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Supplement of

Isotopic insights into the dynamics of soil water pools along an elevation gradient

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S1. Results of the seasonal comparison of the stable isotopic composition from the two remaining sites, TD and RO.

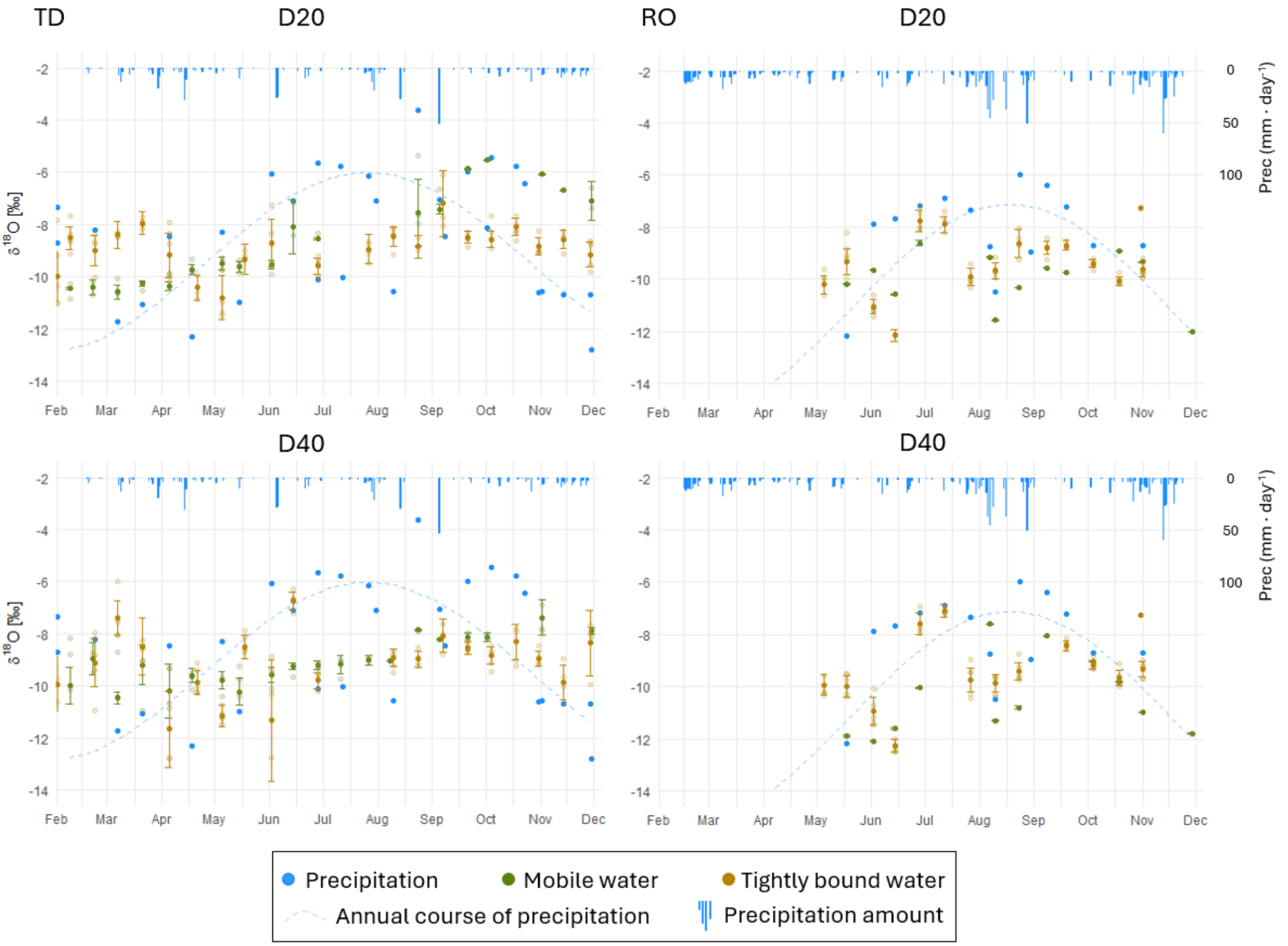

Figure S1. Seasonal comparison of the stable isotopic compositions of mobile and tightly bound soil water at two depths (D20 = 20 cm; D40 = 40 cm) at selected study sites. Left panels: TD (mid-elevation meadow); right panels: RO (montane beech forest at the highest-elevation site). Blue rectangles denote the daily precipitation (mm), and the light-blue dashed sine curve represents the weighted fit of the annual cycle of precipitation isotopic composition.

