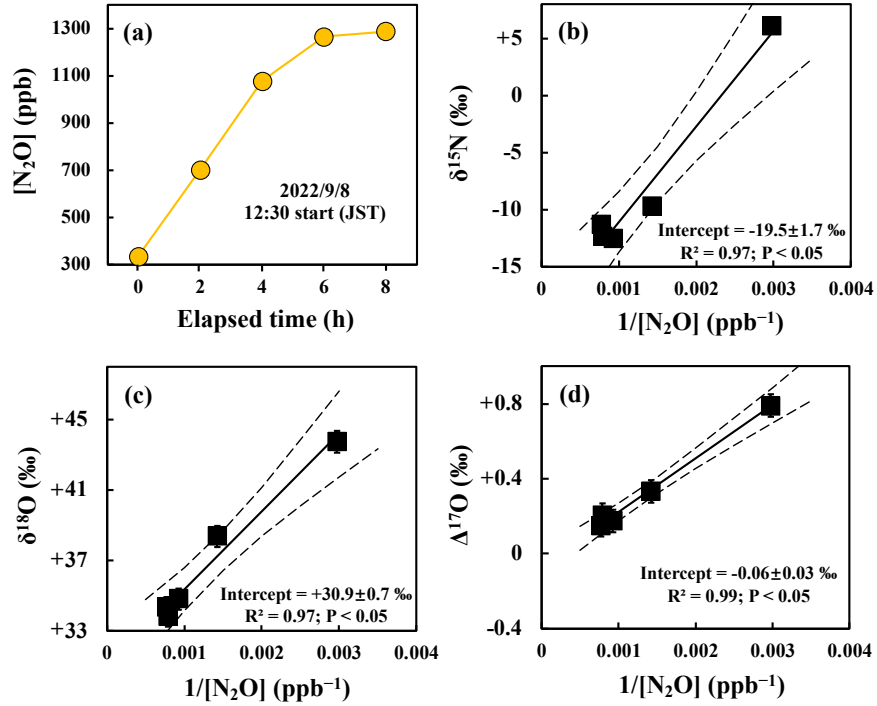
In addition, the sample numbers of N2O gas samples are very limited in this study. For instance, only five data and 18 data are illustrated in Figure 3 and 4.

In Figure 3, the 5 data points represent an example of changes in the concentration and isotopic compositions ($\delta^{15}N$, $\delta^{18}O$, and $\Delta^{17}O$) of N_2O in gas samples during the observation on September 8, 2022. To maintain conciseness, we presented only a subset of data (5 data) in Figure 3 in the main text, while the complete dataset (89 data points) is provided in the supplement (Figure S5).

In Figure 4, 18 data points were estimated from the complete dataset (89 data) using the Keeling plot approach. Importantly, these 18 data points fully support our key findings: (1) N_2O emitted from the soil exhibited significantly higher $\Delta^{17}O$ values on rainy days ($+0.12\pm0.13$ ‰) than on fine days (-0.30 ± 0.09 ‰) and (2) the emission flux of N_2O was significantly higher on rainy days (38.8 ± 28.0 μg N m^{-2} h^{-1}) than on fine days (3.8 ± 3.1 μg N m^{-2} h^{-1}).

The confidence degree of the line fitting and the representative of the results should be further clarified.

We added the confidence degree of the line fitting in Figure 3 in the revised manuscript as follows. We have emphasized that Figure 3 represent an example of changes in the concentration and isotopic compositions of N_2O in gas samples during the observation on September 8, 2022 (P40/L939-943).

The figure shows four panels (a–d):

(a) [N₂O] (ppb) vs Elapsed time (h), with data points increasing and plateauing. Annotation: "2022/9/8 12:30 start (JST)".

(b) $\delta^{15}N$ (‰) vs $1/[N_2O]$ (ppb⁻¹). Intercept = −19.5±1.7 ‰; R² = 0.97; P < 0.05.

(c) $\delta^{18}O$ (‰) vs $1/[N_2O]$ (ppb⁻¹). Intercept = +30.9±0.7 ‰; R² = 0.97; P < 0.05.

(d) $\Delta^{17}O$ (‰) vs $1/[N_2O]$ (ppb⁻¹). Intercept = −0.06±0.03 ‰; R² = 0.99; P < 0.05.

The novelty of the findings and this study should be further highlighted by comparing with prior soil $\Delta^{17}O$ studies.

We highlighted the novelty of this study by comparing it with the prior soil $\Delta^{17}O$ study in the revised manuscript (P5/L86-96) as follows.

