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

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Abstract. Recent intensive research on the soil–plant–atmosphere continuum has introduced novel methodological approaches. These include new in-situ extraction techniques and the application of stable hydrogen and oxygen isotopes in water, which enable tracing of water movement and plant responses at much finer spatial and temporal scales. Such approaches provide detailed insights into soil water dynamics and plant adaptation to changing environmental conditions under climate change. This study aims at an intimate description of dynamics of distinct soil water pools—mobile versus tightly bound water—along an elevation gradient, together with the impact of the absence of snow accumulation in lowland areas on water distribution within the soil profile compared to higher elevations. In contrast to conventional bulk water sampling, the key innovation of this research lies in the novel extraction method that selectively isolates tightly bound soil water for isotopic analysis, combined with a unique experimental design encompassing sites across the elevation gradient. Our results indicate a prolonged residence time of winter-derived soil water in lowland sites, despite limited snow cover, contrasting to a rapid turnover at the highest elevation, where the winter water signal dissipated shortly after snowmelt. Simultaneously, distinct isotopic compositions among water pools—mobile versus tightly bound water—were also found, especially in lowland areas at the edges of the growing season (up to 3‰ and 21‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively), while tightly bound and bulk soil water exhibited—on average—only minor or no isotopic differences. Facing the projected continued decline in snow cover at higher elevations in Central Europe, these findings are critical for improving predictions of soil water storage and, consequently, plant water availability under ongoing climate change.

1 Introduction

Soil drought is becoming increasingly prevalent due to climate change, which alters air temperature, total amount of precipitation and its intra-annual distribution (Gebrechorkos et al., 2025; Samaniego et al., 2018). These shifts have contributed

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to a sustained decline in vegetation-accessible water over the past three decades (Jiao et al., 2021). In response, there has been growing interest in the role of snowpack water storage and runoff generation in snow-dominated catchments, which are essential for groundwater recharge and soil moisture replenishment (Jenicek et al., 2020, 2021; Šípek et al., 2021; Musselman et al., 2017). Numerous studies project a continued decline in snow cover across mountainous regions as a consequence of rising air temperatures (Musselman et al., 2017; Marty et al., 2017; Jenicek et al., 2018; Willibald et al., 2020), accompanied by a shift from snowfall to rainfall during the winter season (Harpold et al., 2017; Safeeq et al., 2016). The implications of these changes in snow storage for the annual water balance remain a critical and unresolved question in hydrological research. Equally important are the downstream consequences for plant-available water in lowland ecosystems, particularly during the latter part of the growing season when drought stress is most acute (Büntgen et al., 2021; Qin et al., 2020; Mankin et al., 2019). A comprehensive understanding of the soil–plant–atmosphere continuum, a concept originally introduced by Gradmann (1928) and later formalized by van den Honert (1948), is therefore crucial for predicting vegetation dynamics and adaptive responses under increasingly frequent and severe drought conditions.

The relationship between plant water use and local hydrology has been studied since the early 20th century (Bates et al., 1921). Pioneering studies on water transport through soils and plants were subsequently summarized in comprehensive reviews (e.g., Tinker, 1976; Weatherley, 1976; Molz, 1981). A major shift in perspective occurred when Dawson and Ehleringer (1991) demonstrated that some riparian trees primarily access deeper groundwater, rather than the more readily available stream water. However, later work by Bond et al. (2002) appeared to challenge this finding by demonstrating diel fluctuations in stream baseflow attributable to plant transpiration, demonstrating clear interactions between transpiration and streamflow. Despite this apparent contradiction, Brooks et al. (2010) showed that mobile water (represented by stream water) and tightly bound soil water (represented by the plant water) are isotopically distinct. Their results suggested that, especially during the dry season, mobile water traveling through macropores or pipes bypasses tightly bound soil water, which is instead more likely to be taken up by plants and not contribute to streamflow.

This conceptual breakthrough formed the basis for the ecohydrological separation framework, later termed the "Two Water Worlds" (TWW) hypothesis (McDonnell, 2014). Since then, the TWW hypothesis has stimulated widespread debate, with numerous studies supporting (e.g., Goldsmith et al., 2012; Evaristo et al., 2015; Hervé-Fernandez et al., 2016) or challenging (e.g., Geris et al., 2015; Vargas et al., 2017; Dubbert et al., 2019) the existence of isotopically distinct water pools for vegetation use and runoff generation. Despite ongoing refinements, the hydrological connectivity between plant-accessible water and mobile water remains a central, unresolved question in ecohydrology.

Due to inconsistencies in the aforementioned studies, a new way forward has been proposed (Berry et al., 2017). Key priorities for future research include investigating internal water cycling within the phloem and xylem, identifying potential sampling and methodological biases, and improving both the spatial and temporal resolution of sampling strategies. Dubbert et al. (2019) further highlight the need to develop a standardized sampling protocol to harmonize methodologies across research groups and thus improve the comparability of results. Such a protocol has recently been proposed by Ceperley et al. (2024).

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However, the vast majority of studies comparing soil water and xylem water (e. g., Zapater et al., 2011; Meunier et al., 2017; Vargas et al., 2017; Barbeta et al., 2019, 2020; Liu et al., 2021; Brighenti et al., 2024; Benettin et al., 2024) rely on mobile and so-called bulk soil water for comparison. Mobile water is typically extracted using suction lysimeters or other vacuum-based systems. The water obtained in this manner—usually under tension of -60 kPa (Brooks et al., 2010; Berry et al., 2017; Sprenger et al. 2018)—originates primarily from macropores and preferential flow paths. In contrast, bulk soil water encompasses the total soil water content, including both gravitational and capillary pore water. During dry periods, when macropores are emptied and suction lysimeters cannot collect water, bulk soil water reliably represents the tightly bound water fraction. However, during wet conditions, when gravitational pores are partially or fully saturated, a significant portion of the bulk soil water may also consist of mobile water. Under such circumstances, bulk water may no longer serve as a representative of the tightly bound fraction and may not be as suitable for direct comparison with xylem water, particularly under the assumption that plants preferentially utilize tightly bound soil water (McDonell, 2014).

This study investigated the distinction between tightly bound soil water (TBW) and mobile soil water (MW). The TBW, extracted using a novel experimental approach, was further compared with the potential composition of bulk soil water (BW) to assess whether these two components can be used interchangeably without significant differences in their isotopic composition. The experiment was conducted simultaneously at four sites spanning an elevation gradient of more than 1000 meters. The focus on different elevations is especially important in areas in the rain-snow transition zone, which are widely affected by changes in snow storage due to increasing air temperature. The site selection aimed to capture the influence of declining snow cover and duration and reduced total precipitation on the isotopic behaviour of soil water. For this purpose, sites with similar soil texture were chosen, as soil texture strongly affects the proportion of macropores and capillary pores. The sampling campaign was conducted at two-week intervals from February to November 2023, except at the highest elevation site, which was accessible only from May onwards. The primary objectives of the study were to:

a. Determine whether there is a significant difference in the isotopic composition of tightly bound and mobile soil water
b. Evaluate the annual course of the isotopic composition of soil water across the elevation gradient with regard to the decrease in snow cover and precipitation in general
c. Identify the sources of tightly bound soil water
d. Determine whether replacing bulk soil water with tightly bound water can lead to different results

2 Study sites

The experiment was conducted in Czechia at four experimental plots (Tab. 1) strategically selected to span a pronounced elevation gradient, thereby capturing corresponding variations in temperature, precipitation, and snow cover extent and duration (Fig. 1). The study sites ranged from the fertile agricultural lowlands of the Elbe River Basin—Zvěřínek (185 m a.s.l.)—through the highlands and foothills—Trhové Dušníky (430 m a.s.l.) and the Liz catchment (870 m a.s.l.)—to the upper

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montane zone of the Bohemian Forest—Rokytka catchment (1,260 m a.s.l.). Throughout the manuscript, these locations are referred to by the abbreviations ZV, TD, LI, and RO, respectively.

Although the soil types varied due to the natural pedogenetic context of each site (Regosol, Gleyc Fluvisol, Cambisol, and Podzol), all plots exhibited similar soil texture (loamy sand or sandy loam). This minimized confounding effects from differences in clay content, allowing for a more direct assessment of elevation-related influences on soil water behaviour. Owing to the pronounced elevation differences, it was not feasible to maintain identical vegetation cover across all sites. However, vegetation at each location was representative of typical plant communities found at the corresponding elevation zones in Central Europe—ranging from agricultural land in the lowlands, to meadows, spruce forest, and beech forest at higher elevations. The agricultural land at the lowest elevation lacked vegetation cover from February to March, with post-harvest stubble remaining from October onward.

