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Triple oxygen isotope evidence for the pathway of nitrous oxide production in a forested soil with increased emission on rainy days

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Text S1. Calculation of WFPS

WFPS was calculated using Eq. S1 (Liu et al., 2007):

$$\text{WFPS}\ (\%) = (\Theta_v/P)*100 \qquad \text{(S1)}$$

where Θ_v denotes the volumetric water content (%) and P represents the total soil porosity.

The volumetric water content (Θ_v) was calculated using Eq. S2:

$$\Theta_v\ (\%) = \Theta_g*B_d \qquad \text{(S2)}$$

where Θ_g denotes the gravimetric water content (%) and B_d denotes the bulk density (g/cm^3). B_d (1.12±0.1 g/cm^3 on average; n = 3) was estimated by dividing the dry weight of each soil sample by the volume of the forested soil. The average value of B_d estimated in this study corresponds well with those determined in forested soils (Han et al., 2016; Jalabert et al., 2010; Teepe et al., 2003).

The gravimetric soil water content (Θ_g) was calculated using Eq. S3:

$$\Theta_g\ (\%) = (M_{soil} - M_{dry}) / M_{dry}, \qquad \text{(S3)}$$

where M_{soil} denotes the initial weight of the sampled soil and M_{dry} denotes the final weight of the soil that had been dried for 48 h in an oven at 80 °C.

Total soil porosity (P) was calculated using Eq. S4:

$$P\ (\%) = (1 - (B_d/P_d))*100 \qquad \text{(S4)}$$

where P_d denotes the soil density. In this study, we used a regular soil density of 2.65 g/cm^3 for P_d (Blake, 2008).

Text S2. Comparative experiment for the extraction of soil NO_2^-

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.

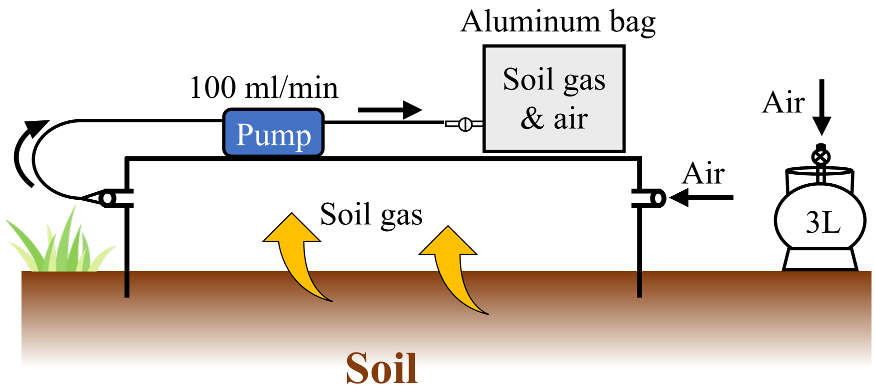
To evaluate this potential issue, we conducted a comparative experiment in April 2022 prior to this study. We collected a soil sample from our study site, which was thoroughly homogenized and divided into two 50 g subsamples. Each subsample was then extracted with either 50 mL of 2M KCl solution or 50 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.

Text S3. Approximating 1/[N_2O] as 0

During the nitrification reaction, only N_2O is the gaseous component. However, because of the further reduction of N_2O to N_2 during denitrification in soils, the emission of N_2O from soil was typically accompanied by N_2 emission (Figure 1). Consequently, the concentration of N_2O ([N_2O]) in the gas emitted from the soil could be diluted by N_2 to some extent; thus, [N_2O] emitted from the soil could be less than 100 %. If [N_2O] emitted from the soil was smaller than 100 %, the $\Delta^{17}O$ value of N_2O emitted from the soil ("true" $\Delta^{17}O$ value) should deviate from that determined using the linear correlation between