Previous studies have identified the elevated $\Delta^{17}O$ values in atmospheric N_2O ($\Delta^{17}O \approx +0.9$ ‰), observed in both stratospheric and tropospheric air (Cliff et al., 1999; Kaiser et al., 2003; Thiemens and Trogler, 1991). Komatsu et al. (2008) subsequently conducted the first $\Delta^{17}O$ measurements of N_2O emitted from a soil to assess whether soil N_2O could be the source of elevated $\Delta^{17}O$ values of atmospheric N_2O. However, the temporal variations of the $\Delta^{17}O$ values for N_2O emitted from soil remain unknown. Besides, whether $\Delta^{17}O$ values of N_2O can be used to identify the pathways of N_2O production in soils has not been discussed. Additionally, the advantages of $\Delta^{17}O$ signature, relative to other natural stable isotopes, for identifying the pathways of N_2O production remain unclear. To address these, in this study, we measured precise $\Delta^{17}O$ values for N_2O emitted from forested soil and those for NO_2^- in the soil.

2. The sample information is missing. The definition of "fine days" and "rainy days" (e.g., precipitation threshold, duration) must be clarified to ensure reproducibility.

In the original manuscript, we have already defined the fine days and rainy days as follows. A fine day is defined as a day without precipitation for 48 hours prior to the end of each sampling. The total precipitation within 12 h at the end of each sampling of the rainy days exceeded 12 mm.

In addition to weather conditions (fine or rainy), the other influencing factors are not

considered and discussed, for instance, soil physical, chemical and microbiological properties, wind speed, air temperature. These factors may affect the implications of the results. For instance, in different seasons, the soil properties, especially soil microorganisms, may largely change and lead to the variation in N2O emission regardless of rainy or fine days. Soil moisture, temperature, and redox data are critical to substantiate the claim that rain-induced anoxia drives denitrification. Their absence weakens causal inferences.

We have discussed the influence of seasons on the variation of N_2O emission in the original manuscript (Lines 293-296 and Lines 299-301). The key soil physical/chemical parameters relevant to determine the pathways of N_2O production in soils, such as bulk density and the concentrations of NH_4^+, NO_3^-, and NO_2^- in soils, were presented in the original manuscript (Lines 105-107) and supplement (Text S1 and Table S1).

In addition, we also have added soil moisture (WFPS) in the manuscript (Section 4.3) and the supplement (Text S1 and Table S1) and included a discussion of redox conditions supporting that rain-induced anoxia drives denitrification.

Finally, in response to your request, while the soil microbiological properties and redox data were unavailable in this study, we added the wind speed and air temperature data to Table S1 in the revised supplement (as follows).

Soil type	Time	T#	Wind speed	P#	WFPS#	[NH$_4^+$]	[NO$_3^-$]	[NO$_2^-$]	Flux-N$_2$O	δ^{18}O (NO$_2^-$)	Δ^{17}O (NO$_2^-$)	δ^{15}N (N$_2$O)	δ^{18}O (N$_2$O)	Δ^{17}O (N$_2$O)
		°C	m / s	mm	%	mg N kg^{-1}			µg N m^{-2} h^{-1}			‰		
Natural soil	2022/4/26	22.3	5.3	0	71.6	11.5	1.2	0.03	3.6	12.03	0.50	-27.5	26.1	-0.32
	2022/6/9	25.2	4.8	0	60.5	7.6	0.9	0.01	0.6	6.72	0.04	-	-	-
	2022/7/11	30.5	3.7	0	77.4	10.1	0.4	0.16	6.9	5.19	0.25	-17.9	37.6	-0.40
	2022/8/8	30.2	3.6	17.5	61.1	8.9	0.4	0.17	6.9	6.98	0.29	-26.6	18.4	0.17
	2022/9/8	26.6	2.1	11.5	92.3	9.5	0.5	0.09	23.7	7.37	0.06	-19.5	30.9	-0.06
	2022/9/13	31.1	3.3	0	69.7	12.5	1.6	0.12	6.0	2.42	0.13	-21	33.2	-0.28
	2022/10/13	20.3	1.5	0	60.9	16.9	1.6	0.21	6.5	3.10	0.09	-21.3	27.6	-0.34
	2022/11/5	17.4	3.7	0	59.6	0.7	5.9	0.03	1.1	4.51	0.21	-21.2	-	-
	2022/12/14	6.4	7.0	0	63.7	9.7	2.2	0.15	0.8	5.84	0.11	-21.1	-	-0.31
	2023/1/29	5.3	3.0	0	74.3	8.5	2.5	0.16	-0.2	6.22	0.24	-	-	-
	2023/3/9	18.9	4.2	0	68.7	8.6	6.0	0.12	2.4	5.55	0.25	-22.4	26.6	-0.26
	2023/3/23	18.4	3.9	16.5	91.5	13.0	3.2	0.45	67.3	5.93	0.29	-25.9	22.7	0.26
	2023/4/7	16.3	5.8	32.5	113.7	11.7	1.2	0.16	77.4	6.91	0.23	-18.5	28.2	0.22
	2023/4/11	19.9	5.2	0	66.2	11.6	1.1	0.23	9.8	6.85	0.20	-21.7	33.4	-0.11
	2023/4/15	13.7	1.9	33.5	108.4	11.7	0.9	0.19	20.0	4.24	0.25	-18.0	31.1	0.18
	2023/5/17	31.2	2.9	0	61.7	10.1	0.7	0.13	3.7	5.75	0.40	-25.3	31.9	-0.34
	2023/6/2	21.4	2.3	137	106.7	6.2	0.03	0.04	37.4	5.79	0.19	-13.8	36.2	-0.03
	2023/7/4	31.1	4.4	0	58.7	7.6	0.1	0.15	3.8	6.25	0.40	-25.8	-	-0.39
Fertilized soil	2023/7/18 NF	34.6	4.1	0	71.9	12.4	2.0	0.20	5.2	2.69	0.42	-17.1	36.1	-0.37
	2023/7/22 NF	30.9	4.7	0	59.4	12.0	2.6	0.26	4.2	1.33	0.35	-12.2	40	-0.32
	2023/7/18 U	34.6	4.1	0	80.3	410.2	5.4	0.10	70.6	7.64	0.31	-39.3	34.4	-0.14
	2023/7/22 U	30.9	4.7	0	62.9	435.9	20.5	0.07	56.7	5.40	0.17	-33.3	25.7	-0.16
	2023/7/18 CS	34.6	4.1	0	47.6	12.9	247.8	0.09	112.3	28.98	8.26	-19.3	54.1	8.22
	2023/7/22 CS	30.9	4.7	0	37.9	18.7	309.0	0.07	39.4	45.24	12.32	-11.3	58.7	7.36

