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RESEARCH ARTICLE

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Key Points:

- Tree water resources changed within a few days from snow dominated to higher share of rainfall as soils wetted up after a dry period
- Compensatory plant water uptake by aspen from the deep layer (90 cm), while uptake from soil depths that became drier (60 cm) declined
- Strong differences between water sources and availability beneath aspen and spruce, respectively

Supporting Information:

Supporting Information may be found in the online version of this article.

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Opportunistic Short-Term Water Uptake Dynamics by Subalpine Trees Observed via In Situ Water Isotope Measurements

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Abstract Variations in tree water sources are important to understand in semi-arid ecosystems because climatic shifts towards lower snowpack and increased drought affect water availability in subalpine forests of the western US. Here, we use daily in situ measurements of stable isotopes (²H & ¹⁸O) in soil and tree stem water, soil matric potential and sap flow to study tree water uptake dynamics. We instrumented three soil profiles down to 90 cm, as well as three aspen and engelmann spruce trees near Gothic, Colorado, in the East River watershed. We observed the fate of natural isotopic variations in rainfall, soil, and plants from June to October 2022, and in August 2023 we conducted a ²H labeled irrigation experiment. Our observations showed that all studied aspen trees compensated for water scarcity in the shallow soil by shifting the dominant water source at 60(±20) cm to 2/3 of uptake from 90 cm within a few days of a dry period. Both species relied on snowmelt stored in the subsoil to sustain transpiration. Intense rainfall caused the plant water uptake to shift partially to top soil layers within 2 days. Spruce transpiration was lower and relied more on snowmelt, because rainfall infiltration was low in the spruce stand due to high canopy interception. Our findings highlight the important role of snowmelt stored in the deep soil layers for subalpine forest drought response and the dominant fate of monsoonal rainfall to become transpiration rather than recharging groundwater and streams in the Upper Colorado River.

Plain Language Summary There is a need to understand how trees in mountainous regions respond to dry conditions that lead to water scarcity, because climate projections suggest that such conditions will become more frequent in the future. Here we present a novel data set of measurements of daily stable isotopes of water across soil profiles and in tree stems of aspen and spruce. Our data show that when the upper soil dried out, aspen trees shifted to using water from deeper layers (beneath 60 cm) to keep transpiring. For spruce trees the uptake pattern is less clear, but both types of trees mainly used snowmelt stored in the deeper soil layers to survive the dry summer. After heavy rain, aspen and spruce trees switched to using water from the top 20 cm of soil. However, for spruce, only some rain reached the soil because the dense tree canopy intercepted it, so spruce trees stayed more dependent on snowmelt and used less water overall. This study shows how important deep snowmelt water is for helping forests survive dry periods and suggests that most summer rain is quickly used by trees rather than replenishing streams and groundwater in the headwaters of the Colorado River.

1. Introduction

Observed and projected reductions in the volume of snowpacks and summer rainfall are impacting and will further impact the water availability in subalpine forests of the Western USA (Harpold, 2016; Siirila-Woodburn et al., 2021; Wainwright et al., 2020). The consequences of these changes for ecohydrological processes are not yet understood. However, as forest health is declining and forests in the Rocky Mountains of Colorado change from carbon sinks to carbon sources (Domke et al., 2023; Vorster et al., 2024), there is an urgent need to understand how trees respond to limited water availability. An adaptive root water uptake strategy by trees in response to changing water availability was shown in several isotope based studies using destructive xylem water

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sampling methods in various climates (Barbeta et al., 2015; Brinkmann et al., 2019; Martin et al., 2018; Muñoz-Villiers et al., 2020; Schwendenmann et al., 2015; Tang et al., 2018). These studies found for several tree species that they shift their relative root water uptake from shallow soil layers to deeper soil layers during dry periods. Therefore, in seasonal climates, winter precipitation was found to play a crucial role for summer transpiration (Allen et al., 2019; Goldsmith et al., 2022). A recent global review by Bachofen et al. (2024) of 49 studies showed an increase of the relative share of water uptake from deeper soil layers where soil moisture remains higher compared to the shallow soil during dry periods. This process where trees shift their root water uptake from the topsoil towards the subsoil to sustain transpiration rates is called “compensatory water uptake” (Javaux et al., 2013).

Understanding the forest response to climate induced shifts in water availability is of particular relevance for the Upper Colorado River basin, where air temperature and evapotranspiration are both increasing, leading to declining flow in the Colorado River (Carroll et al., 2024; Milly & Dunne, 2020). Observations in the Rocky Mountains show a warming of the lower subalpine region in the Upper Colorado River basin by about 1.5°C over the last 50 years, with declines of about 20% (lowered by 306 mm/year) in annual precipitation over the last 30 years compared to the 1980s (Faybishenko et al., 2022). While subalpine forests in the Rocky Mountains are usually water limited (Knowles et al., 2015), the frequency of years in which the potential evapotranspiration exceeds precipitation (aridity index >1) in the East River catchment is increasing (Faybishenko et al., 2022), which indicates increasingly water limited conditions for the prevailing ecosystems. It is unclear how the trends towards increased aridity are affecting forest health. Ecohydrological simulations on the hillslope scale suggested that evapotranspiration in subalpine forests in the East River catchment is partially sourced by recent rainfall during the summer when it is available, but the main contributor to the evapotranspiration flux was snowmelt (Maxwell et al., 2019). Catchment scale stable isotope mass balance analyses for the East River catchment further revealed that most of the summer rainfall becomes evapotranspiration and less than 20% of rainfall contributes to summer discharge (Sprenger et al., 2022). Their findings suggest an effective tree water usage of rainfall as more of this water was lost primarily through evapotranspiration in years of higher summer precipitation.

For subalpine spruce in the East River, a shift from snow towards rain as the dominant water source from the early to the mid growing season was shown to be dependent on the water availability as a result of the year-to-year snowpack depth (Berkelhammer et al., 2020). However, due to the limited few snapshots of isotope sampling during the growing season, it remains unclear for now if there are short-term (i.e., daily to weekly) changes in the water source of subalpine trees in response to sub-seasonal water availability variation and if spruce and aspen respond differently. Newly developed in situ isotope measurement techniques allow for investigation of short term plant responses based on ^2H and ^{18}O observations of the water in soil (Gaj et al., 2016; Volkmann & Weiler, 2014) and plant water (Marshall et al., 2020; Volkmann, Kuhnhammer, et al., 2016). While most in situ measurements were temporally limited during and after ^2H labeling experiments (Kübert et al., 2022; Seeger & Weiler, 2021; Volkmann, Haberer, et al., 2016), natural abundance of stable isotopes of water in soils and plants have only recently been used to study ecohydrological processes over longer periods of time (Gessler et al., 2022; Landgraf et al., 2022). Though, previous stable isotope work suggested that if the seasonal isotopic variation in the precipitation is low, the relatively large water volume of the rooting zone (or “flow path length distribution”) (Seeger & Weiler, 2021) and water storage within trees (Knighton et al., 2020) might limit the detectability of short term changes of plant water sourcing based on natural isotope variations. With regard to short-term plant water source dynamics, Gessler et al. (2022) found, based on in situ isotope measurements, a significant increase in the relative uptake by European beech from deeper soil layers within a week during a drought period. However, they did not observe an increase in the absolute uptake from deeper depths, which would reflect a compensatory effect. Also, Liu et al. (2023) and Kuhnhammer et al. (2023) observed a reduction in the transpiration flux during a drought experiment, and the root water uptake from deeper soil layers could not fully compensate for the lack of water in the more shallow soil layers. Conversely, other in situ xylem water isotope measurements indicated compensatory uptake from deep soil layers for European beech (Seeger & Weiler, 2021) and birch and maple (Ring et al., 2024). Thus, there is a need to test if compensatory uptake is a mechanism that could increase the resilience of subalpine trees to increasing aridity in the East River.

Recent catchment scale water age modeling of 472 catchments across the US showed that the simulated mean water age of root water uptake correlated with several metrics of vegetation drought resilience derived from remote sensing data sets. Trees using older water experienced fewer and less severe drought water limitations, but took longer to recover following drought. In contrast trees using younger water experienced more frequent water

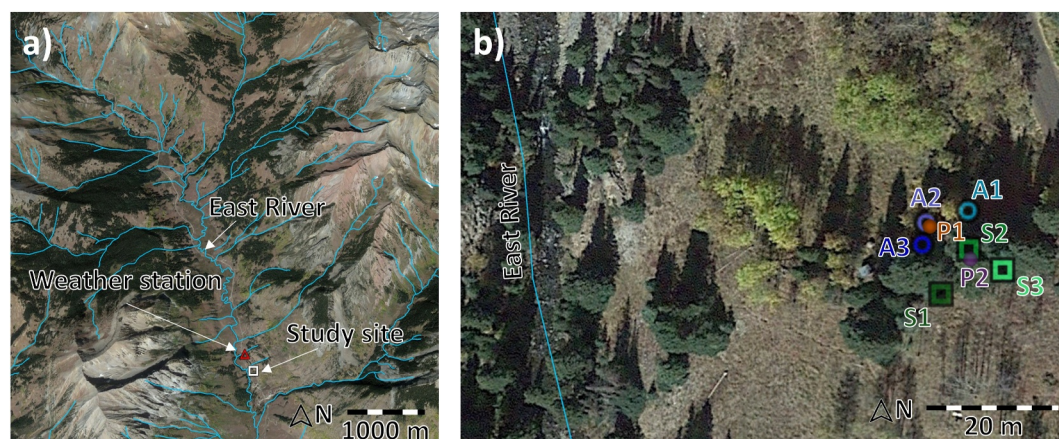


Figure 1. (a) The location of the study site (white square) and its location within the East River catchment (blue river system) and relative to the billy barr meteorological station (red triangle). (b) The study site and the location of the three instrumented aspen (A1, A2, A3) and spruce (S1, S2, S3) trees, as well as the soil profiles instrumented with isotope probes and matric potential sensors beneath aspen (P1) and spruce (P2).

limitations, but returned to pre-drought transpiration rates more rapidly (Knighton & Berghuijs, 2023). Since stable isotopes allow inferring water ages of the plant water (Brinkmann et al., 2018; Sprenger et al., 2019), it is crucial to better understand the information content that in situ measurements can provide to explore the mechanisms that trees use with regard to maximizing water use and drought resilience.