Table 1. Detailed characteristics of selected experimental areas. Climatic data (total annual precipitation and mean annual temperature) are for 2023. Snow data and climate classification (Köppen system) follow Tolasz et al. (2007). Further details for individual locations are provided in the cited references.

Name of the location		Zvěřínek	Trhové Dušníky		Liz		Rokytka	
Coordinates		50° 9' 20" N 15° 0' 37" E	49° 43' 12" N 14° 0' 46" E		49° 4' 0.2" N 13° 40' 49" E		49° 1' 22" N 13° 24' 23" E	
Elevation (m a. s. l.)		185	430		870		1,260	
Total precipitation (mm year^{-1})		631	680		931		1,380	
Average annual temperature (°C)		9.2	7		6.7		4.8	
Max snow depth (cm)		< 15	20 – 30		50 – 70		> 150	
Days with snow cover		< 30	60 – 80		100 – 120		> 160	
Climate classification		Cfb	Cfb		Dfb		Dfc	
Land cover		Agricultural land (*Sinapis alba*)	Meadow (*Agrostis capillaris, Festuca rubra*)		Forest (*Picea abies L.*)		Forest (*Fagus sylvatica L.*)	
Soil type		Regosol	Gleyc Fluvisol		Cambisol		Podzol	
Soil texture		Loamy sand	Sandy loam		Loamy sand		Loamy sand	
Retention curve parameters	Depth	20	20	40	20	40	20	40
	θ_r	0.05	0	0	0.18	0.18	0.10	0.06
	θ_s	0.39	0.50	0.50	0.51	0.52	0.65	0.45
	α	0.05	0.08	0.06	0.05	0.05	0.34	0.50
	n	1.74	1.20	1.18	1.37	1.70	1.45	1.34
	m	0.42	0.17	0.15	0.27	0.41	0.31	0.26

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Reference	Seyedsadr et al. (2022)	Šípek et al. (2019)	Zelíková et al. (2024)	Vlček et al. (2021)

Throughout the year, groundwater levels at most sites remain at least four meters below the soil surface. The only exception is the TD site, where groundwater rises to approximately one meter below the surface during the spring months, potentially influencing the isotopic composition of the overlying soil profile. However, due to the sandy texture of the soil, capillary rise is limited, and this influence is therefore assumed to be confined to the lower soil layer sampled.

Each site was equipped with a meteorological station (Fiedler, Czechia) with rainfall gauges (Meteoservis, Czechia), Palmex precipitation collectors (Palmex Ltd., Zagreb, Croatia), and a tensiometer-regulated vacuum lysimeter system (VS-Pro, UMS, Germany) for mobile soil water sampling. Precipitation amounts, soil moisture, and air temperature were measured in 10-minute steps and calculated on daily basis.

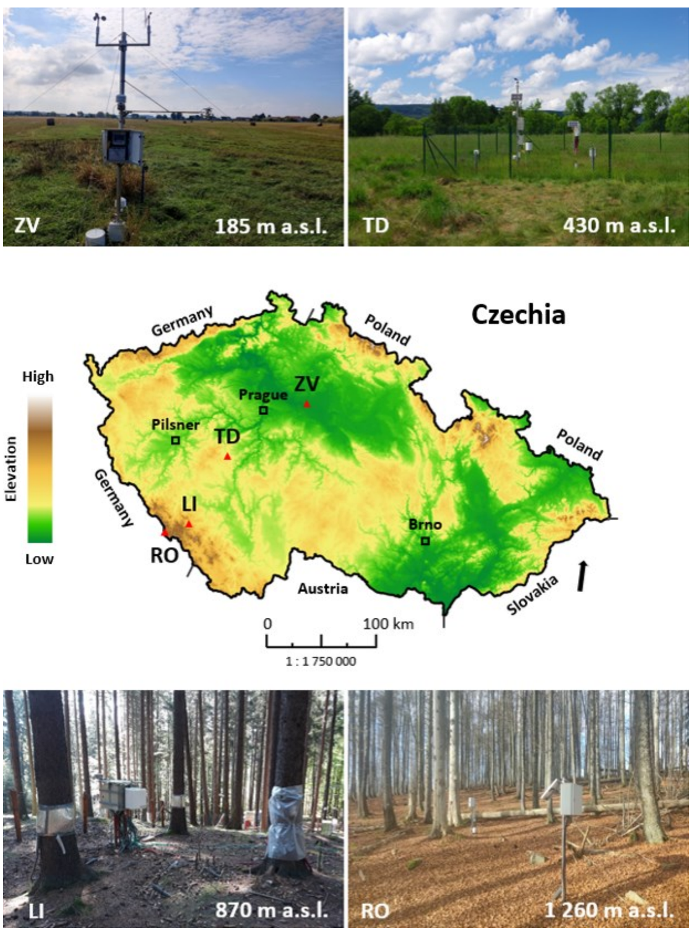

Figure 1. Location of selected experimental areas. Panels: top left – Zvěřínek; top right – Trhové Dušníky; bottom left – Liz; bottom right – Rokytka. Map data: Digital Vector Database of the Czech Republic ArcČR® version 4.3 (ARCDATA PRAHA, s.r.o., 2024).

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3 Data and methods

3.1 Field sampling

At all study sites, samples of precipitation, mobile water (MW), and soil cores were collected at two-week intervals for the stable isotope analysis from February to November 2023. The exception was the RO location, which was only accessible from May 2023 onwards due to its remote location in the heart of the Bohemian Forest National Park, as well as heavy snow conditions during winter and early spring. For the extraction of MW, the tensiometer-controlled vacuum system (VS-Pro) was employed with a maximum applied tension of -60 kPa (Brooks et al., 2010; Berry et al., 2017; Sprenger et al., 2018). The extraction was conducted from two depths, specifically 20 cm and 40 cm, which often play a significant role in root water uptake (Hackmann et al., 2025). Throughout the manuscript, these depths are referred to as shallow and deep layers, respectively. In both cases, the extracted samples represented a composite of water collected over the preceding two weeks.

For the TBW extraction, undisturbed soil cores (100 cm³) were collected from the same two depths, with five replicates per depth. The soil cylinders were wrapped in Parafilm® and stored in a portable refrigerator for transport to the laboratory. A total of 805 soil cores, 329 MW samples, and 108 precipitation samples were collected during the sampling period (Tab. 2).

Table 2. Overview of samples collected at individual locations

	Zvěřínek	Trhové Dušníky	Liz	Rokytka
Soil cores	220	230	215	140
Mobile soil water	101	150	57	21
Precipitation	20	30	44	14
Groundwater	-	9	-	-

3.2 Laboratory processing of soil samples

To obtain TBW the soil cores were inserted into the pressure plate apparatus for retention curve determination (5 Bar Pressure Plate Extractor, Soil Moisture Equipment Corp., CA, USA) with a 1 bar pressure plate cell was used. A pressure of 60 kPa (~ pF 2.4) for a two-week period was chosen to get rid of the MW fraction. Then, the top and bottom of the soil core were removed, and for further extraction, only the inner soil core was used (approx. 50 g of soil sample).

For the subsequent extraction of TBW, the mass balance mixing method was selected due to its accessibility, simplicity and high throughput (Fig. 2). Briefly, the soil sample was placed in a glass vial (volume of 60 mL) with a plastic cap and silicone sealing. Roughly 20-25 mL of a traced water was added to the sample, and the remaining space was filled with glass balls (5 mm in diameter) to remove the presence of air. Then, the samples were placed on a laboratory-constructed rotating device and continuously spun for 16 hours at a fixed speed of 15 rpm. Subsequently, they were stored in a refrigerator to allow sedimentation, after which 0.75 mL of the mixture was collected and filtered through a 0.45 μm mixed cellulose ester

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membrane. The rest of the sample was dried at 105 °C for 48 hours and weighed to calculate the soil water content of the soil sample.

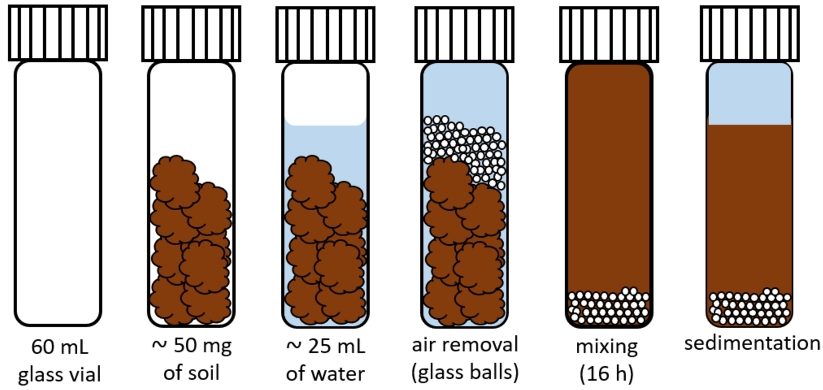

Figure 2. Sample processing procedure during the mixing extraction method.