1/[N_2O] and the $\Delta^{17}O$, assuming that the 1/[N_2O] was 0 in the gas emitted from the soil (Figures 3, 5, and S5). However, the $N_2O/(N_2O + N_2)$ ratios of soil gas were always higher than 3% (Domeignoz-Horta et al., 2015; Scheer et al., 2020; Wang et al., 2020), which corresponds to 1/[N_2O] being less than $3.3 *10^{-8}$ ppb^{-1} for N_2O emitted from the soil. Because almost all slopes found in the linear correlation between 1/[N_2O] and $\Delta^{17}O$ showed were less than 2600 ‰ per ppb^{-1} (Figures 3, 5, and S5), the deviations in the $\Delta^{17}O$ values from the "true" value of each were always less than 0.0001 ‰. Consequently, we ignored the deviation and used a 1/[N_2O] value of 0 for N_2O emitted from the soil.



71 **Figure S1.** Schematic showing the flow chamber system used for sampling gases emitted
72 from soil.

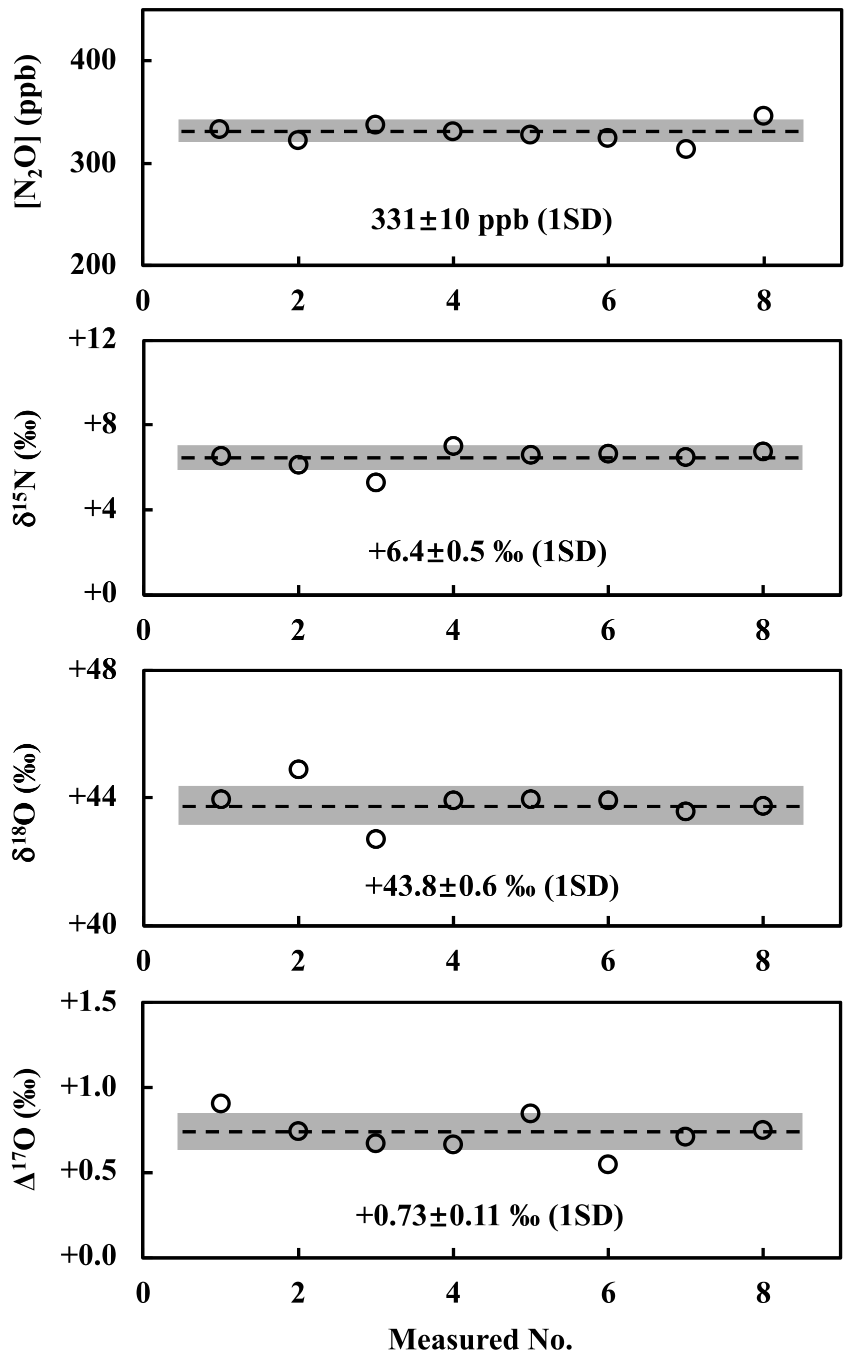


Figure S2. Results of repeated measurements of N_2O in an atmospheric sample collected at Nagoya University.