We therefore test the following hypotheses:

1. Natural variations in stable water isotopes, rather than isotope labeling, can be used to identify the depth of water uptake on a daily timescale over several months.
2. Subalpine trees compensate for lack of water in dry shallow soil layers by sourcing their transpiration from deeper layers during dry spells.

2. Methods

2.1. Study Site

The study site was near Gothic, Colorado (38°57'37.9"N 106°59'27.3"W, Figure 1), where the Rocky Mountain Biological Laboratory is located at about 2,917 m elevation in the lower subalpine (Figure 1a). The forest's climatic conditions are characterized by long, cold winters (avg. air temperature of about −9.2 C) and short, cool summers (avg. air temperature of about +9.8 C) that define a continental, subarctic climate (Hubbard et al., 2018). We studied two tree stands of aspen (*Populus tremuloides*) and engelmann spruce (*Picea engelmannii*), respectively, located directly next to each other, with the spruce stand being elevated by 2.0–2.5 m compared to the aspen stand. These two tree species dominate the lower subalpine ecosystem in the East River catchment. The soils at the study site are classified as Bassel sandy loam (Soil Survey Staff, 2024). The sand, silt and clay content were reported to be 62%, 31%, and 7% and the bulk density is 1.4 g/cm³ (Faybishenko, 2020). At 60 cm depth, we observed a significant increase in the rock content, while the bedrock and groundwater level were deeper than the surveyed 100 cm soil depth with no signs of a perched water table. The water table is several meters below ground surface, as the nearest drainage, the East River stream, is located 80 m west of the study site and at 20 m lower elevation (Figure 1b). Based on measurements at the billy barr (sic) meteorological station (Western Regional Climate Center, 2024), the precipitation in Gothic, CO, is dominated by snowfall (on average 407 mm/year), while summer rainfall is mainly limited to monsoonal showers (on average 295 mm/year). The water year 2022 had close to average (306 mm) growing season rainfall and also average (398 mm) winter precipitation. However, August 2022 was wet (monthly rainfall sum was 30% higher than average), while September 2022 was dry (about 60% of the average September precipitation). The growing season rainfall in 2023 was 30% lower than the long-term average and the three months ahead of an isotope spiking experiment in August 2023 had about 50% of the rainfall relative to the average May to July period, while the winter 2022/23 (Nov–March) precipitation sum (539 mm) was 35% higher than the average (Figure 2a). The studied trees were 33–74 years old according to their

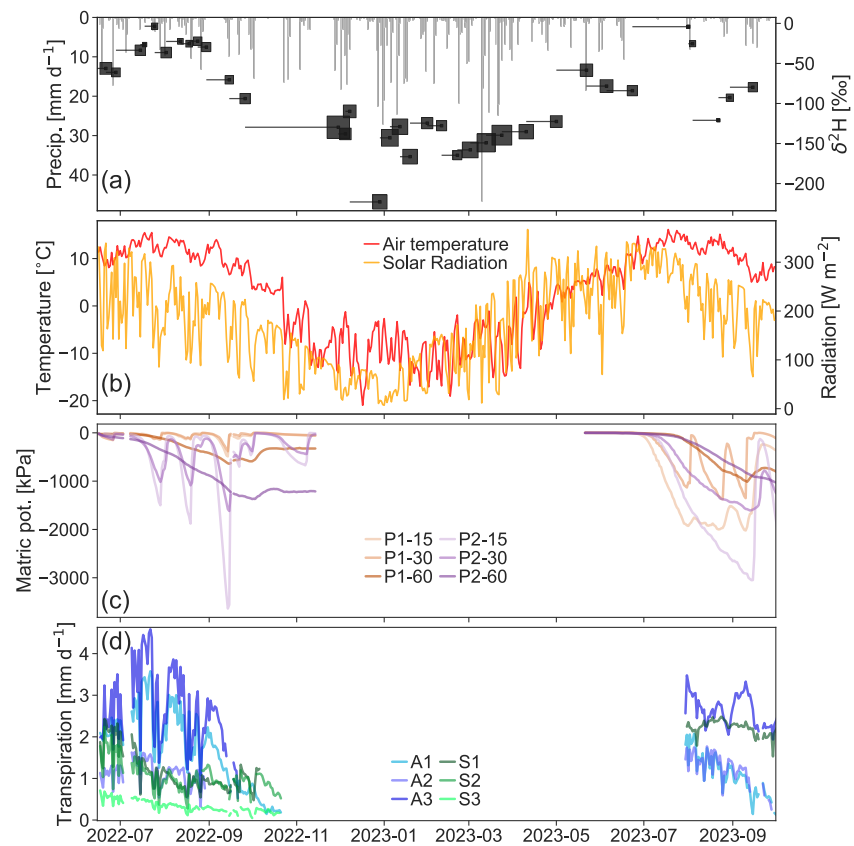


Figure 2. (a) Daily precipitation volumes (bars) and $\delta^2\text{H}$ values of the sampled precipitation. Squares represent the day of taking the precipitation sample and the line to the left represents the period over which the precipitation was sampled. The size of the square represents the precipitation volume. (b) Median daily air temperature and daily sum of solar radiation. (c) Matric potential across the two profiles (P1, beneath aspen, brown colors and P2, beneath spruce, purple colors) at depths 15, 30, and 60 cm. (d) Daily sap flow sum for aspen (blue colors) and spruce (green colors).

tree rings and their circumferences and canopy area varied accordingly (Table 1). The aspen trees' canopy was open, while the spruce trees had a very dense canopy coverage. Allometric equations by Kaufmann et al. (1982) suggest that based on the diameter at breast height and the canopy coverage, the leaf area index of the aspen trees was on average 3.8 and for the spruce 25.5. The root distribution of aspen is reported to be high in the shallow soil layers, but roots can reach down to 2.7 m (DeByle & Winokur, 1985). Similarly, engelmann spruce roots are mainly within the upper 30–45 cm, but can reach 2.4 m depth (Alexander, 1987).

Table 1
List and Characteristics of Instrumented Trees

Tree ID	Species	Diameter at breast height (cm)	Canopy radius (cm)	Sapwood depth (cm)	Age (years)
A1	Aspen	31.74	262.5	9.6	74
A2	Aspen	22.6	237.5	6.7	47
A3	Aspen	13.9	127.5	3.2	58
S1	Engelmann spruce	15.8	145	5.8	33
S2	Engelmann spruce	25.4	140	5.3	68
S3	Engelmann spruce	19.1	140	7.4	67

2.2. Hydrometric Data

We measured the matric potential with sensors (Teros 21, METER, accuracy $\pm(10\%$ of reading $+2$ kPa) from -100 to -5 kPa) installed along two soil profiles at 15, 30, and 60 cm depths, respectively. One soil profile (P1) was located beneath aspen trees and the second one (P2) was in the spruce stand. During installation of the matric potential sensors, a tight connection of the sensor and the surrounding soil was ensured. Three aspen and three engelmann spruce trees, respectively, were instrumented with heat pulse sap flow sensors (Implexx Sap Flow Sensor, Implexx Sense). The sensors were installed at breast height on the south side of the trees and they were covered with aluminum foil to prevent heat impact from the sun. We calculated the sap flux volumes (kg/day) based on the measured sap velocity (cm/day) and sap wood length that we determined from tree cores (Table 1). We determined the transpiration rate (mm/day) by relating the sap flux to the crown area based on the measured canopy widths (Table 1). Matric potential and sap flow measurements started in Mid-June 2022 and were taken every 10 min. Sap flow sensors in 2023 were installed into freshly drilled holes. Daily meteorological data on air temperature, relative humidity, barometric pressure, wind direction and speed, solar radiation, and precipitation were provided by the nearby (ca. 300 m away) billy barr weather station (38.96021739, -106.9911594 , 2,917 m elevation) (Western Regional Climate Center, 2024). For some graphs and analyses, we report the matric potential data as pF-values, which is the decimal logarithm of the pressure in hPa. We used linear regression to relate daily transpiration sums to average daily matric potential given as pF values.