Prior to using this procedure, conventional spike experiments with labelled water were conducted to verify the accuracy of the extraction methods. This allows us to assess the method's performance for specific soil types collected from our study sites. The resulting isotopic shift relative to labelled water (0.35 ± 0.15 and 3.02 ± 1.50 ‰ for $\delta^{18}O$ a $\delta^{2}H$, respectively) was then incorporated into data correction procedures by subtracting this error from the final isotopic values, and the associated standard deviations were incorporated into the reported measurement uncertainties (together with the standard deviation of the real data and the measurement uncertainty of the isotope analyser).

3.3 Isotope analysis and calculations

Stable isotope analyses were performed at the Institute of Hydrodynamics (Czech Academy of Sciences) with the L2140-*i* isotope analyser (Picarro Inc., Santa Clara, US). Standard mode (precision of ± 0.03 ‰ and ± 0.15 ‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively) was used with 6 injections per sample, with the first 3 injections discarded. The isotope ratios are reported in per mil (‰) relative to Vienna Standard Mean Ocean Water (VSMOW) ($\delta^{2}H$ or $\delta^{18}O = (R_{sample}/R_{standard} - 1) \times 1000$ ‰, where R_{sample} is the isotope ratio of the sample and $R_{standard}$ is the known reference value (i.e., VSMOW) (Craig, 1961)).

The isotopic composition of TBW was then calculated according to the mass balance mixing model (Eq. 1, 2).

$$\delta^{18}O_S = \frac{m_M}{m_S} \cdot \delta^{18}O_M - \frac{m_T}{m_S} \cdot \delta^{18}O_T \tag{1}$$

$$\delta^{2}H_S = \frac{m_M}{m_S} \cdot \delta^{2}H_M - \frac{m_T}{m_S} \cdot \delta^{2}H_T \tag{2}$$

where the sub-indices represent mixture (*M*), TBW (*S*), and traced water (*T*), *m* is the weight of those waters and $\delta^{18}O$ and $\delta^{2}H$ represent the stable isotopic composition of the sample.

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Following the estimation of TBW, the same mass-balance mixing model was applied to calculate the potential stable isotopic composition of BW. In this approach, BW was represented as a mixture of MW, obtained from suction lysimeters, and TBW, as derived in the previous step. The relative proportions of these two components were determined based on measurements from the pressure plate apparatus.

Furthermore, the line-condition excess (lc-excess; Landwehr and Coplen, 2006) was calculated (Eq. 3) to identify and exclude data contaminated during the water extraction process.

$$lc - excess = \delta^2H - a \cdot \delta^{18}O - b \tag{3}$$

where a and b are the coefficients of the local meteoric water line (LMWL) from individual experimental plots. This contamination was manifested by abnormally high lc-excess values relative to the rest of the dataset. The methodological nature of the error was further supported by the observation that these anomalous values appeared randomly across different sites and sampling dates, with the only consistent factor being that the affected samples were processed together within the same run of the overpressure apparatus. In total, 6 out of 32 extraction runs of TBW, corresponding to approximately 98 out of 805 soil samples, had to be discarded due to this methodological error and significant sample contamination.

For the seasonal comparison of the stable isotopic composition of mobile and tightly bound soil water, seasons were defined as follows: winter (F), spring (MAM), summer (JJA), and autumn (SON).

3.4 Data fitting

To visualize the stable isotope data and characterize their seasonal variability, a sine function was fitted to the data using the iteratively reweighted least squares (IRLS) regression method with externally supplied weights in case of precipitation data (Eq. 4), a technique commonly applied to characterize the seasonal cycle of precipitation, streamflow, soil water, and groundwater (Kirchner, 2016; Zuecco et al., 2024; Floriancic et al., 2024; Xia et al., 2024). From the Eq. 4, the amplitude can then be determined according to the Eq. 5:

$$c(t) = a \cdot cos(2\pi ft) + b \cdot \sin(2\pi ft) + k \tag{4}$$

$$A = \sqrt{a^2 + b^2} \tag{5}$$

where t is the time, $c(t)$ represents the isotopic time series of the dataset, a and b are the cosine and sine coefficients determined by the IRLS regression, f is the frequency of annual isotopic fluctuation ($f = 1$ yr^{-1} for a seasonal cycle), k is the vertical shift of the sine wave, and A is the amplitude of the fitted sine wave.

Since the soil water and groundwater often lack a consistent seasonal signal, it is hard to fit them with a fixed sine wave, especially when spanning multiple seasons (Xia et al., 2024). Therefore, in some cases a weighted third-degree polynomial fit was used to provide a clear visualization of the stable isotopic composition throughout the year.

To establish the LMWLs and regression lines for soil water isotopic compositions, reduced major axis (RMA) regression was applied (Harper, 2016) instead of conventional linear regression. This approach was selected because

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uncertainties in both δ²H and δ¹⁸O measurements are equally significant, whereas ordinary least squares regression assumes all error is confined to the y-axis and ignores uncertainty in the x-axis.

The slope (Eq. 6) of the RMA regression was calculated as the ratio of the standard deviations of δ²H and δ¹⁸O, scaled by the sign of their Pearson correlation coefficient. The intercept was then calculated according to Eq. 7:

$$\beta_{RMA} = sign(r) \cdot \frac{\sigma_{\delta^2 H}}{\sigma_{\delta^{18} O}} \tag{6}$$

$$\alpha_{RMA} = \delta^{\overline{2}}H - \beta_{RMA} \cdot \delta^{\overline{18}}O \tag{7}$$

where α_{RMA} and β_{RMA} are the intercept and slope of the RMA, respectively, r is Pearson correlation coefficient between δ^2H and δ^{18}O, and $\sigma_{\delta 2H}$, $\sigma_{\delta 18O}$ are their standard deviations. This method minimizes the orthogonal distances between data points and the fitted line, making it more suitable for hydrological isotope data where both variables are subject to analytical and natural variability.

3.5 Seasonal Origin Index (SOI)

To characterize whether the extracted soil water originated from winter or summer precipitation, we calculated the SOI (Eq. 8) (Allen et al., 2019) for individual seasons, as well as for individual months, to provide a more detailed representation of gradual changes in water origin resulting from mixing with newly infiltrating water.

$$SOI = \begin{cases} \dfrac{\delta_x - \delta_{annP}}{\delta_{summerP} - \delta_{annP}}, if\ \delta_x > \delta_{annP} \\ \dfrac{\delta_x - \delta_{annP}}{\delta_{annP} - \delta_{winterP}}, if\ \delta_x < \delta_{annP} \end{cases} \tag{8}$$

where δ_x are the δ^{18}O isotopic values of soil water, and δ_{annP}, $\delta_{winterP}$, and $\delta_{summerP}$, are the δ^{18}O isotopic values of volume-weighted annual precipitation, typical winter (δ_{annP} – fitted amplitude), and typical summer (δ_{annP} + fitted amplitude) precipitation. The SOI ranges from -1 to 1, where values close to -1 represent water predominantly derived from winter precipitation, and values approaching 1 reflecting a dominant contribution from summer precipitation.

3.6 Statistical evaluation

To statistically assess the differences in annual courses of isotopic composition of individual waters (MW, TBW), it was necessary to consider the uncertainty in the coefficients of the regression models. Therefore, following the approach of Davison and Hinkley (1997), bootstrap residual resampling was performed as follows:

1. Regression models f_A and f_B were fitted to datasets A and B (Y; e.g., MW and TBW), and residuals (r_A, r_B) were calculated (Eq. 9, 10).

$$r_{A,i} = f_{A,i} - Y_{A,i} \tag{9}$$

$$r_{B,i} = f_{B,i} - Y_{A,i} \tag{10}$$

2. The following steps were repeated 10,000 times:

a. Synthetic datasets were generated (Eq. 11, 12).

$$Y_{A,boot,i} = f_{A,i} + r_{A,rand} \quad (11)$$

$$Y_{B,boot,i} = f_{B,i} + r_{B,rand} \quad (12)$$

where r_A,rand and r_B,rand are randomly sampled residuals (with replacement).

b. New regression curves f_A,boot and f_B,boot were fitted to the synthetic datasets.

c. The difference between the curves was computed and stored (Eq. 13).

$$d = \int \left| f_{A,boot} - f_{B,boot} \right| \quad (13)$$

3. A 95% confidence interval for the curve difference was derived as a range between the 0.025 and 0.975 quantiles of the resampled differences.
4. The samples were considered significantly different if the confidence interval did not contain zero.