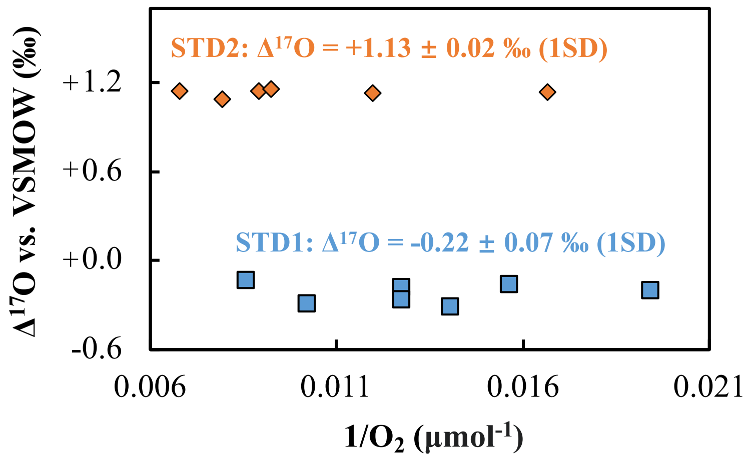


Figure S3. $\Delta^{17}O$ values of N_2O in the standards (STD1 and STD2) determined on the VSMOW scale plotted as a function of the reciprocal of the O_2 quantities evolved through offline reactions with NiO and BrF_5.

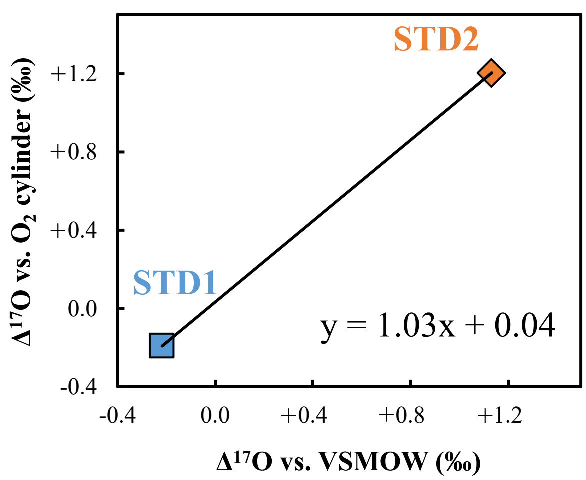


Figure S4. Relationship between the $\Delta^{17}O$ values of the N_2O standards determined using the CF-IRMS system and those of the same N_2O standards determined using the offline method. During the measurements using the CF-IRMS system, pure O_2 in a cylinder with $\Delta^{17}O$ values calibrated to the VSMOW scale was used as the reference gas.