S2. Mass-balance mixing method and error propagation

Principle

Soil samples (20–30g) were transferred into 40 mL glass vials (ND24 (EPA), VERKON s. r. o., Czech Republic) equipped with plastic screw caps fitted with silicone/PTFE septa (VERKON s. r. o., Czech Republic). Subsequently, 20–25 mL of isotopically labelled water (distilled tap water) was added to each sample, and the remaining headspace was filled with 5 mm diameter glass beads to eliminate residual air and to enhance mechanical disaggregation of soil aggregates, thereby promoting efficient mixing (Fig. 2). The vials were then mounted on a laboratory-constructed rotating device (Fig. S2) and continuously rotated for 16 h at a constant speed of 15 rpm. Following mixing, the samples were refrigerated and left to settle to allow sedimentation. Thereafter, a 0.75 mL sample of the clear liquid phase was collected and filtered through a 0.45 μm mixed cellulose ester syringe filter. The remaining sample was transferred into pre-weighed aluminium weighing dishes and oven-dried at 105 °C for 48 h, after which it was weighed to determine the soil water content of the soil sample.

Figure S2. Custom-built laboratory apparatus designed to ensure homogeneous mixing of soil and added water through controlled mechanical motion of sealed sample vials prior to analysis.

In both field measurements and spiking experiments, laboratory-distilled water was used as the labelled end-member. Its isotopic composition lies near the centre of the annual range of soil water $\delta^{18}O$ values, with native soil water typically varying by up to ±5‰ around this value. This minimizes the isotopic contrast between the mixed components. The use of isotopically more distinct labelled water would increase the sensitivity of the mixing equation to weighing errors, leading to larger uncertainties in the estimated soil water isotopic composition, particularly at low soil moisture (Fig. S3).

Accordingly, the isotopic contrast between the labelled and native soil water is explicitly incorporated into the error propagation scheme for each sample, as the associated uncertainty increases with increasing end-member separation. Consequently, higher uncertainty is expected for soil water originating from winter or summer conditions compared to spring or autumn periods.