2.3. Isotope Data

For the isotopic measurements of soil and plant water, we used an in situ system that is based on the isotopic equilibration method as described by Seeger and Weiler (2021). The applied method is based on the isotopic equilibrium between a liquid water pool and the surrounding vapor in a closed system (Wassenaar et al., 2008). At 100% humidity, the phase change from the liquid water to the vapor results in an equilibrium fractionation, which means that the associated change of the isotope ratio is solely a function of the temperature. Therefore, probes with vapor permeable membrane heads (made out of sintered polyethylene with a pore size of 10 μm) were shown to allow for continuous isotopic sampling of soil (Volkman & Weiler, 2014) and plant water (Volkman, Kuhnhammer, et al., 2016) connecting the probes to a cavity ring-down spectroscope (L2140-i, Picarro Inc.). We installed vapor probes along two soil profiles: One beneath an aspen tree (P1) and one beneath a spruce tree (P2). The vapor probes measurement depths were 5, 10, 30, and 60 cm in all profiles and an additional probe at 90 cm beneath aspen and 85 cm (too rocky at 90 cm) beneath the spruce. The P1 and P2 profiles were within 30 cm from where the matric potentials were installed, which is why these two measurement locations are called P1 and P2, respectively. We further installed in each of the six trees a vapor probe at the bottom of the stem and sealed off the surrounding of the probes with silicone to prevent water and vapor from going into the drilled holes after the probes were inserted. In 2023, new vapor probes were installed in new boreholes of the trees. For calibration purposes, we placed three standard water bottles into the soil (covered to prevent any condensation within the bottles) containing each 600 ml of water of known isotopic composition (tap water, snowmelt, ocean water) and measured the vapor isotopic composition of their headspace at least every 4 hr with the same vapor probes as the soil and xylem water. We followed the calibration procedure as outlined in detail by Seeger and Weiler (2021): The isotope measurements of the three standards that were closest in time before and after a measurement with the soil and xylem water vapor probe measurements were used to calculate a calibration equation between the water vapor concentration (ppm H₂O) in the cavity of the laser spectrometer and the isotope measurements. This way, any impact of temperature dependent water vapor concentration that impacts the laser absorption in the cavity as well as a potential drift of the spectrometer was accounted for and we could infer from vapor measurements the liquid water isotope ratios in ‰ relative to the Vienna Standard Mean Ocean Water. We followed the measurement strategy as outlined by Seeger and Weiler (2021): All isotopic measurements with the vapor probes were conducted by first flushing the tubing and probe with dry air and then sampling the vapor at 1 Hz from that probe until a steady water vapor concentration (a plateau in the time series with standard deviation (SD) of <200 ppm) in the cavity was reached, as well as for the $\delta^2\text{H}$ (SD $<1\%$ for $\delta^2\text{H}$) and $\delta^{18}\text{O}$ (SD $<0.2\%$ for $\delta^{18}\text{O}$) values, respectively. Once the plateau was reached, we calculated an average over 2 min and the tubing was flushed with dry air again before switching to the next vapor probe via an automated valve system. We limit our plots to the results based on $\delta^2\text{H}$ values throughout the manuscript and refer to the Supporting Information S1 for graphs showing the results based on $\delta^{18}\text{O}$ data. Our measurements with probes installed in soil, tree xylem and standard bottles all show a linear relationship along the global meteoric water line, which is indicative of isotopic

equilibrium between the liquid and vapor phase of the measured soil-, xylem-, and standard waters. The maximum standard deviation for the repeated isotope measurements of the standard waters corrected for the water vapor concentration in the cavity was 3‰ for $\delta^2\text{H}$ and 0.42‰ for $\delta^{18}\text{O}$, respectively. As a last step, we calculated daily averages of $\delta^2\text{H}$ and, while we did not include $\delta^{18}\text{O}$ for each probe in our analyses due to larger uncertainty related to ^{18}O measurements (Seeger & Weiler, 2021). Rainfall and snow water were sampled for isotope analyses on an event basis at a sampling location at 29 m lower elevation and 3 km south (Williams et al., 2023) and we accounted for a lapse rate ($\delta^2\text{H}$ of $-0.24\text{‰}/100\text{ m}$), which was derived from 33 precipitation events over two years sampled at 2,513.6, 2,885.5, 3,181.5 m elevation, respectively.

The strong temperature differences in the study region lead to pronounced stable isotopic differences between snow and rainfall (Sprenger et al., 2022). In 2022, we used this natural $\delta^2\text{H}$ variation (Figure 2a) to trace the fate of snowmelt and rainfall along the installed soil profiles and into the trees. Based on the findings from the growing season 2022, we decided to conduct a $^2\text{H}_2\text{O}$ labeling experiment during the 2023 growing season (6th and 7th of August in the spruce and aspen stand, respectively). We conducted irrigation experiments around each of the six instrumented trees and arranged four sprayers around the tree to cover the forest floor evenly with the irrigation. Due to limitations by neighboring trees the wetted area varied between 8.4 and 17 m² (Table S1 in Supporting Information S1). After we had conducted the irrigation of the spruce trees on August 6th with a $\delta^2\text{H}$ value of 1,200–2,200‰ (see Table S1 in Supporting Information S1) and a water amount between 3 and 6 mm of rainfall and had not seen much of an isotopic change in the soil water and trees, we increased the irrigation volume to an equivalent of 7.0–8 mm of rainfall on the following day when we irrigated around the aspen trees (Table S1 in Supporting Information S1). We used linear regression to relate daily values of the xylem water $\delta^2\text{H}$ values to average daily matric potential given as pF values.

2.4. Origin of Root Water Sources

For the spatial origin of root water uptake, we used the MixSIAR mixing model (Stock et al., 2018) to infer the distribution of root water uptake depths based on the daily soil water isotope $\delta^2\text{H}$ values at five different soil depths (5, 10, 30, 60, and 90 cm). We made sure that the considered endmembers of the source of the uptake depths had at least a difference larger than the measurement precision of 3‰ in their $\delta^2\text{H}$ values. Whenever the difference between observed $\delta^2\text{H}$ values for two or more probes at neighboring depths was less than 3‰, we grouped the depths and used the average value of the probes as the source water endmember. For days on which probes that were not located next to each other across the profile did not differ at least 3‰ in their $\delta^2\text{H}$ values, the average of all probes between these two depths was used as the water source endmember representing the depth range covered by these two probes.

For example, if the $\delta^2\text{H}$ value measured with the probe at 10 cm did not differ from the measurement of the probe at 60 cm, then the average $\delta^2\text{H}$ value for the probes at 10, 30, and 60 cm was used as an endmember. This grouping of water sources resulted in potential combinations of endmembers that included the following ranges: 0–7.5 cm (vapor probe at 5 cm), 0–20 cm (vapor probes at 5 and 10 cm), 7.5–20 cm (vapor probe at 10 cm), 0–45 cm (vapor probes from 5 to 30 cm), 7.5–45 cm (vapor probes from 10 to 30 cm), 20–45 cm (vapor probe at 30 cm), 7.5–75 cm (vapor probes from 10 to 60 cm), 20–75 cm (vapor probes from 30 to 60 cm), 45–75 cm (vapor probe at 60 cm), 45–90 cm (vapor probes at 60 and 90 cm), 75–90 cm (vapor probe at 90 cm). For days on which only one group remained after the outlined grouping of the sources, no mixing analyses was conducted. The daily xylem $\delta^2\text{H}$ values measured at the six trees were the mixtures with the $\delta^2\text{H}$ values measured across profile 1 being the source for the aspen and across profile 2 the source of the spruce, respectively. We did not apply any weighting for the different endmembers (“uninformative prior,” $\alpha = 1$) and defined the MCM parameters as follows: chainLength = 100,000, burn = 50,000, thin = 50, chains = 3, which led the mixing model to converge (Gelman diagnostic <1.05).

We report the mean values of the mixing results that represent the relative share of the water source from different depths to the root water uptake. By multiplying the relative share of root water uptake based on the MixSIAR model with the tree's transpiration rate, we inferred the absolute contributions from the different depths in mm per day. We filled the gaps, for periods in which sap flow data was missing, based on linear regression of the sap flow data from one tree with the average sap flow data of the two other trees of the same species. To see the effect of drought and re-wetting on the relative and absolute water uptake depths, we defined a pre-drought period (17–20 Aug 2022), a drought period (10–13 Sept 2022), and after rainfall had occurred again a post-drought period (30

Sept–3 Oct. 2022). These periods were chosen based on the relative differences in the soil matrix potential data and the availability of mixing model results. The pre-drought period covers the earliest available data for the mixing model and is prior to a 2 week dry period in early September. The end of this dry period is characterized by the lowest matric potential values and therefore defines the drought period. The post-drought period is defined by the time after the soil was rewetted due to rainfall. The average matric potential for the three periods (pre-drought, drought, and post-drought) are for profile 1 at 15 cm -107 , -349 , -15 kPa, respectively, at 30 cm, -126 kPa, -257 kPa, -13 kPa, at 60 cm -316 , -577 , -580 kPa, respectively and for profile 2 at 15 cm $-1,805$, $-2,786$, -263 kPa, at 30 cm $-1,044$, $-1,226$, -311 kPa, at 60 cm -693 , $-1,095$, $-1,368$ kPa, respectively. We used Pearson correlation to relate daily source water depths to average daily matric potential given as pF values and we applied the non-parametric Mann-Whitney U Test to identify significant differences of the relative and absolute water uptake from the different depth classes during pre-drought, drought, and post-drought periods.