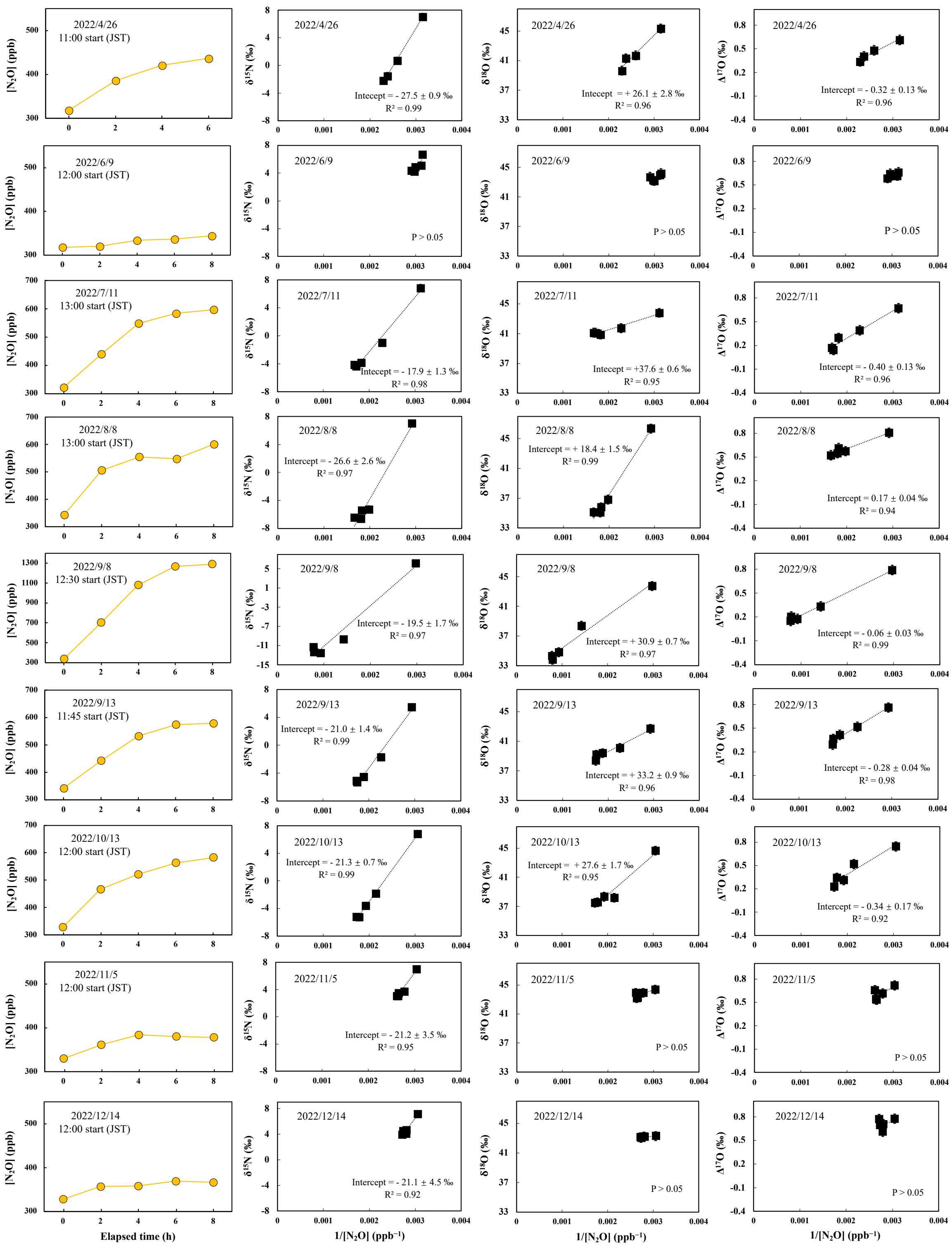


Figure S5. Changes in $[N_2O]$ of the gas samples collected from the forested soil plotted as a function of the elapsed time since the deployment of the flow chamber, and the $\delta^{15}N$, $\delta^{18}O$, and $\Delta^{17}O$ values of N_2O plotted as a function of the reciprocal of $[N_2O]$ ($1/[N_2O]$).