4 Results

4.1 Precipitation and soil water data

Given the geographical proximity of the study sites, the slopes of the LMWLs were similar across all locations (Fig. 3), ranging from 7.7 to 8. In contrast, the y-intercepts varied considerably, from 4.2 to 11.5. Both parameters reflected the elevation gradient, with the lowest values recorded at the lowland ZV site and the highest at the mountainous location RO. The narrower isotopic range observed at the RO site was likely due to the incomplete dataset, as sampling was limited to the period between May and November.

Mobile soil water (MW) closely tracked the LMWLs, with slopes ranging from 7.4 to 8.1 and intercepts from 2 to 14, suggesting minimal isotopic alteration by evaporation or condensation. However, its isotopic range was narrower and often more depleted than that of precipitation. This depletion likely resulted from the absence of winter precipitation data (December and January), which typically exhibits more negative isotopic values. Similar to precipitation, an elevation trend was also apparent in the isotopic composition of MW.

In contrast, no consistent elevation pattern was observed for TBW. Unlike MW and precipitation, TBW exhibited clear signs of evaporative enrichment and isotopic modification due to condensation and internal mixing processes within the soil matrix. These effects were reflected in lower slopes (ranging from 5.9 to 7.5) and a broader dispersion of data around the respective soil water evaporation lines.

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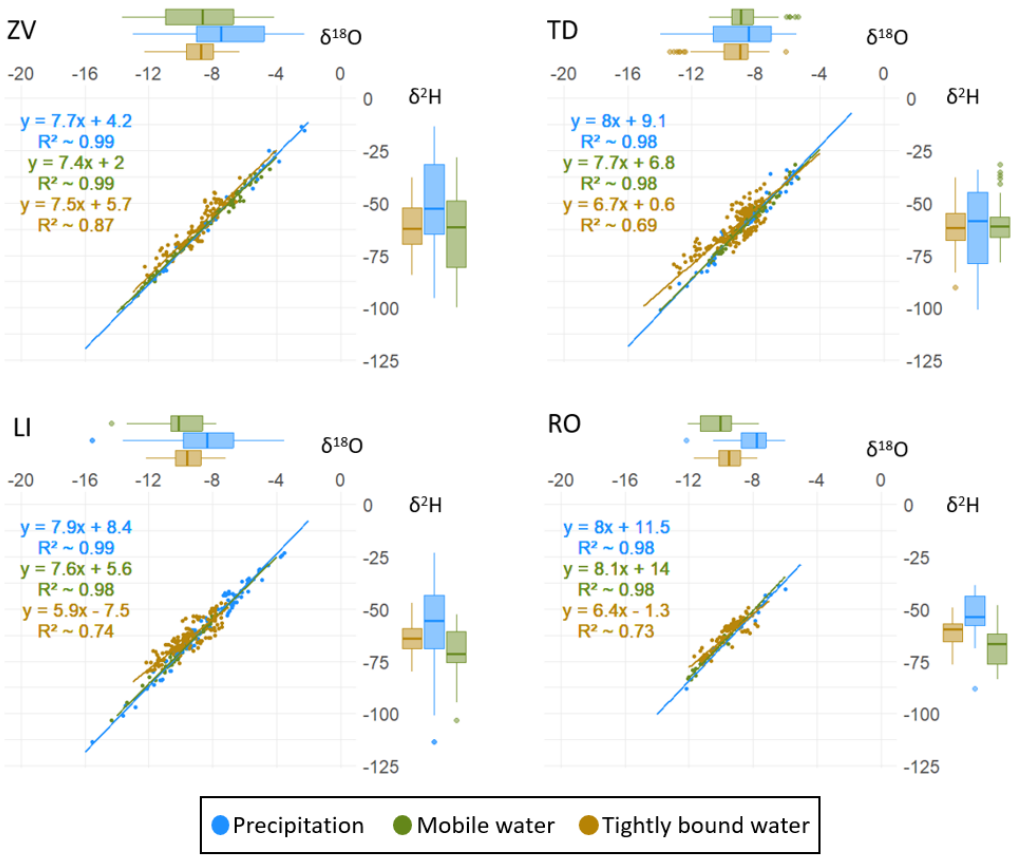

Figure 3. Dual-isotope plots of all water samples collected in this study, with corresponding regression lines. Panels: top left – Zvěřínek; top right – Trhové Dušníky; bottom left – Liz; bottom right – Rokytka. Precipitation is shown in blue, mobile soil water in green, and tightly bound soil water in brown.

4.2 Comparison of mobile and tightly bound soil water

A difference in stable isotopic composition of MW and TBW was observed across experimental sites (Fig. 4). Among all components, shallow MW exhibited the greatest annual isotopic variability. However, this variability diminished with increasing elevation. In lowland areas, deep soil water tended to be more isotopically depleted compared to shallow layers, but this trend weakened or even reversed at higher elevations.

The most pronounced contrasts between MW and TBW were recorded in spring and autumn, and to a lesser extent during summer at the TD site. At the ZV site, isotopic differences between shallow and deep soil water, both MW and TBW, became evident primarily in the second half of the year when shallow soil water was being replaced by the summer precipitation. In contrast, at the mountainous sites (LI, RO), the largest differences between shallow and deep MW occurred in the first half of the year, while the isotopic composition of TBW remained relatively stable throughout the study period.

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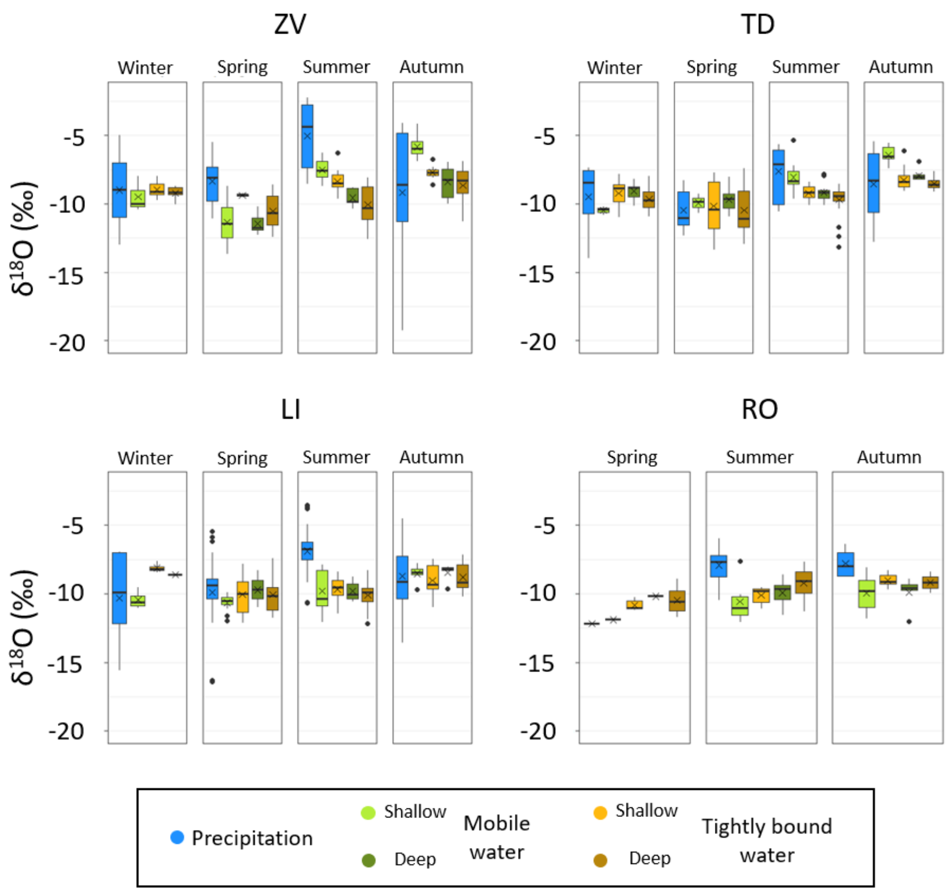

Figure 4. Seasonal comparison of the stable isotopic compositions of mobile and tightly bound soil water at the study sites. Panels: top left – Zvěřínek; top right – Trhové Dušníky; bottom left – Liz; bottom right – Rokytka. Seasons are defined as follows: winter (F), spring (MAM), summer (JJA), and autumn (SON). Boxplots are presented in the same order across panels, except for winter at the Liz site, where deep tightly bound soil water data are missing.