3. Results

3.1. Transpiration Flux and Soil Water Storage

The hydrometric measurements show a very clear seasonality with matric potential sensors indicating that soil layers were generally close to or at saturation after snowmelt and became drier from late June on due to transpiration, which is reflected in the sap flow data. Sap flow sensors recorded the highest transpiration rates in mid-July with a declining trend towards September (Figure 2).

The matric potential dynamics of drying down and wetting up at 15 and 30 cm were pronounced in 2022 beneath aspen and spruce due to the interplay of relatively frequent monsoonal rainfall events and transpiration throughout the growing season. In 2023, when very few rainfall events occurred during the summer, the shallow soil layers did not wet up beneath the spruce, rather they dried down continuously. At 60 cm soil depth, there was a general drying trend throughout the growing season in both years beneath aspen and spruce. Soils in 2023 were generally drier than in 2022 with the exception of 60 cm beneath spruce (Figure 2c). Soils beneath the spruce trees approached pF 4.2 ($=1,500$ kPa), which is often associated as the matric potential limit for root water uptake (Kirkham, 2014). Only when intense rainfall in late September 2022 happened, a general wetting up close to saturation at the 15 and 30 cm depths beneath both aspen and spruce trees occurred and, at 60 cm the drying trend stopped (Figure 3e). The observed matric potential gradients in the upper 60 cm of the soil profiles indicated that the soil beneath 60 cm was generally more moist than above, as soils dry from above.

The transpiration by aspen trees was higher in 2022 than in 2023, while measurements of spruce trees were very limited due to sensor failure, which did not allow for an inter-annual comparison for spruce. In 2022, the transpiration rates of aspen trees were higher than those of spruce, but for both species the transpiration rates generally decreased as the water availability decreased over the growing season (Figure 3c). For all studied trees, the transpiration rates significantly declined with declining matric potential at 60 cm (Figure 4c, Pearson correlation significant at $p < 0.05$). The spruce trees showed a similar significant relationship between transpiration rate and matric potential at both 15 and 30 cm, while the aspen transpiration rates were not sensitive to matric potential at the 15 and 30 cm soil depth (Figures 4a and 4b). Our sap flow measurements showed that the trees remained transpiring throughout the study period (see Figure S1 in Supporting Information S1 for green leaves on photo taken on 24 September 2022), but the rates were reduced after September 15, when temperatures dropped.

3.2. Isotope Dynamics of Plant and Soil Water

Throughout 2022, we observed that $\delta^2\text{H}$ values were generally higher beneath aspen (P1, Figure 3g) than beneath spruce (P2, Figure 3h). Soil water isotope ratios beneath aspen showed a clear response to rainfall infiltration and the water in soil pores at 5, 10, and 30 cm depth were dominated by isotopically enriched summer precipitation with the isotopic signal of snowmelt greatly diminished in the shallow soil layers in mid-August. The vapor sensors at 60 and 90 cm soil depth did not detect any changes in July 2022 and only slight changes throughout August in the stable isotopic composition. Only after several rainfall events in late August 2022, isotope ratios of soil water beneath aspen were enriched at 60 cm, while the pore water at 90 cm retained the isotopic composition of snowmelt throughout the entire observation period. The soil water beneath spruce remained more similar to snowmelt $\delta^2\text{H}$ values (-100 to -150‰) and only at 5 and 10 cm soil depth the pore water isotopes became slightly enriched due to isotopically enriched rainfall infiltrating the soil and/or kinetic fractionation due to soil evaporation.

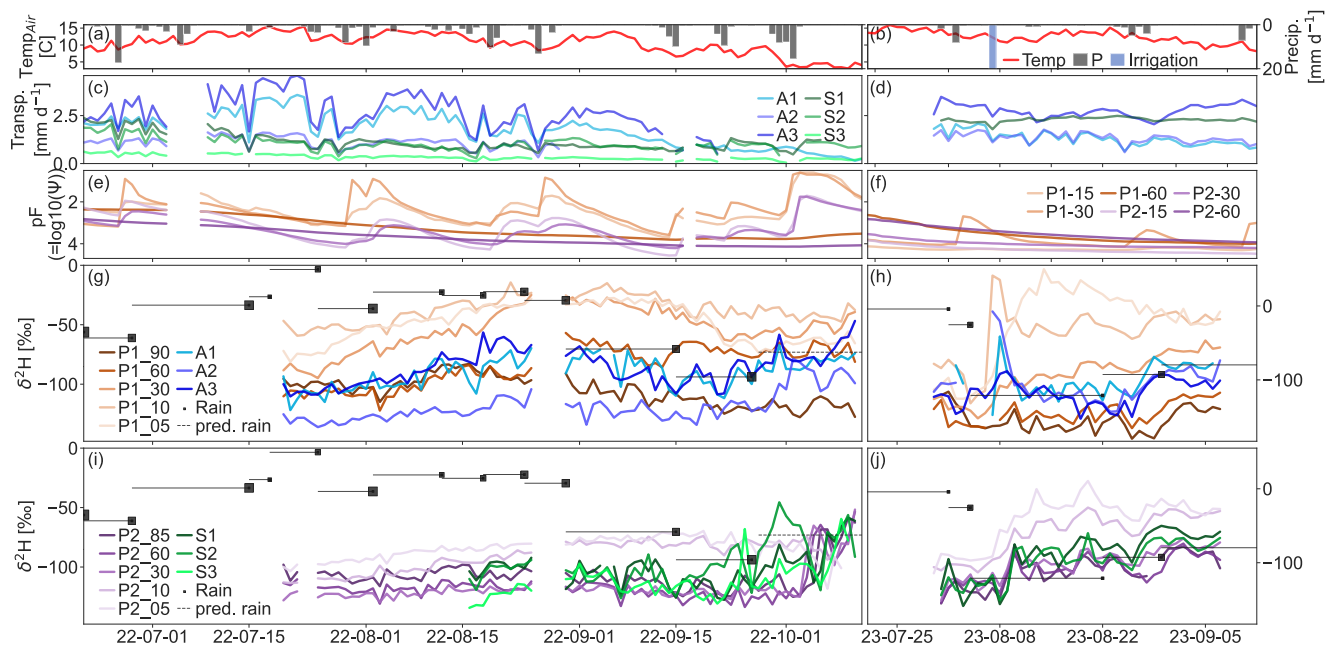


Figure 3. Time series covering the in situ isotope measurement periods in 2022 (left column) and 2023 (right column), respectively. (a) and (b) Daily precipitation shown as bars and median air temperature as red line, respectively. Note that irrigation in early August 2023 is shown as a blue bar. (c) and (d) Daily transpiration sum with different colors representing different trees. (e) and (f) Daily median matric potential in logarithmic scale ($\log_{10}(\Psi)$ [hPa]) with different colors representing different sites and soil depth. (g)–(j) Stable isotopes of hydrogen ($\delta^2\text{H}$) shown for the precipitation (black) and daily averages for the soil water at different soil depths of profiles P1 (beneath aspen) and P2 (beneath spruce), as well as for the six different trees (aspen, A1, A2, A3 and spruce, S1, S2, S3). The dashed horizontal line for rainfall isotopes in October 2022 shows predicted $\delta^2\text{H}$ values based on humidity and air temperature data due to a lack of rainfall sampling. Note that for 2023, the $\delta^2\text{H}$ has a larger scale due to the ^2H irrigation and no data for S3 was recorded due to a probe failure. Black horizontal lines represent sampling periods for individual rainfall samples and the size of the black squares reflects the rainfall amount accumulated over that time span. We refer to Figure S4 in Supporting Information S1 for the same graph showing the $\delta^{18}\text{O}$ values.

Xylem $\delta^2\text{H}$ values were generally more similar to deeper soil layers (60 and 90 cm in aspen, 30–90 cm in spruce), but $\delta^2\text{H}$ values for stem water in aspen increased throughout the relatively wet August 2022 and decreased again during the dry September 2022. The intense rainfall in late September 2022 (31 mm over three days) led to a considerable increase of $\delta^2\text{H}$ values in xylem water isotope ratios of spruce (Figure 3i). The $\delta^{18}\text{O}$ values showed very similar dynamics in 2022 as shown in the dual isotope plot (Figure S2 in Supporting Information S1) and $\delta^{18}\text{O}$ time series (Figure S3 in Supporting Information S1).