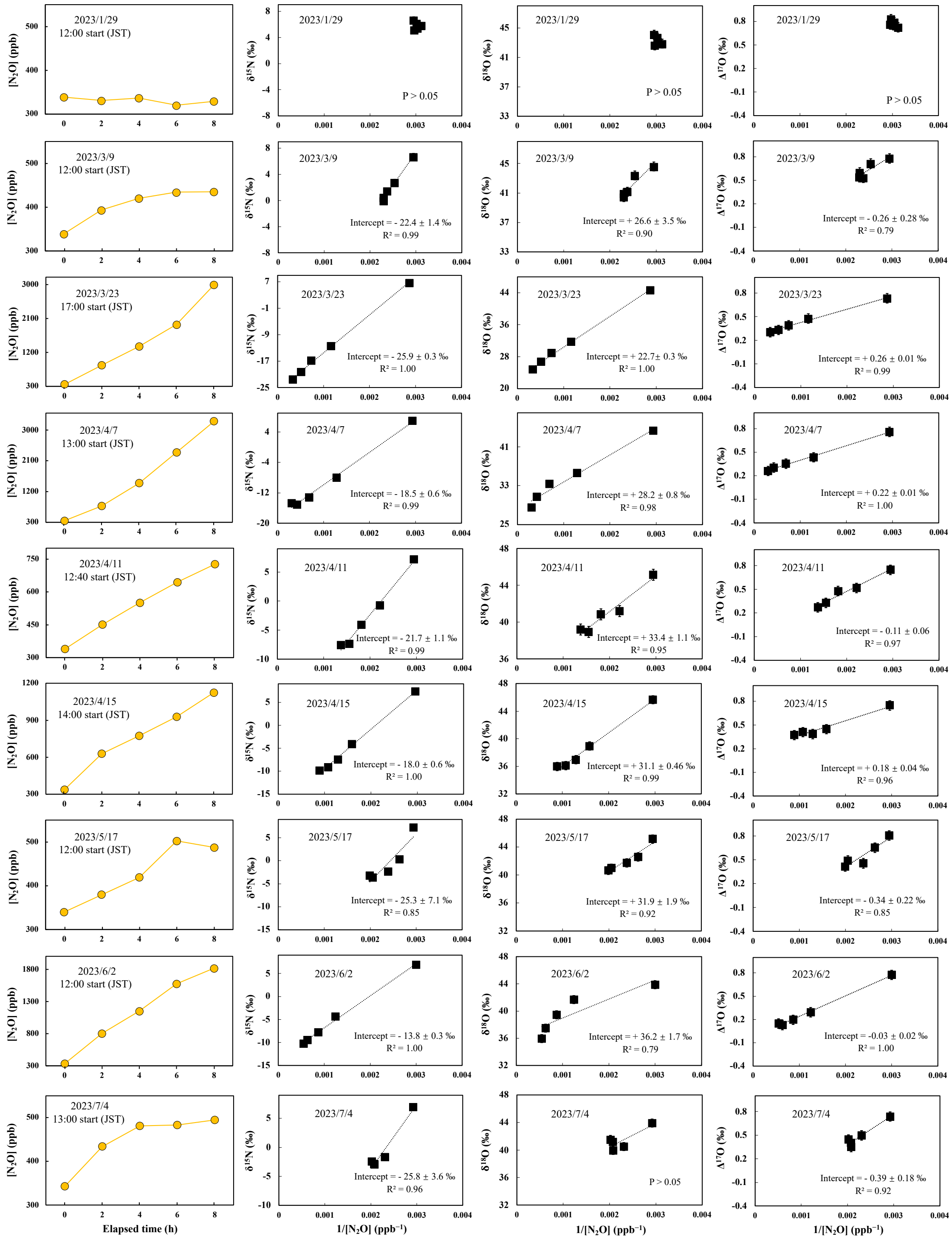


Figure S5. (continued). Changes in [N_2O] of the gas samples collected from the forested soil plotted as a function of the elapsed time since the deployment of the flow chamber, and the $\delta^{15}N$, $\delta^{18}O$, and $\Delta^{17}O$ values of N_2O plotted as a function of the reciprocal of [N_2O] (1/[N_2O]).

Table S1. Data obtained in this study.

Soil type	Time	T[#]	Wind speed	P[#]	WFPS[#]	[NH_4^+]	[NO_3^-]	[NO_2^-]	Flux-N_2O	$\delta^{18}O$ (NO_2^-)	$\Delta^{17}O$ (NO_2^-)	$\delta^{15}N$ (N_2O)	$\delta^{18}O$ (N_2O)	$\Delta^{17}O$ (N_2O)
		°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

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