T#: Air temperature; P#: Precipitation; WFPS#: Water-filled pore space

Variability in N₂O fluxes (e.g., ±28.0 μg N m⁻² h⁻¹ on rainy days) warrants discussion (e.g., soil heterogeneity, rain intensity).

While the present results show no significant relationships between N₂O flux and soil moisture (WFPS), precipitation amount, temperature, and wind speed on rainy days (Figures R1a, R1b, R1c, and R1d), we recognize that further rainy-day monitoring, incorporating assessment of factors such as soil heterogeneity and rain intensity, will be needed in the future to explain the observed variability in N₂O flux on rainy days.
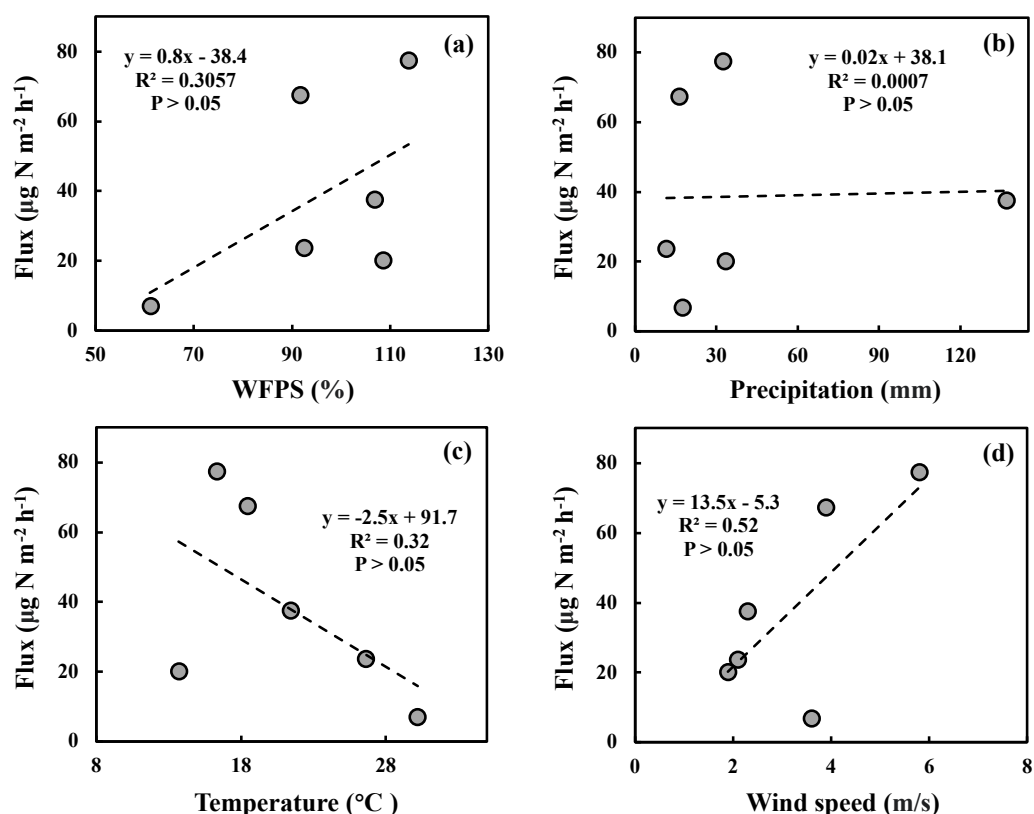
Figure R1. The flux of N₂O on rainy days plotted as a function of the WFPS (a), that of amount of precipitation (b), that of air temperature (c), and that of wind speed (d).