In most cases, the isotopic composition of soil water closely reflected that of precipitation during the corresponding time period. A notable exception was observed at the ZV site, where the response time of soil water to precipitation exceeded three months, making it the slowest among all sites. Another distinct anomaly was detected at the LI site during the summer, where soil water showed no clear isotopic response to recent rainfall. This lack of response is attributed to an intense precipitation event (>100 mm in two hours), which had an isotopic composition of approximately -10‰ for $\delta^{18}O$, and effectively stabilized the isotopic signature of soil water around this value.

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4.3 Influence of the elevation gradient on the lag in soil water pool dynamics

Significant differences between MW and TBW were observed across the elevation gradient, both in their absolute quantities and their stable isotopic composition. With increasing elevation and precipitation amounts, the volumetric soil water content increased across the study sites (Fig. 5). At the lowest elevation sites (ZV, TD), the average water content was approximately 23 g per 100 cm^3 of soil at both, 20 cm and 40 cm depths, with slightly higher values in 20 cm, whereas at the highest site (RO) it reached up to 47 g per 100 cm^3 at both depths, again with slightly higher values in the upper layer. Furthermore, the influence of snow cover, increased precipitation, and lower temperatures (resulting in a delayed onset of the growing season and generally reduced evapotranspiration) was reflected in a gradual increase in soil water content during spring and early summer at higher-elevation sites. In contrast, in lowland areas, soil water content tended to plateau after the winter season due to full saturation of the soil profile. Following the onset of the growing season, accompanied by increased evapotranspiration and progressive soil drying, the soil water content began to decline at all sites.

In terms of stable isotopic composition, significant phase shifts between precipitation and various soil water pools were observed both among the study sites and within individual locations over the course of the year. The most pronounced lag between precipitation and shallow MW occurred at the ZV site, where the temporal offset exceeded three months (Fig. 5). This lag decreased along the elevation gradient by shortening to approximately six weeks at the mid-altitude site and becoming negligible at the highest-elevation site.

Furthermore, at all locations except the RO site, a distinct phase shift between shallow MW and TBW was observed between February and May, with the magnitude of this shift decreasing with increasing elevation. Deep soil water compartments generally exhibited similar temporal dynamics and phase lags as shallow tightly bound water, although they tended to be more isotopically depleted, hence contained more water from winter precipitation. An exception was found at the TD site, where the isotopic compositions of deep MW and TBW were different. Moreover, we hypothesize that the seasonal pattern of shallow TBW at the RO site would resemble that of the deep soil water pools. However, due to the limited number of data points available, all fitting approaches applied (both weighted and unweighted sine functions, as well as polynomial models) yielded comparable results, as illustrated in Fig. 5.

Following the onset of the dry season, the isotopic differences among soil water pools gradually diminished. By the end of the year, amplitude peaks became synchronized, resembling the pattern of shallow MW, though with attenuated isotopic signals. This attenuation likely resulted from mixing between newly infiltrated precipitation and residual water from previous periods. Despite this homogenization, a temporal offset between precipitation and MW remained evident. The negligible lag observed at the highest site was likely due to the high annual precipitation (~1,400 mm), which rapidly flushed the saturated soil profile.

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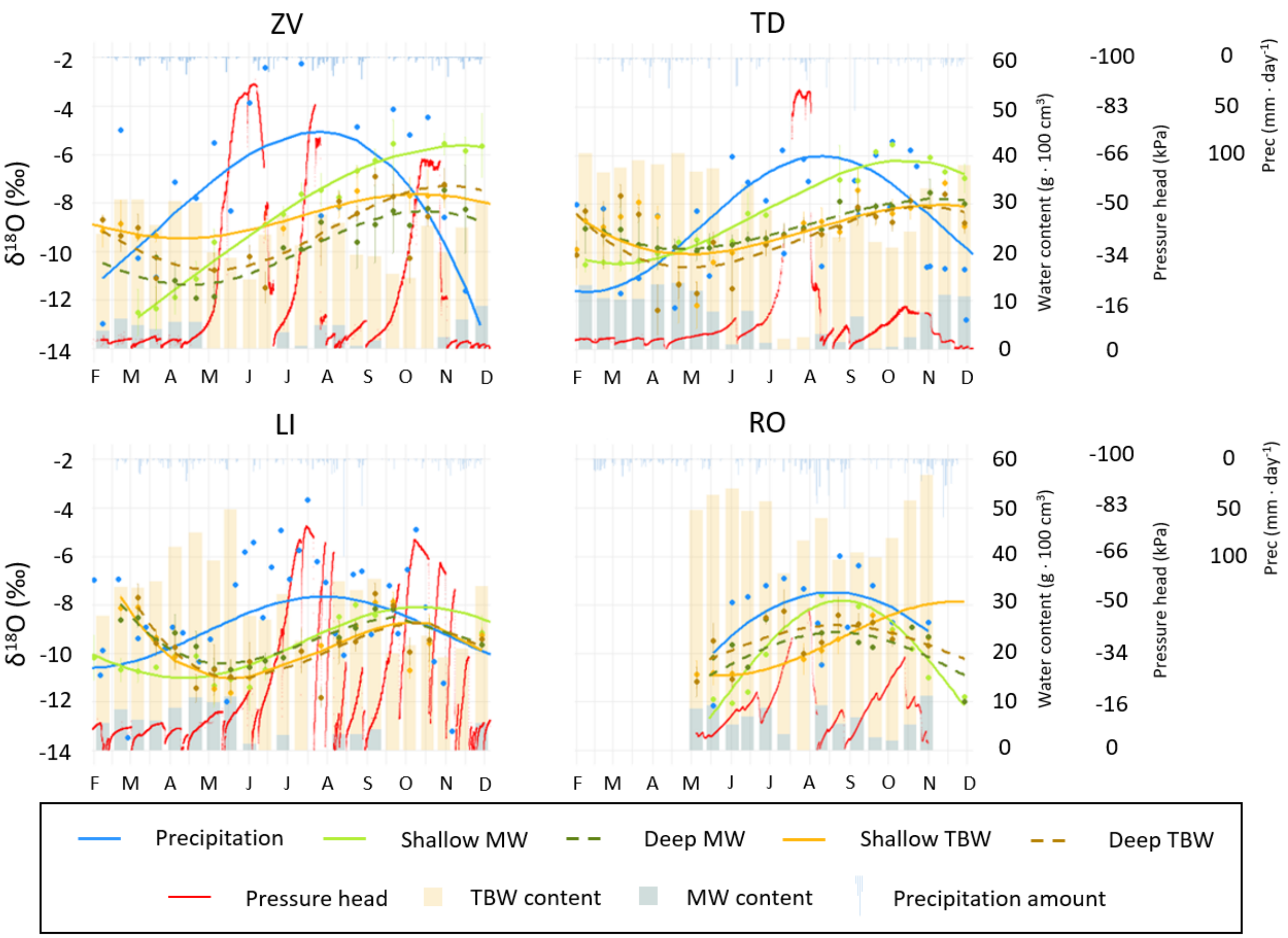

Figure 5. Annual variation in the isotopic composition of soil water in relation to soil water content, pressure head, and precipitation amounts. Both soil water content and pressure head refer to a depth of 20 cm. Panels: top left – Zvěřínek; top right – Trhové Dušníky; bottom left – Liz; bottom right – Rokytka.

The influence of the elevation gradient was further supported by the bootstrap residual analysis, which revealed statistically significant differences in the seasonal dynamics of the stable isotopic composition of soil water pools. These differences were more pronounced in lowland areas (Table 3). Among the four pairwise comparisons assessed (shallow MW vs. TBW; deep MW vs. TBW; shallow vs. deep MW; and shallow vs. deep TBW), the most consistent differences were observed between shallow and deep MW. This contrast was statistically significant in three out of four cases, with the exception of the LI site, where—despite apparent differences early in the year—the statistical test did not confirm significance.

For the comparison between deep MW and TBW at the ZV and TD sites, no statistically significant differences were detected; however, the null hypothesis was rejected only marginally, with the critical zero threshold exceeded by just -0.07 and 0.03, respectively.

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In the case of shallow MW vs. TBW at the ZV site, a statistical difference was not confirmed, despite clearly distinct isotopic signatures (Fig. 5). This outcome likely reflects a limitation of the applied statistical method, which lacks sensitivity to detect differences in symmetrically distributed data. A similar limitation may also affect the comparison between shallow and deep MW at the RO site. However, the two instances confirming the null hypothesis at this site were more likely due to a lack of the TBW data and their unrepresentative interpolation using a sine curve (Fig. 5).

Table 3. The results of the bootstrap residual resampling analysis. H_0: Fitted sine functions are statistically different. The H_0 is rejected if the interval between q025 and q975 contains zero.