The ^2H irrigation in early August 2023 onto a dry soil led to quick isotopic response in the soil water of the upper 10 cm beneath aspen and spruce. However, only two aspen trees (A1 and A2) showed a rapid uptake of the irrigation water, while one aspen (A3) did not show a measurable isotopic response to the irrigation water input (Figure 3h). Interestingly, the 5 cm vapor probe beneath aspen responded in delay compared to the immediate response at 10 cm in that profile. For the soil profile in the spruce stand, there were no signs of deuterated water infiltrating beneath 30 cm. Irrigation volumes beneath spruce were lower than for the aspen stand (Table S1 in Supporting Information S1) and $\delta^2\text{H}$ response was delayed and moderate at 5 and 10 cm depth, while xylem isotopes did not increase much after irrigation (Figure 3j).

3.3. Temporal Plant Water Origin Dynamics (Snow vs. Rain)

The xylem water $\delta^2\text{H}$ values significantly decreased for all trees as the soil dried at 15 and 30 cm (i.e., pF values increased, Figures 4d and 4e). Thus, when the shallow soil was drier, the plant water generally had a higher share of winter precipitation in the transpiration flux. The xylem water $\delta^2\text{H}$ values increased as the soil at 60 cm got drier (Figure 4f). However, since the soil layer at 60 cm was already dry when isotope measurements started in July 2022, there was relatively little variation in wetness. The plant water $\delta^2\text{H}$ values indicated more rain water uptake when the soil at 60 cm was at its driest condition during the late growing season and shallow soil pores were getting refilled by intense rainfall at that time (30 September 2022). Therefore we see a positive statistical relationship between xylem water $\delta^2\text{H}$ values of all trees and soil dryness at 60 cm soil depth (Figure 4f), which is

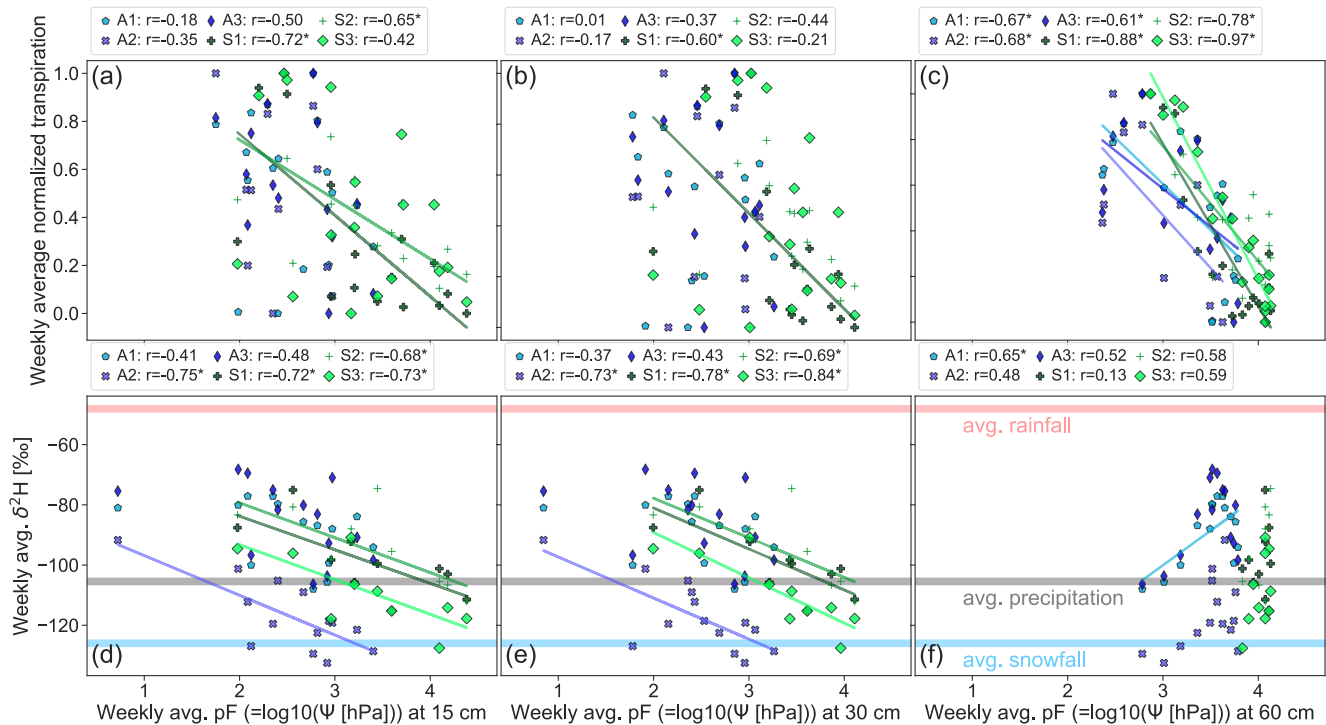


Figure 4. Weekly averages of the normalized transpiration of three aspen (in blue) and three engelmann spruce (in green) as a function of the weekly averages of matric potential measured in soil profiles beneath the trees at 15 cm (a), 30 cm (b), and 60 cm (c), respectively. The transpiration flux was normalized to allow for comparison of the dynamics on the same y-axis despite differences in absolute values. Weekly averages of xylem water $\delta^2\text{H}$ values in the six studied trees as a function of the matric potential measured in soil profiles beneath the trees at 15 cm (d), 30 cm (e), and 60 cm (f). Legends show the Pearson correlation for each tree. Significant correlations are indicated by an asterisk and a linear regression line in the graphs. Note that the isotope measurements started one and two months later for aspen and spruce, respectively, compared to the sap flow measurements. All data shown are limited to 2022 to prevent effects of the ^2H labeling experiment in 2023. The red, gray, and blue horizontal lines represent the volume weighted average of $\delta^2\text{H}$ for rainfall, total precipitation, and snowfall, respectively. A plot showing daily data is provided in Figure S4 in Supporting Information S1.

not a causal relationship. Thus, since the drying trend at 60 cm soil depth was not interrupted by any rewetting due to rainfall during the summer, the stable isotopes did not change much at 60 cm depth, which is why any changes of the xylem water $\delta^2\text{H}$ values reflected mainly the water availability dynamics within the upper 30 ± 20 cm of the soil profiles.

3.4. Spatial Plant Water Origin Dynamics (Uptake Depth)

The general pattern of the relative water uptake depths as derived by the MixSIAR mixing model showed that aspen trees adapted their water uptake throughout the growing season based on the water availability (Figure 5). The dominant uptake depth of the aspen trees was 60 ± 15 cm during July and August 2022. In late August, the uptake from soil layers beneath 80 cm depth became dominant until late September, when the shallow soil layers became more important due to a rewetting via intense rainfall events. As soils at 60 cm continuously dried throughout the growing season, the relative contribution to root water uptake from depths beneath 60 cm (based on the isotope measurements with the probe at 90 cm depths) increased, because we expect higher water availability in soil layers deeper than 60 cm. The spruce root water uptake depth variations did not show as clear a temporal pattern as the aspen trees due to the lower differentiability of the potential plant water sources (i.e., many depths had to be grouped). The dominant water uptake of spruce trees in August was between 20 and 75 cm for S3 and between 7.5 and 75 cm for S1 and S3. The share of shallow water uptake by S1 and S2 declined by Mid-September. Only after the rainfall events in late September, the shallow soil layers (0–20 cm) became the dominant water source for spruce. However, the uncertainty of the MixSIAR results for the spruce trees was relatively high (average standard deviation for spruce between 15% and 19%, Table S2 in Supporting Information S1).