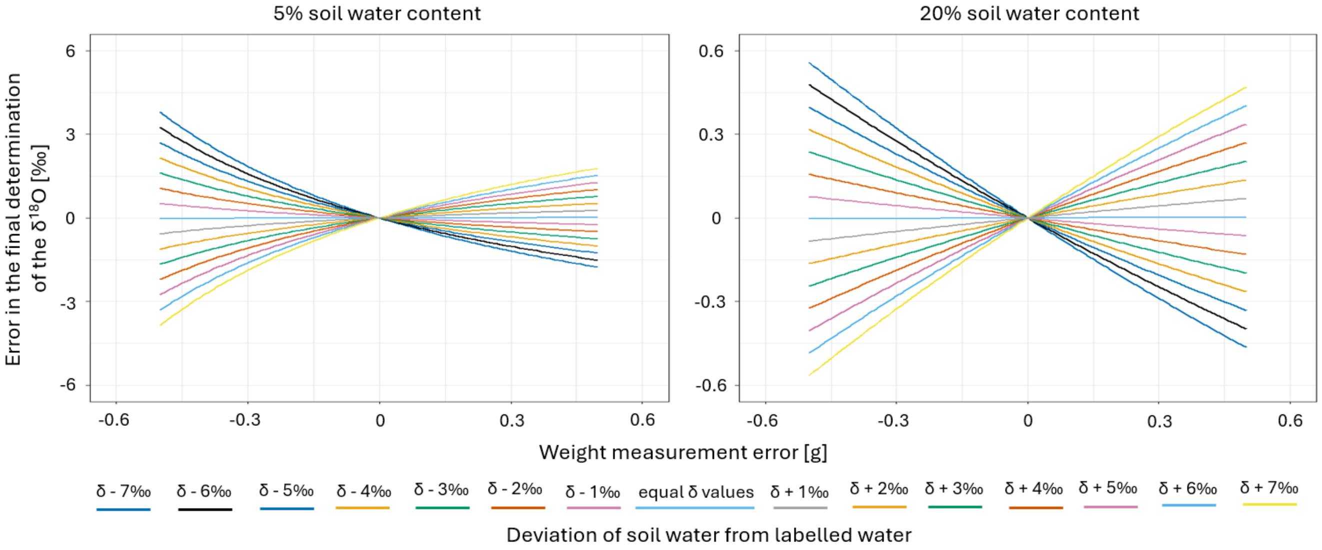

Figure S3. Sensitivity of the mixing method to weighing errors under different water contents and varying isotopic differences between the mixed components. Y-axes differ in scale.

Table S1. Measured variables and their measurement error

Variables	Variable description	Measurement error
m_1	Weight of empty vial	± 0.01 g
m_2	Weight of empty vial + soil	
m_3	Weight of empty vial + soil + labelled water	
m_4	Weight of empty vial + soil + labelled water + glass beads	
m_5	Weight of empty aluminium weighing dish	
m_6	Weight of the oven-dried aluminium weighing dishes with dry sample	± 0.03 g*

* *An increased weighing uncertainty was considered to account for unstable balance readings caused by weighing the hot sample immediately after removal from the oven.*

Table S2. Key relationships for mixing method calculation

Weight of labelled water	$m_L = m_3 - m_2$
Weight of tightly bound soil water	$m_{TBW} = (m_2 - m_1) - \big(m_6 - m_5 - (m_4 - m_3)\big)$ $m_{TBW} = m_2 - m_1 - m_6 + m_5 + m_4 - m_3$
Weight of the mixture	$m_M = m_L + m_{TBW}$
Mixing model equation	$\delta_{TBW} = \frac{m_M \delta_M - m_L \delta_L}{m_{TBW}}$
Estimation of error propagation	
GUM uncertainty calculation	$\sigma_{\delta_{TBW}} = \frac{1}{\lvert m_{TBW} \rvert} \sqrt{[3(0.01)^2 + (0.03)^2](\delta_M - \delta_{TBW})^2 + 2(0.01)^2(\delta_{TBW} - \delta_L)^2 + m_M^2 \sigma_{\delta_M}^2 + m_L^2 \sigma_{\delta_L}^2}$
Final error estimation	
Observed SD	$\sigma_{obs} = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2}$
Predicted SD	$\sigma_{pred} = GUM\ uncertainty\ calcultion$
Final error estimation	$if\ \sigma_{obs}^2 > \sigma_{pred}^2\ then\ \sigma_{final} = \sqrt{\sigma_{obs}^2 + \sigma_{pred}^2}$ $if\ \sigma_{obs}^2 < \sigma_{pred}^2\ then\ \sigma_{final} = \sigma_{pred}$

Table S3. Final results presentation

Inverse-Variance Weighted Mean	$\hat{X} = \frac{\sum_{i=1}^{n}\omega_i x_i}{\sum_{i=1}^{n}\omega_i}$ and $\omega_i = \frac{1}{\sigma_{i_{final}}}$
Standard Error of $\hat{X}$	$\sigma_{\hat{X}} = \sqrt{\frac{1}{\sum_{i=1}^{n}\omega_i}}$ $\chi^2 = \sum_{i=1}^{n}\omega_i\left(X_i - \hat{X}\right)^2$ $\upsilon = n - 1 \qquad \chi^2_{red} = \frac{\chi^2}{\upsilon}$ $SE = \begin{cases} \sigma_{\hat{X}}\sqrt{\chi^2_{red}}\,, & if\ \chi^2_{red} > 1 \\ \sigma_{\hat{X}}\,, & otherwise \end{cases}$
Weighted Standard Deviation	$SD_\omega = \sqrt{\frac{\sum_{i=1}^{n}\omega_i\left(X_i - \hat{X}\right)^2}{\sum_{i=1}^{n}\omega_i}}$
95 % Confidence Interval	$CI_{95} = 1.96 \cdot SE$

All soil water samples are then presented in the form of $X = \hat{X} \pm SE \pm SD$ *or* $\hat{X} \pm CI_{95}$.