Compared combinations	Quantiles	Locations			
		Zvěřínek	Trhové Dušníky	Liz	Rokytka
Shallow MW vs. Shallow TBW	q025	-0.44	-1.19	-0.20	1.42
	q975	0.40	-0.29	1.40	2.62
	Result	False	True	False	True
Deep MW vs. Deep TBW	q025	-0.07	-0.83	-0.69	-0.82
	q975	1.11	0,03	0.76	1.25
	Result	False	False	False	False
Shallow MW vs. Deep MW	q025	0.65	0.33	-1.27	-2.33
	q975	1.68	0.80	0.23	-0.67
	Result	True	True	False	True
Shallow TBW vs. Deep TBW	q025	0.12	-0.36	-0.69	-0.58
	q975	1.15	0.83	0.83	1.13
	Result	True	False	False	False

4.4 Origin of the soil water

The results of water origin analyses revealed distinct temporal patterns in the SOI across the study sites. Among the soil water components, shallow MW showed the highest isotopic variability, with the magnitude of fluctuations decreasing along the elevation gradient. Within the soil profile, the lowest SOI values, indicating a predominant contribution of winter precipitation, were typically recorded in spring, whereas the highest values, reflecting a dominant influence of summer precipitation, occurred in autumn (Fig. 6).

The influence of winter precipitation remained detectable in the soil profile until late summer at all sites except the highest-elevation site, RO. This pattern was evident across all soil water compartments except shallow MW, which was already affected by summer precipitation during the summer months. At the RO site, the data obtained indicate that influence of winter precipitation had already diminished by May. Although this assessment may be influenced by the absence of data from previous months.

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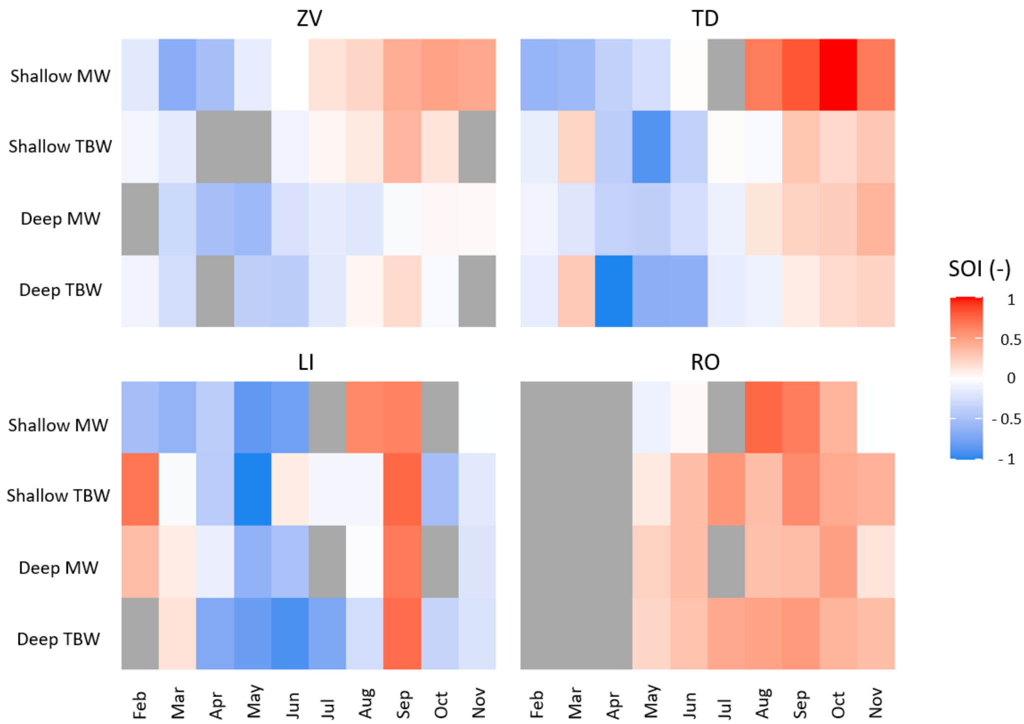

Figure 6. Seasonal origin index (SOI) values for individual sites and months. The abbreviations MW and TBW refer to mobile and tightly bound soil water, respectively. Values near -1 indicate a predominant contribution from winter precipitation, whereas values approaching 1 reflect a dominant contribution from summer precipitation. Grey areas indicate missing data for the respective period. Panels: top left – Zvěřínek; top right – Trhové Dušníky; bottom left – Liz; bottom right – Rokytka.

The highly positive SOI values observed in autumn at the TD site were attributed to a period of severe drought in July, which desiccated the upper soil layers (Fig. 5), followed by rewetting from late-summer rainfall. SOI values increased steadily from August to October and subsequently declined, closely mirroring the isotopic composition of precipitation during this period. Conversely, the strongly negative SOI values recorded in spring were likely caused by a rising groundwater table. This would explain their earlier appearance in deeper soil layers and delayed onset in shallower horizons. Notably, despite the fact that the proportion of MW in the deeper soil layer reached up to 64% relative to TBW at the beginning of the year, there was no isotopic homogenization between these two water pools, and both components remained isotopically distinct. Furthermore, we attribute the alternation of negative and positive SOI values at the TD site early in the year to preferential flow, which may have influenced the February samples of tightly bound water. We further hypothesize that the isotopic composition during this period would resemble that at the LI site, where water from the previous summer and autumn was retained and delayed within the soil profile.

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The markedly positive SOI values observed in September at the LIZ site were linked to the second most intense precipitation event of the year, nearly 50 mm, characterized by an isotopic composition of -6.55‰ for $\delta^{18}O$ and -43.36‰ for δ^2H, which saturated the otherwise relatively dry soil profile.

4.5 Bulk soil water

Although BW was not directly sampled in this study, we present a conceptual illustration of its potential isotopic composition based on the obtained data. The results showed, that the stable isotopic composition of BW, estimated indirectly using a mass-balance mixing model (Eq. 1, 2), may vary statistically from TBW for both $\delta^{18}O$ and δ^2H. Unpaired t-test ($P < 0.05$) revealed this difference on at least one sampling date at both lowland study site (ZV, TD), with no difference observed at higher elevations. During the summer drought period, however, when soil desiccation removed almost all MW from the soil profile, BW effectively represented TBW alone.

Since the isotopic signature of BW depends on both, the relative proportions of MW and TBW and the isotopic contrast between them, the greatest deviations were observed during the spring and autumn seasons. During these periods, both the isotopic difference between water pools and the proportion of MW in the soil reached their annual maxima (Fig. 5). At the ZV site, the discrepancy between BW and TBW was primarily driven by the pronounced isotopic contrast between mobile and tightly bound fractions, despite the low proportion of MW in the profile. In contrast, at the TD site, the difference was mainly attributed to a higher proportion of MW, while the isotopic contrast between the components was less pronounced.

In cases where BW and TBW differed, the average isotopic offset was 0.41‰ for $\delta^{18}O$ and 2.29‰ for δ^2H, with maximum differences reaching 1.48‰ and 7.99‰, respectively. When BW values were used to calculate the SOI, only minimal or no differences between BW and TBW were observed from a broader perspective (Fig. 7). Notable deviations, however, occurred during individual sampling campaigns in April and May. In these instances, the SOI difference between BW and TBW reached up to 0.3.

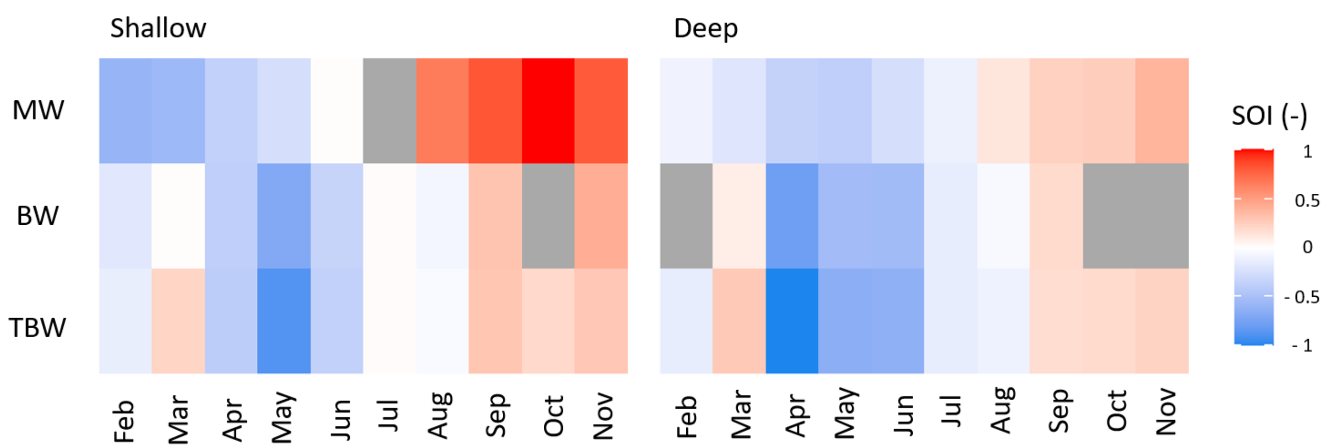

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Figure 7. Comparison of the difference in SOI calculations using mobile (MW), bulk (BW) and tightly bound (TBW) soil water at the Trhové Dušníky site. Values near -1 indicate a predominant contribution from winter precipitation, whereas values approaching 1 reflect a dominant contribution from summer precipitation. Grey areas indicate missing data for the respective period.