3. The Δ¹⁷O of N₂O on rainy days (+0.12‰) is lower than that of NO₂⁻ (+0.23‰). The authors should address whether this reflects mixing of oxygen sources (e.g., H₂O, O₂) or kinetic fractionation during denitrification.

We have quantitatively assessed the effect of kinetic fractionation during denitrification on the $\Delta^{17}O$ of N₂O in the original manuscript (Lines 388-398) and concluded that the possible range of variations in the $\Delta^{17}O$ value of N₂O from that of NO₂⁻ to be less than 0.075 ‰. Thus, the lower $\Delta^{17}O$ of N₂O on rainy days (+0.12 ‰) compared to that of NO₂⁻ (+0.23 ‰) mainly reflects mixing of oxygen sources derived from O₂ (−0.44 ‰).

On fine days, the $\Delta^{17}O$ of N_2O (−0.30‰) differs from O_2 (−0.44‰). Potential contributions from H_2O ($\Delta^{17}O \approx 0$‰) during nitrification should be discussed.

We have already discussed the potential contributions from H_2O during nitrification (Lines 445-454 in original manuscript) and concluded that the contribution of O atoms derived from soil H_2O was minor during the oxidation of NH_4^+ to produce N_2O.

4. A more in depth comparison with complementary isotopic compositions (e.g., $\delta^{15}N$, $\delta18O$) would strengthen pathway discrimination.

We have already compared the $\delta^{15}N$ and $\delta^{18}O$ of N_2O with $\Delta^{17}O$ for the pathway discrimination in original manuscript as follows.

Although the $\delta^{18}O$ values of N_2O emitted from the soil were significantly higher than those of the sources of O atoms in N_2O (NO_2^-, O_2, and H_2O; Figures 4e and 6a) due to the fractionations of oxygen isotopes during the production and/or reduction of N_2O, the $\Delta^{17}O$ values of N_2O remained within the range of these sources. This indicates that $\Delta^{17}O$ primarily reflects the pathways of N_2O production, providing information distinct from the $\delta^{18}O$ signature because $\Delta^{17}O$ is stable during the processes of biogeochemical isotope fractionation (Lines 517-523). Moreover, while N_2O emission from the forested soil did not show significant differences in $\delta^{15}N$ and $\delta^{18}O$ values between fine and rainy days due to the fractionations of nitrogen and oxygen isotopes (Figures 4f and 4h), the significant difference in the $\Delta^{17}O$ values of N_2O between fine and rainy days (Figure 4d) highlights $\Delta^{17}O$ to be a promising natural signature for identifying the pathways of N_2O production in soils (Lines 524-528).

Specific comments:
1. **Line 56, 59, spell out "SP" for its first appearance**

We spelled out "SP" (^{15}N site preference) in the revised manuscript (P3/L56).

2. **Line 150, which kind of autoanalyzer**

We revised the sentence in the revised manuscript (P8/L153).

3. **Line 205, spell out "VSMOW" for its first appearance**

We spelled out "VSMOW" (Vienna Standard Mean Ocean Water) in the revised manuscript (P9/L184).

4. **Line 357, Identification of pathways of N2O production in forested soil using Δ17O signature, the subhead can be changed to "Identification of N2O production pathways in forested soil using Δ17O signature.**

We changed the subhead to "Identification of N_2O production pathways in forested soil using $\Delta^{17}O$ signature" in the revised manuscript (P17/L369).

5. Section 4.2, this section only reports experimental results and has no discussion.

In Section 4.2, in addition to presenting experimental results, we have also included discussion regarding the possible contributions of O atoms to soil N_2O derived from soil H_2O during denitrification and nitrification processes.

6. The figures appear to be crudely constructed, seemingly pieced together, with text added afterward. Subfigures are misaligned, and axis labels are inconsistently positioned. It is recommended to use professional illustration tools to improve the clarity and precision of the figures.

We checked the figures including the proper alignment of subfigures, consistent positioning of axis labels, and visual clarity in the revised manuscript.