Spike experiments

To verify the accuracy of the method, a spike experiment with ~29g of soil, and known amounts and stable isotopic compositions of soil water, and labelled water was conducted. Loamy sand from the Liz site was selected, as it is representative of the soil cover across the study sites included in this study. Oven-dried soil was spiked and stored at 4 °C for 24 h to allow equilibration. Subsequently, different amounts of labelled water were added to simulate a range of mixing ratios. In contrast to the extraction of field samples, all input variables were known; therefore, the oven-drying step was omitted. Accordingly, the error propagation scheme was modified to reflect this experimental setup.

Table S4. Measured variables and their measurement error within spike experiment

Variables	Variable description	Measurement error
m_1	Weight of empty vial	± 0.01 g
m_2	Weight of empty vial + soil	
m_3	Weight of empty vial + soil + soil water	
m_4	Weight of empty vial + soil + soil water + labelled water	
m_5	Weight of empty vial + soil + soil water + labelled water + glass beads	

Table S5. Key relationships for mixing method spike experiment calculation

Weight of labelled water	$m_L = m_4 - m_3$
Weight of tightly bound soil water	$m_{TBW} = m_3 - m_2$
Weight of the mixture	$m_M = m_L + m_{TBW}$
Mixing model equation	$\delta_{TBW} = \dfrac{m_M \delta_M - m_L \delta_L}{m_{TBW}}$

Estimation of error propagation

GUM uncertainty calculation

$$\sigma_{\delta_{TBW}} = \frac{1}{|m_{TBW}|}\sqrt{2(0.01)^2[(\delta_{TBW} - \delta_L)^2 + \delta_{TBW}^2] + m_M^2 \sigma_{\delta_M}^2 + m_L^2 \sigma_{\delta_L}^2}$$

Results of spike experiments

In total, five spike experiments, with 8 repetition each, were performed to simulate various mixing ratios of soil and labelled water, specifically 2.5, 5, 6, 7, and 10. Furthermore, additional spike experiments involving the pressure plate apparatus were performed. For all spike experiments, the isotopic composition of soil and labelled water was as follows: for δ^{18}O, −12.52 and −9.35 ± 0.03‰, and for δ^2H, −90.04 and −64.82 ± 0.15‰, respectively. The results of these experiments are summarized in Table S6.

Table S6. Spike experiments result of the mixing method and the use of pressure plate apparatus.

Mixing ratio	N	$\delta^{18}O$				$\delta^{2}H$			
		Average shift	SE	SD	CI_{95}	Average shift	SE	SD	CI_{95}
2.5	8	0	0.10	0.05	0.19	0.17	0.35	0.90	0.69
5	8	0.09	0.15	0.13	0.30	0.18	0.76	2.01	1.49
6	8	0.08	0.16	013	0.32	-0.04	0.79	2.10	1.56
7	8	0.08	0.16	0.14	0.32	-0.36	0.97	2.56	1.89
10	8	0.12	0.23	0.32	0.44	0.77	1.9	5.03	3.73
5^*	2	-0.30	0.29	0.10	0.57	-2.40	1.54	1.54	3.03
6^*	10	-0.40	0.14	0.13	0.27	-3.56	0.46	1.37	0.89
7^*	9	-0.30	0.14	0.10	0.28	-2.73	0.38	1.02	0.74
8^*	3	-0.43	0.23	0.08	0.44	-3.08	1.21	1.71	2.37

N: number of samples used in the test. Average shift: difference between the calculated and the initially known soil water. SE: standard error. SD: weighted standard deviation. CI_{95}*: 95% confidence interval. All δ values are reported in ‰. Results marked with* * *indicate tests involving the pressure plate apparatus. All study samples processed in the pressure plate apparatus were corrected for the average shift by -0.36 ‰ and -2.94 ‰ for* $\delta^{18}O$ *and* $\delta^{2}H$*, respectively. Samples that could not be processed in the overpressure apparatus due to low moisture content (no MW) were corrected using values obtained from tests of the mixing method itself.*