5 Discussion

5.1 Isotopic changes due to soil properties and precipitation amount

All soil samples obtained in this study fell close to or directly on the LMWL, indicating their meteoric origin and suggesting minimal influence of the selected extraction procedures on isotopic composition. Consistent with previous studies (e.g., Hervé-Fernández et al., 2016; Sprenger et al., 2018), MW was closely aligned with the LMWL, whereas TBW exhibited a lower slope and greater variability. This reflects its longer residence time in the soil profile and mixing with water of varying ages. Interestingly, the isotopic characteristics of TBW in our study were similar to those reported for xylem water in several studies (Oliveira, et al., 2025; Floriancic et al., 2024; Brighenti et al., 2024; Yang et al., 2023; Goldsmith et al., 2019).

Despite the occurrence of extreme drought during the sampling year, which should leave an isotopically enriched signal in soil water (Dubbert et al., 2019), no such enrichment was observed at any of the study sites, regardless of precipitation regime or land cover. This was most likely due to the sampling depth (20 and 40 cm), as isotopic enrichment from evaporation typically occurs at shallower depths, between 5 and 15 cm (Dubbert et al., 2019). However, while Floriancic et al. (2024) reported no evaporative effect even at 10 cm depth, Brooks et al. (2010) observed significant evaporative enrichment down to 30 cm. This discrepancy may be attributed to differences in soil texture or extraction methodologies and their associated, often unquantified, errors, particularly under low soil moisture conditions during drought periods.

In agreement with Kleine et al. (2020), we observed a longer mean transit time, inferred from the phase shift of individual isotopic data, in non-forested areas. This phase shift also increased with soil depth, particularly for MW. In contrast, the phase shift observed in TBW remained similar between shallow and deep layers. The greater lag observed in non-forested areas is likely driven by two main factors. The first is precipitation amount (Hervé-Fernández et al., 2016) for which higher rainfall can enhance leaching, thereby diminishing the isotopic distinction between MW and TBW. The second is vegetation cover, because, both soil texture and vegetation significantly influence the velocity of the wetting front (Xue et al., 2024). Preferential flow pathways promote deeper and more rapid infiltration in forested areas, whereas under bare soil or grass, water infiltration proceeds more slowly and diffusively.

The unexpectedly rapid turnover in isotopic composition at our highest-elevation site (RO) contrasts with results from other studies. For example, Floriancic et al. (2024) reported significant differences in soil water isotopic composition within the top 40 cm, even at forested sites with vegetation cover and precipitation amounts similar to those at our highest-elevation site. These discrepancies may be explained by differences in elevation, mean annual temperature, and soil texture. Lower elevations combined with higher temperatures can prolong the vegetation growing season, thereby increasing interception and

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evapotranspiration and reducing the infiltration of precipitation into the soil profile. In addition, the slightly higher silt content at their site likely enhances capillary water retention. Such capillary pores can hold water more effectively and may be bypassed by preferential flow paths, in contrast to the coarse sandy soils at our sites. This comparison suggests that vegetation cover and soil properties may exert a stronger influence on soil water dynamics than precipitation amount alone.

5.2 Bulk soil water

Bulk soil water is commonly used as a proxy for the immobile fraction of soil water and is frequently employed in comparisons with xylem water (e. g. Oliveira et al., 2025; Floriancic et al., 2024; Brighenti et al., 2024; Benettin et al., 2024; Barbeta et al., 2019, 2020; Goldsmith et al., 2019; Dubbert et al., 2019). Our results demonstrate that BW can differ significantly from TBW at certain times of the year. This isotopic divergence was observed primarily at the onset of the growing season. Following this period, all four study sites experienced substantial drought, which led to progressive drying of the soil profile and a consequent reduction or complete depletion of the MW fraction.

Historical data of MW from one of the experimental sites (Fig. 8, left), however, indicate that in years without severe drought, isotopic phase shifts within the soil profile, particularly in the MW fraction, can persist throughout the whole year. This persistence may result in subtle but consistent differences between BW and TBW even outside the early spring transition period.

Surprisingly, at the highest elevation RO site (where an unexpectedly rapid replacement of water with predominant contribution from winter precipitation was observed), previously collected mobile water data from 2021 to 2023 revealed a consistent pattern (Fig. 8, right), regardless of interannual variability in precipitation and temperature. Assuming that TBW exhibits similar behaviour, the difference between TBW and BW across years is expected to be negligible.

From a long-term perspective, substituting TBW with BW does not lead to substantially different isotopic interpretations. Furthermore, due to the methodological challenges associated with TBW extraction, this approach is generally unsuitable for routine applications. However, it may be considered in short-term or one-off experiments (e.g., Lehmann et al., 2024), especially when the soil type and environmental conditions indicate a higher proportion of MW. In such cases, isotopic differences can occur—as demonstrated by the 0.3 difference in SOI between BW and TBW—which may result in underestimation of the role of winter precipitation.

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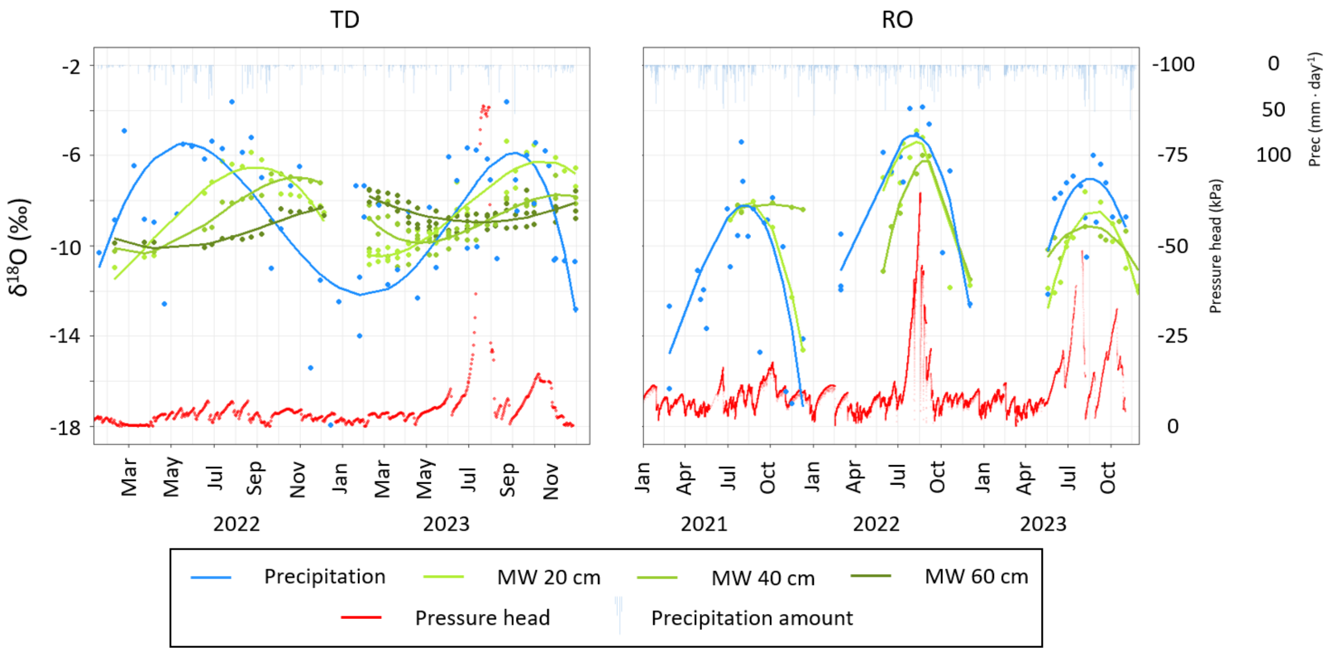

Figure 8. Annual cycle of isotopic composition of precipitation (blue) and mobile soil water (shades of green) at the Trhové Dušníky site in 2022-2023 (left) and the Rokytka site in 2021-2023 (right). MW was sampled up to three depths (20, 40, and 60 cm), where darker colour corresponds to greater depths. The red colour represents the suction pressure head at 20 cm depth, and the blue columns at the top of the graph show total precipitation.