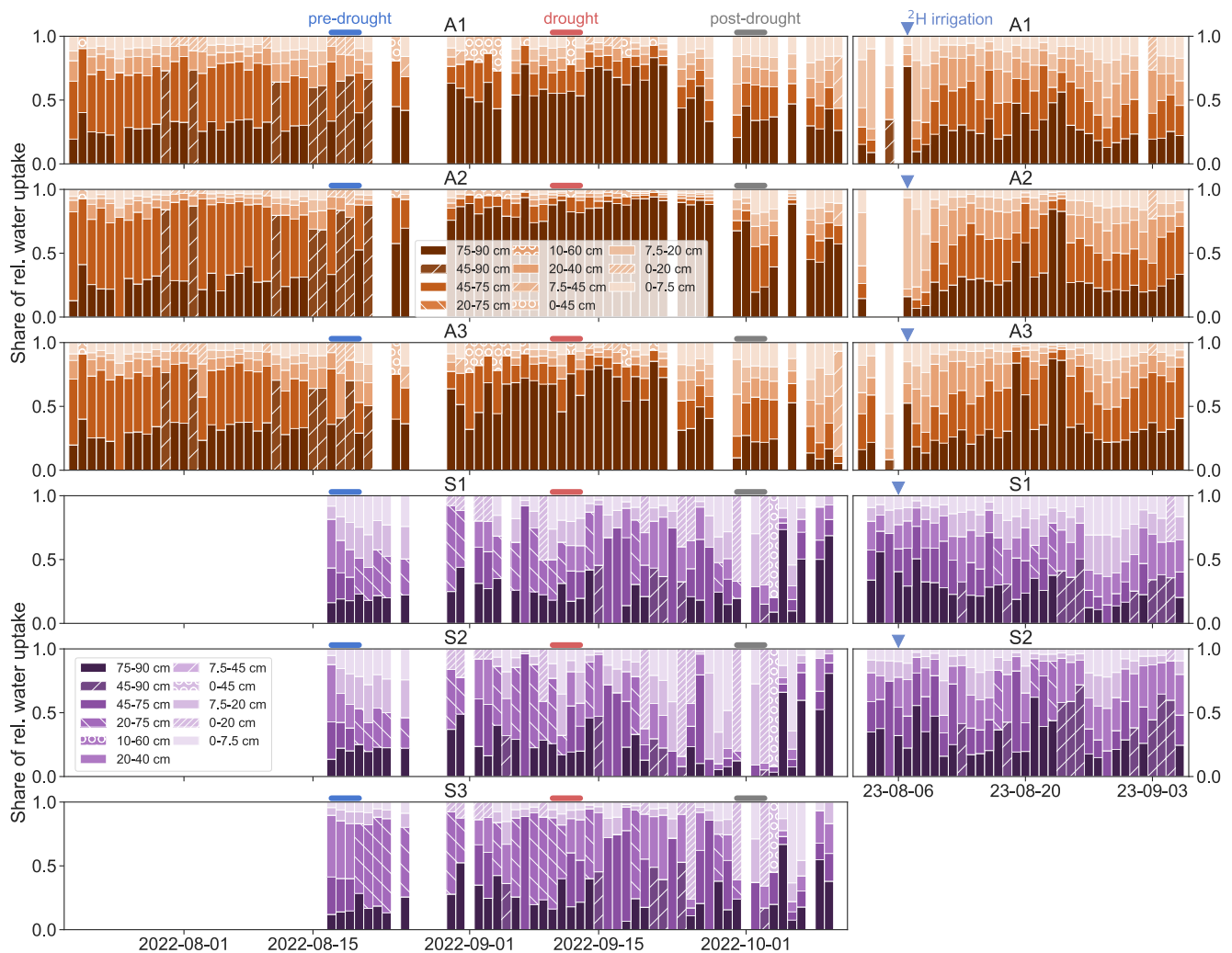


Figure 5. Daily relative share of root water uptake from different depth ranges resulting from the MixSIAR mixing model based on $\delta^2\text{H}$ for each tree (rows) for 2022 and 2023 (columns), respectively. Darker tones indicate deeper depths, brown represents P1 beneath aspen and purple represents P2 beneath spruce. Horizontal lines for 2022 in blue, red, and gray represent the pre-drought, drought, and post-drought periods as defined for Figure 7. The blue triangles indicate the timing of the irrigation experiments in 2023. Plots showing the MixSIAR results based on $\delta^{18}\text{O}$ values (Figure S5 in Supporting Information S1), with added error bars (Figure S6 in Supporting Information S1), and the absolute water uptake for 2022 (Figure S7 in Supporting Information S1) are provided in the Supporting Information S1.

The temporal dynamics of the relative share of water uptake from different soil depth classes by the trees strongly correlated with the soil water availability in 2022 (Figure 6). As soil pores at 60 cm depth dried down, the uptake from 60 ± 20 cm depth became significantly less important for aspen trees (negative correlation coefficients at p -values < 0.05 in Figure 6), while their root water uptake from beneath 80 cm increased significantly. Spruce trees also tended to increase their uptake from the deepest observed depths as the soil dried at 60 cm. The matric potential changes at 15 and 30 cm soil depths did not have strong effects on the share of aspen water uptake from 40 to 80 cm depth. However, the relative share of water uptake from the topsoil (0–40 cm) decreased as the soil at 15 and 30 cm became drier. The spruce tree water uptake from the upper 40 cm was generally low due to the dry conditions and their uptake from 60 ± 20 cm further increased as the top 40 cm of the soil became drier.

In addition to a shift from the relative share of root water uptake from 60 ± 20 cm to deeper soil layers as the aspen's response to drought, we also see an increase in their absolute water volumes taken up from beneath 80 cm soil depth during the drought (Figure 7j). While the relative share of aspen root water uptake from beneath 80 cm soil depth increased by a factor of five from pre-drought to drought conditions (Figure 7e), the absolute uptake from beneath 80 cm increased by a factor of seven during the drought. After the drought, aspen trees shifted their uptake to shallow soil layers by doubling the absolute uptake from the upper 20 cm compared to the drought

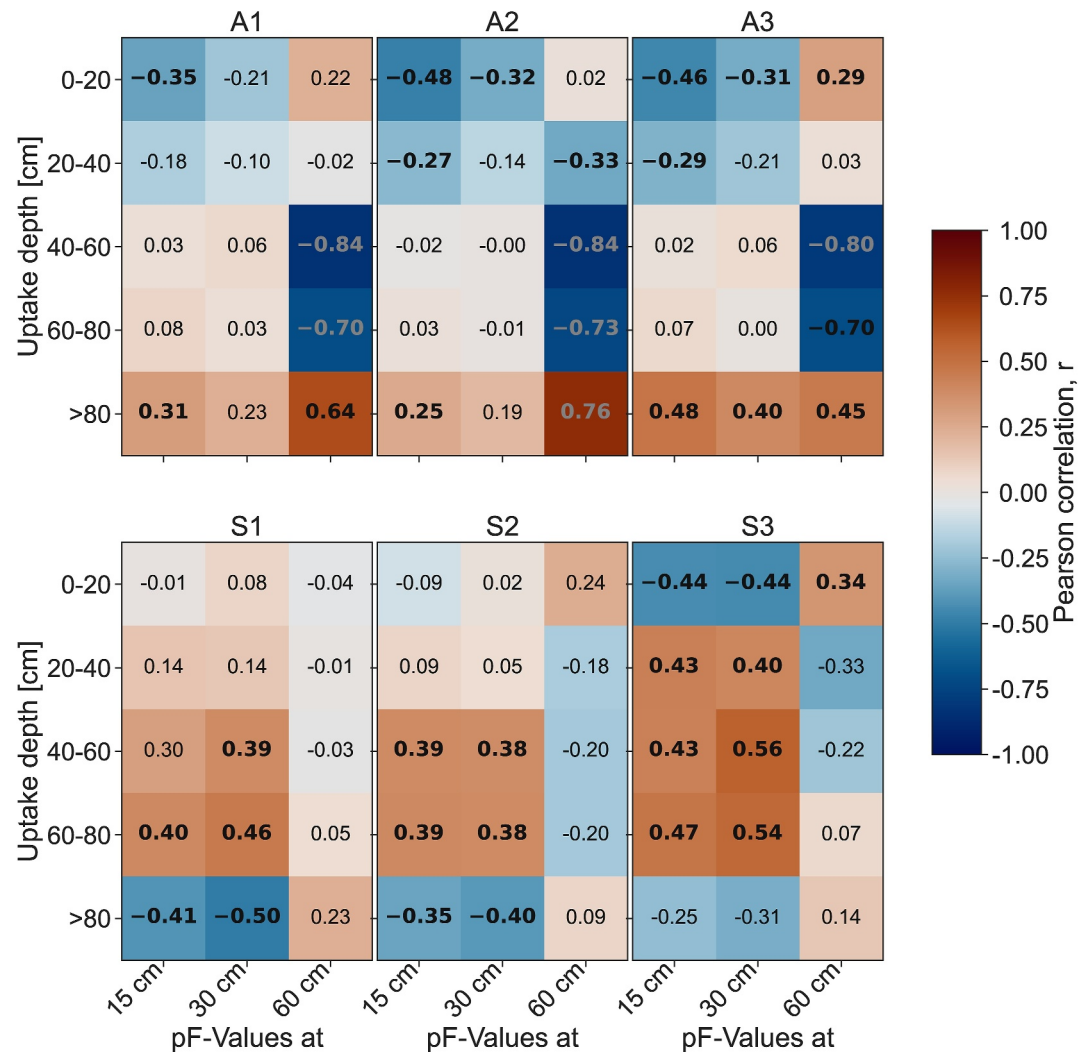


Figure 6. Relationship between the daily relative root water uptake from different soil depths (rows) and the soil's matric potential at different depths (columns) in 2022. Each subplot shows individual trees (aspen, A1, A2, A3 and spruce, S1, S2, S3) and the color code represents the Pearson correlation coefficient. Statistically significant correlations are indicated by bold font. Note that higher pF-values represent drier soils and that a negative r-value (blue colors) therefore indicates a decline of the water uptake in response to soil drying. The uptake depths were grouped according to the depth ranges shown in Figure 5. For depth groups used in the MixSIAR analyses that exceeded the 20 cm range, the depth of largest overlap was chosen in this analysis. Scatterplots of these relations (Figure S8 in Supporting Information S1) and the relation of matric potential and absolute uptake estimates (Figure S9 in Supporting Information S1), as well as the same graph based using MixSIAR results based on $\delta^{18}\text{O}$ values (Figure S10 in Supporting Information S1) are provided in the Supporting Information S1.

conditions (Figure 7f). Transpiration fluxes were generally lower for spruce trees than for aspen and the spruce tree water uptake from the deepest observed soil layer did not change significantly between pre-drought and drought conditions. Once rainfall had rewetted the shallow soil, the relative share of root water uptake from the upper 20 cm tripled and doubled from drought to post-drought conditions for aspen and spruce, respectively (Figure 7a). Thus, while the relative contribution to root water uptake from beneath 60 cm soil depth was high (>50%) throughout the study period for all trees, we observed a swift increase in the relative uptake from the upper 20 cm when rainfall re-wetted the topsoil.

