

Dear Referee #2

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

Major comments:

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.

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

While the current work emphasizes temporal variations of $\Delta^{17}\text{O}$ 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 $\Delta^{17}\text{O}$ 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 N_2O 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 ($\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$) 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 $\Delta^{17}\text{O}$ values on rainy days ($+0.12 \pm 0.13 \text{ ‰}$) than on fine days ($-0.30 \pm 0.09 \text{ ‰}$) and (2) the emission flux of N_2O was significantly higher on rainy days ($38.8 \pm 28.0 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$) than on fine days ($3.8 \pm 3.1 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$).

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

We would like to add the confidence degree of the line fitting in Figure 3 in the revised manuscript. We would also like to emphasize 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.

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

Thank you for your advice. We would like to further highlight the novelty of this study by comparing it with the prior soil $\Delta^{17}\text{O}$ study in the revised manuscript as follows.

While Komatsu et al. (2008) first estimated the $\Delta^{17}\text{O}$ of N_2O emitted from a soil to assess whether soil N_2O could be the source of atmospheric N_2O 's high $\Delta^{17}\text{O}$ ($\Delta^{17}\text{O} = +0.7\text{‰}$), our study provides the first report of temporal variations of the $\Delta^{17}\text{O}$ values of soil N_2O . Furthermore, we first propose that $\Delta^{17}\text{O}$ can serve as a natural signature for identifying the main N_2O production pathways.

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 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 (Lines 114-116).

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 N_2O 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 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 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 (Lines 466-470).

Finally, in response to your request, while the soil microbiological properties and redox data were unavailable in this study, we would like to add the wind speed and air temperature data to Table S1 in the 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., $\pm 28.0 \mu\text{g N m}^{-2} \text{h}^{-1}$ 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. We would like to add this information to the revised manuscript.

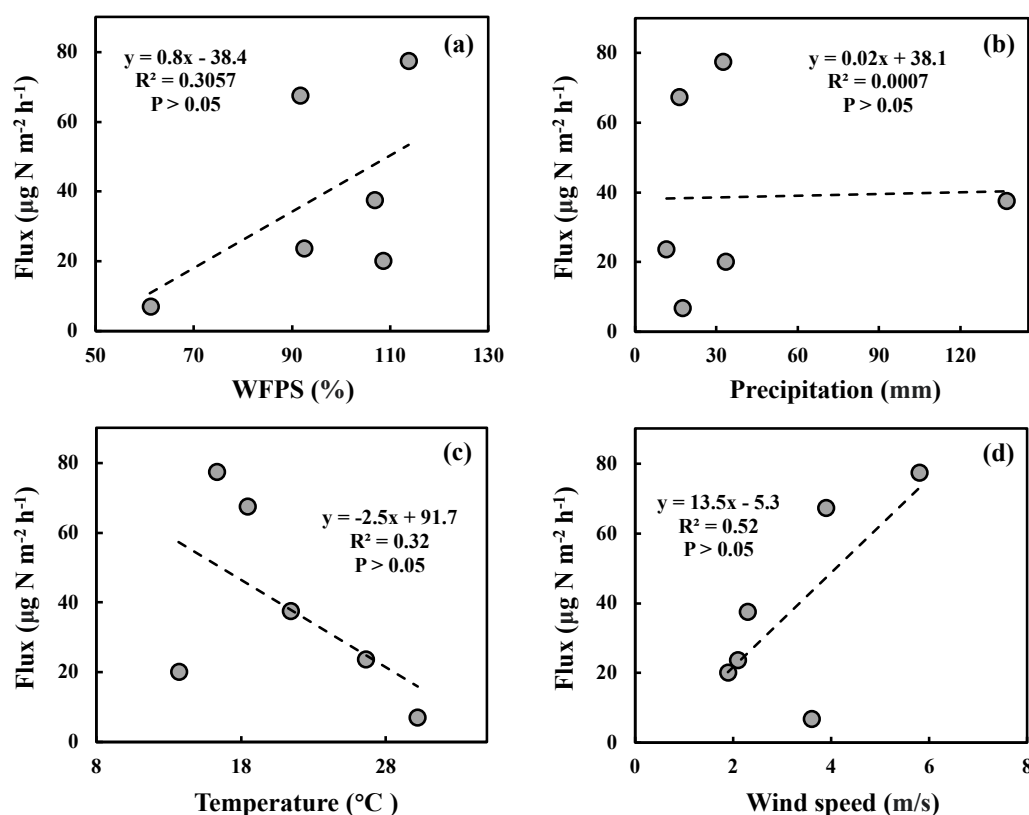


Figure R1. The flux of N₂O on rainy days plotted as a function of the WFPS (a), that of amount of precipitation (b), that of air temperature (c), and that of wind speed (d).

3. The $\Delta^{17}\text{O}$ 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 $\Delta^{17}\text{O}$ of N₂O in the manuscript (Lines 388-398) and concluded that the possible range of variations in the $\Delta^{17}\text{O}$ value of N₂O from that of NO₂⁻ to be less than 0.075 ‰. Thus, the lower $\Delta^{17}\text{O}$ of N₂O on rainy days (+0.12 ‰) compared to

that of NO_2^- (+0.23 ‰) mainly reflects mixing of oxygen sources derived from O_2 (−0.44 ‰).

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

We have already discussed the potential contributions from H_2O during nitrification (Lines 445–454) 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., $\delta^{15}\text{N}$, $\delta^{18}\text{O}$) would strengthen pathway discrimination.

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

Although the $\delta^{18}\text{O}$ 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 $\Delta^{17}\text{O}$ values of N_2O remained within the range of these sources. This indicates that $\Delta^{17}\text{O}$ primarily reflects the pathways of N_2O production, providing information distinct from the $\delta^{18}\text{O}$ signature because $\Delta^{17}\text{O}$ 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 $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values between fine and rainy days due to the fractionations of nitrogen and oxygen isotopes (Figures 4f and 4h), the significant difference in the $\Delta^{17}\text{O}$ values of N_2O between fine and rainy days (Figure 4d) highlights $\Delta^{17}\text{O}$ 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

Thank you for your comment. We would like to spell out “SP” (site preference) in the revised manuscript

2. Line 150, which kind of autoanalyzer

Thank you for your comment. We would like to revise the sentence as follows.

Their concentrations were determined using a high performance microflow analyzer (QuAAtro 39 Autoanalyzer, BLTEC, Osaka, Japan) (Lines 149–151).

3. Line 205, spell out “VSMOW” for its first appearance

Thank you for your comment. We would like to spell out “VSMOW” (Vienna Standard Mean Ocean Water) in the revised manuscript

4. Line 357, Identification of pathways of N₂O production in forested soil using $\Delta^{17}\text{O}$ signature, the subhead can be changed to “Identification of N₂O production pathways in forested soil using $\Delta^{17}\text{O}$ signature.

Thank you for your comment. We would like to change the subhead to “Identification of N₂O production pathways in forested soil using $\Delta^{17}\text{O}$ signature” in the revised manuscript.

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 N₂O derived from soil H₂O 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.

Thank you for your suggestion. We would like to carefully revise all figures using professional illustration software to ensure proper alignment of subfigures, consistent positioning of axis labels, and overall improved visual clarity in the manuscript.

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

Sincerely,

Weitian Ding

Graduate School of Environmental Studies,

Nagoya University

Furo-cho, Chikusa-ku, Nagoya,

464-8601, JAPAN

E-mail: dwt530754556@gmail.com

Cc: Drs. Urumu Tsunogai and Fumiko Nakagawa