5.3 The importance of winter precipitation

We hypothesized that winter-derived water would persist longest in soil profiles at higher elevations, where thicker snowpack melts later, compared with lowland areas characterized by transient snow cover and predominantly rainfall-based winter precipitation. This hypothesis was supported by soil water content (SWC) patterns: in lowland sites, SWC remains relatively constant during the first quarter of the year until the onset of the vegetation period or the occurrence of the first dry spells, whereas at higher elevations a distinct snowmelt-induced saturation signal is observed. In the latter case, increasing SWC coincides with gradual snow cover depletion and minimal or absent transpiration, conditions that limit soil moisture loss. Our results show that, with increasing elevation (except at the RO site), the presence of winter-derived water in the soil profile is progressively delayed into later months of the year.

The above elevation-dependent delay was also evident in the isotopic signatures of various soil water pools. The most rapid response was observed in MW, which exhibited its most isotopically depleted values in lowland sites as early as March, whereas the minimum values in high-elevation sites occurred approximately one month later. This was followed by changes in shallow TBW, with isotopic minima occurring in April in the lowlands and in May at higher elevations. Winter water appeared last in the deeper soil layers (both MW and TBW), with signals emerging from April to May at low elevations and from May to June at the LI site.

However, at the highest-elevation site, which receives the greatest total precipitation and maintains the most persistent snow cover, we observed, despite limited data and a less pronounced dry season relative to other sites, a consistent pattern in which the isotopic composition of soil water closely mirrors that of precipitation. In this setting, winter water is rapidly displaced from the soil profile by subsequent rainfall events. While isotopically light values of shallow soil water in May and June reflect residual snowmelt, the influence of summer precipitation becomes increasingly evident during the vegetation period. This finding, at least with respect to water in deeper soil layers, contradicts our original hypothesis, which posited that summer precipitation would infiltrate into these layers only during the late summer and autumn months, due to elevated interception and evapotranspiration earlier in the season.

5.4 Tightly bound water extraction

To obtain TBW, the mobile fraction first had to be removed. There are several studies, that tried to extract soil water held in the soil matrix at different tensions (Geris et al., 2019; Bowers et al., 2020; Orlowski et al., 2020). The results of these studies show different isotopic compositions of individual water pools, both with laboratory-prepared (Orlowski et al., 2020; Bowers et al., 2020) and real soil samples (Geris et al., 2015). In this study, we use the pressure plate apparatus, similar to Orlowski et al. (2020), but with a different procedure. In their study, a spike experiment was performed, after which a pressure of 15 bar was applied, and the outflowing water was collected for isotopic analysis. Although labelled water was recovered during a specific time window, the initial and final stages of the experiment yielded water with isotopic signatures differing from the input. This method presents several limitations that hinder its applicability to natural soil samples. First, the true isotopic composition of soil water is typically unknown, making it difficult to determine whether the observed isotopic composition already corresponds to soil water. This ambiguity arises from mixing between the soil water and the water used to saturate the ceramic plates within the apparatus, making the collected outflow likely a composite of both sources. Second, for the method to be valid, each ceramic plate would have to be conditioned exclusively with samples from a single location and soil depth, to prevent internal mixing of different water sources. This requirement greatly reduces the practicality and scalability of the approach.

In our study, the pressure plate apparatus was employed in a modified configuration. Collected soil samples were subjected to a pressure of 0.6 bar, corresponding to the operational threshold for mobile water typically targeted by field-based suction lysimeters (Brooks et al., 2010; Berry et al., 2017; Sprenger et al., 2018). Unlike previous approaches that rely on collecting the outflow water (Orlowski et al., 2020), our method involved a subsequent extraction from pre-dried soil samples. This modification enabled the simultaneous processing of up to 24 samples from various depths and locations within a single run.

In line with the work of Geris et al. (2015), we observed (until the onset of drought) different isotopic compositions between MW and TBW, especially in the upper part of the profile, with these differences decreasing or disappearing with

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depth due to longer residence times. Despite the absence of data on cation exchange capacity (CEC), given the very low clay content at all four sites, it can be assumed that fractionation due to high clay CEC (Araguás-Araguás et al., 1995; Meiβner et al., 2013; Oerter et al., 2014) was negligible and that differences in isotopic composition were mainly due to different water retention times in macro- and capillary pores.

To eliminate the influence of the pressure apparatus on the isotopic composition, another potential approach would involve extracting the BW from a subset of samples, while using the pressure plate apparatus on the remaining samples to determine the proportion of MW and TBW. Based on the known isotopic composition of the BW (laboratory extraction) and MW (obtained via suction lysimeters), the isotopic composition of the TBW could then be estimated using the same mixing equation.

5.5 Data correction

Numerous studies have attempted to compare soil water (including both BW and MW) with xylem water (e.g., Zapater et al., 2011; Meunier et al., 2017; Vargas et al., 2017; Barbeta et al., 2019, 2020; Liu et al., 2021). To enable a meaningful comparison between soil water and xylem water, it is essential to employ an extraction technique that minimizes isotopic alteration of the sample. However, it is well known that no currently available extraction method can extract soil water from all soil types and moisture contents without introducing some degree of isotopic bias (Sprenger et al., 2015; Orlowski et al., 2018; Kocum et al., 2025).

For this reason, various corrections are often applied to the measured data, although not universally. These include, for instance, adjustments to account for the presence of organic compounds (Martín-Gómez et al., 2015), or corrections based on Rayleigh-type fractionation models (Araguás-Araguás et al., 1995).

There is, however, another critical yet frequently overlooked limitation. While each newly developed extraction method is typically validated using soils of different textures and moisture contents (Dalton, 1988; Revesz and Woods, 1990; Leaney et al., 1993; Scrimgeour, 1995; Wassenaar et al., 2008), the outcomes of these validation efforts often show a wide range of method-specific inaccuracies. These inaccuracies are commonly expressed as the shift ± standard deviation in ‰ from the known isotopic value of labelled water used in the test. Although useful for method comparison, these deviations are generally not applied as corrections to real measured data.

The correction employed in this study, which we consider essential for enabling meaningful comparison between individual water samples, relies on conducting spike experiments using the selected extraction method. This allows us to assess the method's performance for specific soil types collected from our study sites. Given that soil texture and moisture content significantly influence extraction outcomes (Hendry et al., 2015; Orlowski et al., 2016), such validation experiments should be performed separately for each soil type involved, ideally across a range of moisture conditions. The resulting isotopic deviations (relative to labelled water) should then be incorporated into data correction procedures for actual samples.

Ultimately, the use of different extraction methods across laboratories, each associated with varying degrees of systematic error, does not necessarily constitute a critical limitation. If these methods produce results with low standard deviations, even in the presence of systematic offsets, such biases can be quantified and subsequently corrected. Such calibration should allow meaningful comparisons across studies and research groups.

6 Conclusions

This study demonstrated that soil water stable isotope dynamics vary systematically along an elevational gradient. Despite the absence of snow cover at lowland sites, these areas exhibited longer residence times, whereas high-elevation sites—with substantial winter snow accumulation—showed more rapid isotopic turnover. All soil water samples plotted close to the respective local meteoric water lines, confirming their meteoric origin and indicating minimal isotopic bias introduced by the applied extraction methods. Mobile soil water most closely mirrored the isotopic composition of precipitation, while tightly bound water showed slight signatures of evaporative enrichment and potential mixing with older water, likely due to its longer residence time. Although tightly bound and bulk soil water exhibited only minor isotopic differences on average, seasonal variability increased—particularly in spring and autumn—when differences in isotopic composition and the proportion of mobile to tightly bound water were most pronounced. These findings highlight the importance of accounting for such variability, especially in short-term or single-time-point studies comparing soil and xylem water for plant source attribution. Future research should further explore how these dynamics interact with vegetation type, rooting depth, and changes in precipitation regimes under ongoing climate change.

Code availability. The code is available from the corresponding author upon request.

Data availability. Tha data from this study are available from the corresponding author upon request.

Author contributions. Concept: JK, LV. Methodology: JK, LV, MS. Investigation: JK, KF, VS, LV. Formal analysis: JK, JH. Resources: VS, MJ, LT, LV. Visualization: JK, KP. Writing (original draft preparation): JK, KF, VS. Writing (review and editing): JK, KP, KF, VS, JH, MJ, MS, LT, LV. Supervision: LV.

Competing interests. The authors declare that they have no conflict of interest.

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