This opportunistic response of short-term adjustments of water uptake depths in response to soil water availability was supported via irrigation with deuterated water onto a relatively dry soil in 2023. Our observation showed a rapid water uptake from shallow soil depths by aspen after the irrigation (Figure 5). However, within a few days,

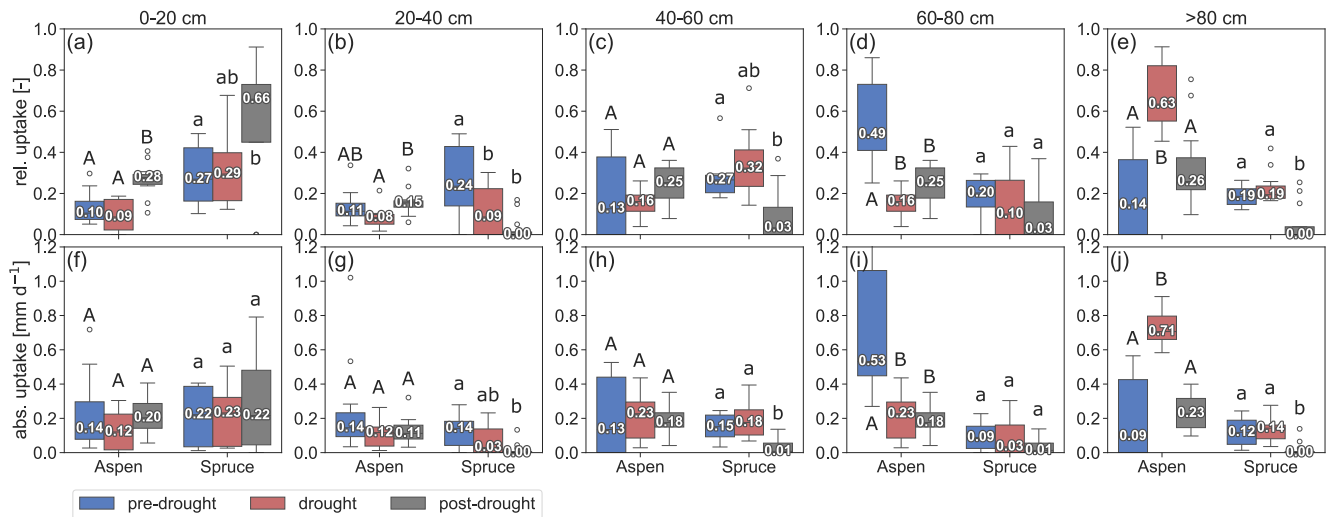


Figure 7. Boxplots comparing the relative root water uptake based on the MixSIAR model results (first row) as well as the absolute root water uptake based on the product of the relative uptake and the tree's transpiration flux (second row) from the five different soil depth classes (0–20, 20–40, 40–60, 60–80 cm, and beneath 80 cm) during four days prior the September 2022 drought (17–20 Aug, pre-drought shown in blue), during the drought (10–13 Sept, red), and after rainfall had occurred again (30 Sept–3 Oct, post-drought shown in gray). These weeks are highlighted with the same colors in Figure 5. The median values are shown in white font on the median line of each box. Letters indicate significant differences of root water uptake for the three drought conditions for aspen (capitalized) and spruce (lowercase), respectively, according to the Mann-Whitney-U test. The same graph based on MixSIAR results derived from $\delta^{18}\text{O}$ values is shown in Figure S11 in Supporting Information S1.

when most of the irrigated water was taken up by roots or had evaporated, the subsequent dominant uptake depths were again the soil layers between 45 and 90 cm. In response to monsoon rainfall in late August 2023, root water uptake shifted towards shallower soil depths; but, uptake from the 45–90 cm soil layer still accounted for more than 50% of total uptake for most of the time, likely due to the very dry summer in 2023. For spruce, the irrigation water amount (Table S1 in Supporting Information S1) was not sufficient to significantly change the isotope ratio of the soil water and thus, also the isotope ratio of the plant water did not change much in response to the deuterium irrigation.

4. Discussion

4.1. Short-Term Plant Water Uptake Source Dynamics Are Identifiable

Our daily observations of $\delta^2\text{H}$ values in soil and tree xylem water show how rainfall water infiltrates into the shallow soil layers (upper 40 cm) and how the water source of tree transpiration changes in response to water availability. Observed relationships between changes in water availability based on matric potential measurements and the isotope-based temporal and spatial origins of the plant water uptake were detectable on scales of a few days (i.e., uptake shifted to deeper soil layers for moisture during dry spell in September 2022) to seasonal (i.e., uptake shifted from predominantly winter to summer precipitation during July and August 2022). Previous in situ measurements have been effective for observing rapid responses to experimental deuterium pulses (Beyer et al., 2020; Seeger & Weiler, 2021; Volkmann, Haberer, et al., 2016), however natural abundances of stable isotopes have shown limited insights in applications of in situ measurements (Gessler et al., 2022; Kinzinger et al., 2024; Landgraf et al., 2022) due to a lack of isotopic variability in both plant water and soil water. The success of our experimental setup (in a semi-arid sub-alpine aspen stand) is based on the following five favorable conditions for a stable isotope approach to study soil-plant water interactions:

- Within the East River catchment, water limited conditions ($\text{PET} > \text{P}$) generally prevail during the summer time, requiring that plants adapt and respond to overcome water scarcity (Berkelhammer et al., 2020).
- There is a strong isotopic difference between summer rainfall (enriched in heavy isotopes) and snowmelt (depleted in heavy isotopes) resulting from the winter snowpack (Sprenger et al., 2022). While rainfall in the East River catchment has $\delta^2\text{H}$ values that are $>-50\text{‰}$, the snowfall is found to be as depleted as -150‰ (Figure 2a).
- Because the water balance in the East River catchment is dominated by snowfall, spring snowmelt drives an

intense infiltration pulse into this hydrological system leading to fully water saturated pores in the soil profile in May (Figure 2b). Therefore, all infiltration happening after the snowmelt of (isotopically enriched) summer rainfall will have a distinct isotope ratio compared to the (isotopically depleted) soil water, of snowmelt origin, at the beginning of the growing season. (d) The dry down of soil moisture in the East River catchment leads to strong changes in soil water isotope ratios in response to monsoonal rainfall, due to a larger share of the newly infiltrating “event water” that mixes with a relatively small amount of “pre-event water” in dry soil (Sprenger et al., 2016). (e) The more deterrent spruce canopy (and litter) results in rainfall interception that's more impedant than the aspen canopy. This differentiating property was a beneficial factor which allowed for detecting species specific processes that impact water infiltration and plant water uptake (Berkelhammer et al., 2025). However, the lack of rainfall infiltration into the soil beneath the spruce trees led to limited soil water $\delta^2\text{H}$ (endmember) variation, which prevents clear identifiability of root water uptake depths in our study. Independent of the study site characteristics, the choice of installing the vapor probes at the bottom of the tree trunks allowed for a reduced travel time of xylem flow after root water uptake, which directly benefits the detectability of variation in short-term plant water uptake (Seeger & Weiler, 2023).

The benefits of a strong isotopic difference between snow and rainfall and the resulting strong variation of isotope ratios in the soil water (across the profile) beneath aspen trees allowed overcoming the following limitations of the in situ approach: (a) The measurements in the field have a lower precision than measurements in the laboratory, and strong tracer differences between endmembers lowers uncertainty in mixing models. (b) We expect relatively high subsurface heterogeneity of water storage and flow and their related isotope ratios (Penna & van Meerveld, 2019; Sprenger et al., 2017). However, the in situ setup limits our ability to install replicate soil profiles due to a trade off between the number of vapor probes across one profile and the installation of several profiles with fewer probes. Because we wanted to capture the potential endmembers of deep versus shallow soil layers for root water uptake during dry and wet periods, we decided to invest in capturing depth variation rather than lateral variation. Our findings showed strong vertical isotopic variation across the depth profile beneath the aspen trees resulting from the above outlined dynamics of infiltration of snowmelt and rainfall. The isotopic variation beneath the spruce trees was much lower due to a lack of rainfall infiltration because of the spruce's canopy interception discussed above. There were several days at which tree A2 and few days at which S3 showed more depleted $\delta^2\text{H}$ (and $\delta^{18}\text{O}$) values than any of the measured soil waters. We interpret these low isotope ratios as a sign for snowmelt sourced from soil layers beneath our observation depth.

We did not include potential foliar water uptake by the trees in our analyses, because we only observed in two of the three spruce and one of the three aspen trees negative sap flow velocities (indicative of downward flux) during some nights. These fluxes were much lower than the positive fluxes during the daytime, which is why we expect any foliar uptake to be much lower in volume than water sourced from root water uptake. We can therefore exclude any impact of potential leaf water uptake on the isotope measurements via the vapor probes installed at the bottom of the tree trunks.

One limitation of the deuterium irrigation experiment in 2023 was that the irrigated area did not cover the entire potential-area occupied by roots of the individually studied trees. Aspen trees, for example, are known to have expansive lateral rooting systems. This shortcoming might explain the limited detection of the irrigated water in the transpiration flux. The differences between the isotopic response observed for spruce and aspen trees suggest that the irrigation water (applied to the dryer spruce profile) was not sufficient to induce a distinct plant uptake signature (of the irrigation water) by spruce trees.

4.2. Compensatory Uptake, as Root Water Temporal and Spatial Origin, Depended on the Water Availability

The subalpine aspen trees we studied compensated for the lack of water in the soil's upper 60 cm by increasing their absolute water uptake to deep soil layers during the observed dry spell. Our data does not support that spruce trees increased their share of neither relative nor absolute uptake from the deepest studied soil layers. However, due to the lack of endmember variability for the potential spruce water uptake depths, we cannot definitively reject the hypothesis that subalpine spruce use compensatory uptake as a drought response mechanism. Our data generally show that the trees' physiological response to the lack of plant available water in the upper 60 cm of soil for the aspen and upper 40 cm for spruce was a shift of the temporal (snowmelt vs. rain) and spatial (soil depth) origin of root water uptake. The subalpine trees were highly adaptable to ambient water scarcity, which led to a

considerably opportunistic response in this water limited environment. A compensatory behavior of the aspen trees is contrary to findings by Gessler et al. (2022) on beech trees during drought, and more aligned with the observations by Ring et al. (2024) of a drought response by birch and maple trees in an urban setting.

Our data revealed that during periods when there was sufficient water available in the upper 30 cm (first and second columns in subplots of Figure 6), the aspen trees took up more water from the upper 30 cm soil depth. This relationship was less clear for the spruce trees, because the soil beneath these trees was much drier and remained relatively dry ($pF > 2$) throughout the growing season. Experimental work by Lopushinsky and Klock (1974) has shown that engelmann spruce reduce their transpiration rate at about 1 bar soil pressure ($=pF 3$) and our sap flow measurements support this, as we saw a strong decline in transpiration rates at $pF > 3$ (Figures 4a–4c). The decline in transpiration as a function of soil pressure explains why root water uptake from the shallow soil is low for spruce, and since the entire upper 60 cm soil was generally dry (approaching $pF 4.2$), spruce transpiration was generally lower than for the aspen. The spruce trees were therefore potentially more adapted to the dry soil, they had a reduced transpiration and did not need to compensate for lack of water in the shallow soil with uptake from deeper soil layers. While there was a decline in the aspen transpiration as a response to the lower water availability, which indicates that root water uptake was not fully compensated by deep soil layers, the soil layers deeper than 60 cm became more important in sustaining the transpiration flux. We can expect higher water availability (lower pF values) in depths below 60 cm, because there is no evaporation loss happening from these depths and snowmelt will be stored in the subsoil.

The opportunistic behavior of the studied trees resulted in an efficient use of rainfall water, which ended up entirely in the evapotranspiration flux, as there were no signs for any percolation of rainfall reaching 60 cm. Our site-specific finding that there was a rapid root water uptake of recently infiltrated water is in good agreement to catchment scale estimates of water sources of evapotranspiration based on isotope mass balance mixing analyses. These endmember splitting evaluations were based on the catchment scale water balance and stable isotope observations of precipitation and streamflow. They revealed that on average about half of summer precipitation in the East River becomes evapotranspiration, and that generally more of the rainfall will sustain evapotranspiration with higher tree canopy coverage in a catchment (Sprenger et al., 2022). Our combination of natural abundance of stable isotopes and artificial tracer experimental work showed that at least for monsoonal rainfall events that occurred during the measurement period of no more than 17 mm/day, no rain water reaches 60 cm soil depth at the forested study site. In other regions of the Western US monsoonal rain was found to infiltrate the 30–60 cm soil layer and provide an essential water resource for aspen and junipers during the late growing season (Samuels-Crow et al., 2023). In contrast, our xylem isotope measurements showed aspen trees rapidly switching their uptake from subsoil layers to recent infiltrate during rewetting in late September 2022 and during the 2H labeling experiment in August 2023.

Also other studies have found a quick shift of the relative contribution from deep to shallow soil layers in response to rainfall after a dry period. e.g., Tang et al. (2018) sampled stable isotopes in soil and plant water on rainfall event basis and showed with that sampling strategy that the trees on an experimental plantation entirely limited their uptake to the upper 20 cm of soil after a heavy rainfall. Similarly to our findings, Martin et al. (2018) found a higher share of shallow soil water in the plant water in the semi-arid snow-dominated Lubrecht Experimental Forest. Volkmann, Haberer, et al. (2016), Volkmann, Kuhnhammer, et al. (2016) showed, based on in situ measurements, a quick increase in the relative uptake from the upper 20 cm after an irrigation had ended an experimental rainfall exclusion.

While we observe that the variation of root water uptake depths from one day to another is limited, our daily measurements show that occasional snapshot sampling of soil and plant water isotopes are not necessarily representative of seasonal differences of dominant plant water uptake depths throughout the growing period. Our observations of short-term response of trees to sub-seasonal water availability cannot be detected with monthly sampling campaigns. However, targeted event-based sampling of soil and plant water isotopes to infer the plant's response to recent rainfall (e.g., Tang et al., 2018) or ongoing drought conditions will potentially allow for considering short-term plant water uptake variation. As such, targeted sampling of soil and xylem material for subsequent isotope analysis in the laboratory could be a valuable alternative to installation of a laser spectrometer in the field.

Our result that the transpiration rate of aspen trees declines during drought conditions, despite a strong increase of their absolute root water uptake volume from 90 cm soil depth during drought, confirms that the shift towards

deeper soil layers and accessing older water is not sufficient to *fully* compensate for lack of plant available water in the shallow soil (see also discussion in Mas et al., 2024). A lack of monsoonal rainfall in the East River catchment will therefore likely affect the health of aspen and spruce trees negatively making the subalpine forest sensitive to climate change and potentially vulnerable to increasing aridity in the region. As such, our findings support the suggested mechanisms by Knighton and Berghuijs (2023) that young water uptake from shallow soil layers will increase forest drought resilience due to efficient use of sparse rainfall, while old water taken up from deeper soil layers will help maximizing the transpiration due to compensatory effects when shallow soil water availability is limited. The concept of water age as a means to assess important water sources is therefore an important tool to understand water availability within the soil-plant-atmosphere continuum in a changing climate which alters the hydrological cycle in mountainous environments particularly.

5. Conclusion

Our study is the first to present short term plant water source dynamics that reveal how subalpine trees change their dominant water sources (within days) in response to variation in water availability. We found that compensatory root water uptake from deep soil layers was an important mechanism by aspen trees during dry periods to sustain their transpiration flux. In particular, we see this compensatory effect in the increased absolute share in the aspen's transpiration flux of water that has an isotopic composition of soil water at 90 cm depth. The lack of isotopic variability along the soil profile beneath the spruce trees prevented an identification of compensatory water uptake mechanism by spruce. Our in situ measurements allow for observing shifts in the temporal and spatial origins of root water uptake, as a result of changes in daily water availability. While strong isotopic differences between snow and rainfall helped to reduce sources of uncertainty in our approach based on natural abundance of isotope ratios, in the first year, the ^2H labeling experiment in the second year further corroborated our findings of opportunistic water use by subalpine aspen trees. We conclude that snowpack meltwater stored in the subsurface serves as the major uptake source for transpiration, and that rainfall is rapidly used by subalpine trees as it becomes available, following summer monsoon rains. A reduction in snowpack depth due to climate change (low-snow future) will unlikely impact water availability for subalpine trees as long as spring snowmelt will still fully saturate the soil profile. However, a potential no-snow future in the Rocky Mountains (Siirila-Woodburn et al., 2021), one in which soil pores would not be entirely filled before the onset of the growing season, would make the subalpine forest highly dependent on monsoonal rainfall to ensure sufficient water availability. Nevertheless, in the current climate, a lack of monsoonal rainfall during the growing season generally lowers water availability within the upper 60 cm of soil, reducing transpiration rates as a result, which slowly diminishes the forest's carbon sequestration capability (Martínez-Sancho et al., 2022) and resilience to bark beetle infestations (Franceschi et al., 2005). At our investigated subalpine forest stands, compensatory root water uptake by aspen trees from deeper soil layers cannot fully replace the lack of water in shallow soil under dry conditions. Consequently, the forest becomes stressed during severely dry summer months in the East River catchment.

Data Availability Statement

The in situ observations of stable isotopes, sap flow and matric potential, as well as the MixSIAR results were made publicly available by Sprenger et al. (2024), the precipitation isotope data was published by Williams et al. (2023), and the meteorological measurements were provided by the Western Regional Climate Center (2024). All data are in the public domain as referred to in the citations.

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